MONSANTO

# Petition for the Determination of Non-Regulated Status for MON 87460

The undersigned submits this petition under 7 CFR Part 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

February 18, 2009
(Revised November 20, 2009)

OECD Unique Identifier: MON-87460-4
Monsanto Petition Number: 07-CR-191U

**Submitted by**

William R. Reeves, Ph.D.
Regulatory Affairs Manager
Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167
Phone: (314) 694-2662
Fax: (314)-694-3080
E-mail: william.r.reeves@monsanto.com

**Prepared by**

Todd A. Pester, Ph.D.

**Contributors and/or Principal Investigators**

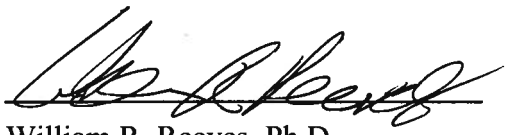
R. Alba, Ph.D., G. Bannon, Ph.D., L. Burzio, Ph.D., C. Carrera, K. Glenn, Ph.D., G. Harrigan, Ph.D., G. Hartnell, Ph.D., M. Horak, Ph.D., L. Lahman, J. Masucci, Ph.D., S. McClain, Ph.D., T. Nickson, Ph.D., J. Petrick, Ph.D., W. Reeves, Ph.D., E. Rice, Ph.D., B. Sammons, Ph.D., A. Silvanovich, Ph.D., Q. Tian, Ph.D., M. Wideman

## Release of Information

Monsanto is submitting the information in this petition for review by the USDA as part of the regulatory process. By submitting this information, Monsanto does not authorize its release to any third party. In the event the USDA receives a Freedom of Information Act request, pursuant to 5 U.S.C., § 552 and 7 CFR Part 1, covering all or some of this information, Monsanto expects that, in advance of the release of the document(s), USDA will provide Monsanto with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, e.g., responsiveness, confidentiality, and/or competitive concerns. Monsanto expects that no information that has been identified as CBI (confidential business information), will be provided to any third party. Monsanto understands that a CBI-deleted copy of this information may be made available to the public in a reading room and by individual request, as part of a public comment period. Except in accordance with the foregoing, Monsanto does not authorize the release, publication or other distribution of this information (including website posting) without Monsanto's prior notice and consent.

## Certification

The undersigned certifies that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioner that are unfavorable to the petition.



William R. Reeves, Ph.D.
Regulatory Affairs Manager

Address:
Monsanto Company
800 North Lindbergh Blvd.
Saint Louis, MO 63167

Phone: (314) 694-2662
Fax: (314)-694-3080
E-mail: william.r.reeves@monsanto.com

**Petition for the Determination of Non-Regulated Status for MON 87460**

## Summary

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has responsibility under the Plant Protection Act (PPA) (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. The APHIS regulation at 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of non-regulated status for the new biotechnology-derived corn (*Zea mays* L.) product, MON 87460, any progeny derived from crosses between MON 87460 and conventional corn, and any progeny derived from crosses of MON 87460 with other biotechnology-derived corn which has been granted non-regulated status under 7 CFR Part 340.

*Drought Tolerance Trait*

Corn is a versatile crop that provides food, feed, and fuel to a global economy. Recently, a surge in demand for corn has been created by growing economies in the developing world and its use as an alternative fuel source in the developed world. These demands are exceeding production, leading to diminished grain reserves worldwide. In addition, climate change may have variable impacts on crop yields, potentially creating further supply disruptions. The combination of these factors places a premium on corn yield stability in sub-optimal environments.

Drought stress is the major cause of yield reduction in corn and its effects have far reaching global socio-economic implications. In North America alone, it is estimated that 40% of annual crop losses are due to sub-optimal water availability. Consequently, increasing drought tolerance in corn is a major goal of breeding selection and biotechnology. Advances in conventional plant breeding and agronomic practices have made contributions to increasing corn drought tolerance and yield potential. Nearly all conventional corn hybrids currently on the market have been bred to exhibit some degree of drought tolerance. Biotechnology provides a significant new tool that can be used in combination with conventional breeding and agronomic practices to increase corn yield stability under water-limited conditions.

Monsanto has developed MON 87460 that reduces yield loss under water-limited conditions compared to conventional corn. Under well-watered conditions, grain yield for MON 87460 is not different from conventional corn. Like conventional corn, MON 87460 is still subject to yield loss under water-limited conditions, particularly during flowering and grainfill periods when corn yield potential is most sensitive to stress

by disrupting kernel development. Under severe water deficit, corn grain yield for MON 87460, as well as conventional corn, can be reduced to zero.

MON 87460 expresses a cold shock protein B (CSPB) produced from the inserted *Bacillus subtilis*-derived gene. In bacteria, the CSPB protein helps preserve normal cellular functions during certain stresses by binding cellular RNA and unfolding non-translatable secondary structures affecting RNA stability and translation. As in bacteria, the CSPB protein in MON 87460 binds RNA and appears to help maintain plant cellular functions. Data suggest that MON 87460 reduces yield loss, primarily through increased kernel number per ear, under water-limited conditions by minimizing the effect of water limitation on photosynthesis, stomatal conductance, and carbon fixation. On a plant level, corn yield losses associated with drought stress occur as a result of reduced synchrony between anthesis and silking, embryo loss, and/or reduced grain filling in viable kernels. Studies on conventional germplasm with enhanced drought tolerance show that yield improvements are attained through improvements in all of these endpoints. Therefore, the enhanced yield stability of MON 87460 under water-limited conditions, conferred by the expressed CSPB protein, appears to be the result of improvements in the natural stress response mechanisms in conventional corn.

*Water Management Regimes Employed for Field Studies*

Because MON 87460 reduces yield loss under water-limited conditions, field studies were designed to assess the plant pest potential of MON 87460 across a broad range of soil moisture and environmental conditions relevant to where commercial production would be expected. In each study, MON 87460 was compared to a conventional control, which had a genetic background similar to MON 87460 but did not possess the drought tolerance trait. In addition, multiple conventional corn hybrids (references) that possess a range of drought tolerance imparted through conventional breeding were included in the analysis to establish a range of natural variability for each measured characteristic. The commercial reference hybrids selected for each study were adapted to the geographic region relevant to each study site; as such, each reference hybrid included some degree of drought tolerance conferred through conventional breeding.

Field studies were established using three different water management regimes: (1) well-watered, (2) both well-watered and water-limited treatments established in the same field, or (3) water managed according to typical or standard agronomic practices, which included typical amounts of supplemental irrigation at relevant sites. Field studies established under well-watered conditions allowed an evaluation of MON 87460 in the absence of trait bias, where no statistical differences were expected between MON 87460 and the control. Field studies managed to establish both well-watered and water-limited conditions allowed an evaluation of MON 87460 under limited soil moisture conditions where it is expected to provide a yield benefit. Finally, field studies managed according to typical agronomic practices allowed an evaluation of MON 87460 under a natural range of environmental conditions relevant to commercial corn production regions. Data and plant tissue samples generated from these field studies with various water management regimes were used to assess the safety of MON 87460 under different soil moisture conditions including: (1) expression levels of the inserted proteins in several

tissue types, (2) compositional analysis of forage and grain, (3) phenotypic and agronomic characteristics at several plant development stages, and (4) environmental interactions. The variation in yield response observed among MON 87460, the control, and commercial reference hybrids in these field studies is within normal levels expected for conventional corn. Therefore, the data from these field studies are relevant to the plant pest potential assessment of MON 87460. In the water-limited sites, the data are also relevant to demonstrate the efficacy of MON 87460. MON 87460 is expected to provide reduced yield loss under water-limited conditions compared to conventional corn. Reduced yield loss is a desirable agronomic characteristic and is not, *per se*, considered to be associated with plant pest potential. In the case of MON 87460, the data and analysis presented in this petition support the conclusion that MON 87460 is unlikely to be a plant pest.

*Data and Information Presented to Assess Plant Pest Potential of MON 87460*

The data and information presented in this application demonstrate the familiarity of MON 87460 as compared to conventional corn and, moreover, show that MON 87460 is unlikely to be a plant pest. This conclusion is based on eight major lines of evidence. The first is that modern corn has inherently low plant pest potential because it is poorly suited to survive without human assistance and is not capable of surviving as a weed due to intense selection for domestication purposes during its evolution as a crop. The second is the molecular characterization of the inserted DNA in MON 87460 which confirms the insertion of a single functional copy of the *cspB* and (neomycin phosphotransferase II (*nptII*) expression cassettes at a single locus within the corn genome. The third is a detailed biochemical characterization of the CSPB and NPTII proteins produced in MON 87460, which shows very low levels of these proteins and demonstrates that the proteins have a history of safe use. The fourth line of evidence is an assessment of the allergenicity and toxicity potential of the CSPB and NPTII proteins based on extensive information collected and studies performed on the two proteins. The results demonstrate with reasonable certainty that the CSPB and NPTII proteins are unlikely to be allergens or toxins. The fifth is the compositional and nutritional assessment which confirms that MON 87460 forage and grain are not compositionally different from conventional corn. The sixth line of evidence is the extensive evaluation of the MON 87460 phenotypic and agronomic characteristics and environmental interactions, which demonstrates that MON 87460 poses no increased plant pest potential, including weediness potential, and no adverse environmental impact compared to conventional corn. The seventh is an assessment on the potential impact on non-target organisms (NTO) and threatened or endangered species, which concludes that MON 87460 is unlikely to have any effect on these organisms under the conditions of use. Finally, the eighth line of evidence is an assessment of agronomic practices confirming that the introduction of MON 87460 is no more likely to have an impact on land use, cultivation practices, or the management of weeds, diseases, and insects than the use of conventionally bred drought tolerant corn.

*Plant Pest Potential of Modern Corn*

In the U.S., corn is not listed as a weed in the major weed references, nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). Modern corn cannot survive as a weed due to intense selection for domestication

purposes during its evolution as a crop. During domestication of corn, traits often associated with weediness such as seed dormancy, a dispersal mechanism, or the ability to form reproducing populations outside of cultivation, have not been selected. For example, the corn ear is enclosed with husks. Consequently, seed dispersal of individual kernels is limited. Even if individual kernels of corn were distributed within a field or along transportation routes from the fields to storage or processing facilities, sustainable volunteer corn populations are not found growing in fence rows, ditches, and road sides. Although corn seed can overwinter and emerge as volunteer plants in rotational crops, the populations do not persist, and agronomic management practices, including mechanical and chemical measures, can be used to control the volunteer plants. Therefore, the plant pest potential of modern corn is inherently low.

*Molecular Characterization of the Inserted DNA*

MON 87460 was produced by *Agrobacterium*-mediated transformation of corn with PV-ZMAP595, a binary vector containing a single transfer DNA (T-DNA). The T-DNA contains two expression cassettes. The first expression cassette produces CSPB and the second expression cassette produces NPTII, a selectable marker that was used during product development. During transformation, the T-DNA was inserted into the genome. Molecular characterization of MON 87460 by Southern blot analyses demonstrate that the DNA inserted into the corn genome is present at a single locus and contains one functional copy of the *cspB* and the *nptII* expression cassettes. All genetic elements are present in the inserted DNA as expected. No backbone plasmid DNA sequences were detected. PCR and DNA sequence analyses provided the complete DNA sequence of the insert and confirmed the organization of the elements within the insert.

The stability of the integrated DNA is demonstrated by the fact that the Southern blot fingerprint of MON 87460 was maintained for seven generations tested in the breeding history. Furthermore, these generations have been shown not to contain any backbone sequence from plasmid PV-ZMAP595. The stability was further confirmed by the fact that the inheritance of the T-DNA in MON 87460 follows Mendelian patterns of segregation.

*Characterization of the CSPB and NPTII Proteins*

The expression levels of CSPB and NPTII proteins were determined in MON 87460 tissues produced during two growing seasons from multiple field sites in the major corn production regions of the U.S. and Chile. In the U.S. during 2006, six sites were established using typical water management practices as a standard assessment under a natural range of environmental conditions. In Chile during 2006/2007, the experimental design at three sites included well-watered and water-limited treatments to evaluate the effect of soil moisture level on CSPB and NPTII protein levels. The results demonstrate that both CSPB and NPTII proteins were expressed in all tissues collected, including leaf, root, forage, silk, pollen, grain, and stover, the expression levels declined over the growing season, and were similar under both well-watered and water-limited conditions. Results from the U.S. 2006 study show the mean CSPB protein levels (µg/g dwt) across all test sites were 0.072 in grain, 0.10 in forage, 13 in pollen, 3.1 in leaves of plants at V2-V4 stage, 0.47 in leaves of plants at pre-VT stage, 0.029 in forage root, and 0.042 in

stover. In tissues harvested throughout the growing season, mean CSPB protein levels (µg/g dwt) across all test sites varied from 0.47-3.1 in leaf, 0.24-1.4 in root, and 0.67-2.8 in whole plant. In general, levels of the CSPB protein declined over the growing season. The mean NPTII protein levels (µg/g dwt) across all test sites were less than the limit of quantitation in grain, 0.12 in forage, 2.6 in leaves of plants at V2-V4 stage, and 0.47 in roots of plants at V2-V4 stage. Results from the Chile 2006/2007 study show the mean CSPB protein levels (µg/g dwt) across all test sites under well-watered and water-limited treatments, respectively, were 0.048 and 0.038 in grain, 0.11 and 0.15 in forage, 25 and 27 in pollen, 2.8 and 2.8 in leaves of plants at V2-V4 stage, 0.39 and 0.44 in leaves of plants at pre-VT stage, 0.039 and 0.076 in forage root, and 0.033 and 0.072 in stover. In tissues harvested throughout the growing season, mean CSPB protein levels (µg/g dwt) across all test sites under well-watered and water-limited treatments, respectively, varied from 0.39-2.8 and 0.44-2.8 in leaf, 0.31-1.3 and 0.40-1.5 in root, and 0.67-3.2 and 0.70-2.9 in whole plant. In general, levels of the CSPB protein declined over the growing season and were similar between the well-watered and water-limited treatments. The mean NPTII protein levels (µg/g dwt) across all test sites under well-watered and water-limited treatments, respectively, were less than the limit of quantitation in grain, 0.16 and 0.17 in forage, 2.4 and 2.6 in leaves of plants at V2-V4 stage, and 0.51 and 0.48 in roots of plants at V2-V4 stage. The results show that the level of CSPB protein was very low in all tissue types, the level declined over the growing season, and there were no obvious differences in expression level in tissues collected from plants grown under well-watered or water-limited conditions. The level of NPTII protein in grain was below the Limit of Quantitation (LOQ) of the method, and there were no obvious differences observed in NPTII protein levels in the other three tissue types collected from plants grown under well-watered or water-limited conditions.

Detailed safety assessments of CSPB and NPTII and their respective donor organisms establish that these proteins are safe for human consumption. CSPB has a history of safe consumption and NPTII is present in several biotechnology-derived crops that have undergone previous safety assessments. The history of safe use and data from multiple studies demonstrate the safety of MON 87460 and the CSPB and NPTII proteins. The donor organism of the CSPB protein, ***B. subtilis***, is not pathogenic, is present in many fermented foods, and has a history of safe consumption. Proteins containing cold shock domains are ubiquitous in nature, being present in many plants and common bacteria including species that are normally present in gastrointestinal flora. Cold shock proteins (CSPs) have no known toxicity and are not associated with pathogenicity. The CSPB protein is homologous to the CSPs found in *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, and *E. coli*, which are normally present in gastrointestinal flora and, therefore, considered to be safe. The amino acid identity ranges from 35% to 98.5% across different plant and bacterial species. The strains of lactic acid bacteria, *Bifidobacterium* and *Lactobacillus*, are the most common type of bacteria used in the dairy industry for preparation of probiotic products containing live bacterial cultures. In addition, *Bacillus*, *Lactobacillus, and Lactococcus* species containing CSPs are involved in many food fermentation processes of milk, meats, cereals, and vegetables.

*Allergenicity and Toxicity Potential of the CSPB and NPTII Proteins*

CSPB protein does not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which have adverse effects on mammals. This has been shown by extensive assessments with bioinformatic tools, such as FASTA sequence alignment search and an eight-amino acid sliding window search. Digestive fate experiments conducted with the CSPB protein demonstrate that the full-length protein is rapidly digested in simulated gastric fluid (SGF), a characteristic shared among many proteins with a history of safe consumption. A small transiently stable CSPB protein fragment was very quickly degraded during short exposure to simulated intestinal fluid (SIF). Rapid digestion of the full-length CSPB protein in SGF and SIF, together with rapid degradation of the small transiently stable fragment in SIF, indicates that it is highly unlikely that the CSPB protein and its fragment will reach absorptive cells of the intestinal mucosa. Proteins that are rapidly digestible in mammalian gastrointestinal systems are unlikely to be allergens when consumed. Finally, the CSPB protein represents no more than 0.00007% of the total protein in the grain of MON 87460. Acute oral toxicity studies with mice demonstrate that CSPB protein is not acutely toxic and does not cause any adverse effects even at the highest dose levels tested, which was 4.7 mg/kg body weight. The dietary safety assessment of CSPB based on the acute toxicity data and corn product dietary pattern establishes that the margin of exposure (MOE) for the overall U.S. population is 26,700. For children aged 1-6 years old, an age group with the highest corn consumption on a body weight basis, the MOE was greater than or equal to 11,400 for CSPB. Dietary exposure in animals will also be low with chickens, swine, and dairy cows consuming only nanogram quantities of each protein per kilogram of body weight. Taken together, these data indicate that food and feed derived from MON 87460, which contain the CSPB protein, are safe for consumption.

The safety of NPTII has been extensively evaluated through several lines of experimental evidence, and several products containing NPTII have been approved by regulatory agencies on a global basis. NPTII is the most commonly used antibiotic resistance marker in several commercially grown biotechnology-derived crops including YieldGard® Rootworm corn (MON 863), Bollgard® cotton (MON 531), Bollgard®II cotton (MON 15985), and Roundup Ready cotton (MON 1445). FDA evaluated NPTII as part of a petition for FLAVR SAVR® tomatoes and approved its use as a food additive. Additionally, EPA established an exemption from the requirement of a tolerance for NPTII for use as a selectable marker in raw agricultural commodities. In 2007, the European Food Safety Authority (EFSA) affirmed its conclusion that the presence of *nptII* does not pose a threat to human health or the environment. Extensive bioinformatic assessments with tools such as FASTA sequence alignment search and an eight-amino acid sliding window search demonstrate that NPTII does not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which have adverse effects on mammals. Numerous studies have suggested that the presence of this antibiotic-resistance gene in any crop or crop products will have no impact on food safety. Studies using purified NPTII protein revealed that NPTII degrades rapidly in simulated gastric and intestinal fluids suggesting that the protein is unlikely to cause an allergic response. Acute oral toxicity studies with mice demonstrate that NPTII is not acutely toxic and does not cause any adverse effects even at the highest dose levels

tested, 5,000 mg/kg body weight. A dietary safety assessment for MON 87460 based on the acute toxicity data and a corn product dietary consumption pattern established that the MOEs are very high for the overall U.S. population and for children aged 1-6 years old, an age group with the highest corn consumption on a body weight basis. NPTII is ubiquitous in *E. coli,* and, therefore, is normally present within the human gastrointestinal tract. Moreover, USDA previously evaluated the safety of NPTII in other biotechnology-derived products, including corn. Similar to these products, there is negligible risk for the production of NPTII in MON 87460 to result in a plant pest risk. A published assessment of the ecological impact of NPTII in crops reported that the amount of free kanamycin accumulating in soils, through the action of microorganisms or animal feces, is restricted by absorption to soil components so that no direct selection pressure for kanamycin resistant plants can occur. Also, enhanced physiological fitness resulting from potential pleiotropic effects of *nptII* gene expression has not been observed. Thus, based on all the available evidence, it can be concluded that the NPTII protein is safe for use as a selectable marker in biotechnology-derived plants.

*Composition and Nutrition of Forage and Grain*

A detailed compositional assessment of MON 87460 confirmed that it is as safe and nutritious as conventional corn. The composition of MON 87460 was determined from forage and grain tissues produced during two growing seasons from multiple field sites in the major corn production regions of the U.S. and Chile. In each assessment, MON 87460 was compared to an appropriate conventional control, which had a genetic background similar to MON 87460 but did not possess the drought tolerance trait. In addition, multiple conventional corn hybrids (references) that possess a range of drought tolerance imparted through conventional breeding were included in the analysis to establish a range of natural variability for each analyte, where the range of variability is defined by a 99% tolerance interval for that particular analyte. In the U.S. during 2006, six sites were established using typical water management practices as a standard assessment under a natural range of environmental conditions. In Chile during 2006/2007, the experimental design included well-watered and water-limited treatments to evaluate the effect of soil moisture level on component levels. Across both years of data there were no consistent differences between MON 87460 and the control. Within the U.S. 2006 combined-site analysis, there were no significant differences ($p>0.05$) for 59 (95.2%) of 62 comparisons. Furthermore, results from the U.S. 2006 study show that there were no significant differences ($p>0.05$) for 407 (93.7%) of the 434 total comparisons made between the MON 87460 test and the control. The 27 detected differences were not consistent across sites, were small in magnitude and the mean component values of MON 87460 and the control were within the 99% tolerance interval established from the commercial reference hybrids that were produced at the same time and field sites as MON 87460 and the control. Within the combined site analysis of the well-watered plots from the Chile study, there were no significant differences ($p>0.05$) for 59 (96.7%) of 61 comparisons. Furthermore, results from well-watered plots in the Chile study show that there were no significant differences ($p>0.05$) for 230 (94.3%) of the 244 total comparisons made between MON 87460 and the control. The 14 detected differences were not consistent across sites, were small in magnitude, and the mean component values of the test and control substances were within the 99% tolerance

interval established from the commercial reference hybrids that were produced at the same time and field sites as MON 87460 and the control. Within the combined site analysis of the water-limited plots from the Chile study, there were no significant differences ($p>0.05$) for 59 (96.7%) of 61 comparisons. Furthermore, results from the water-limited plots in the Chile study show that there were no significant differences ($p>0.05$) for 233 (95.5%) of the 244 total comparisons made between MON 87460 and the control. The nine detected differences were not consistent across sites, were small in magnitude and the mean component values of the test and control substances were within the 99% tolerance interval established from the commercial reference hybrids. Samples from the Chile study were also analyzed for 11 secondary metabolites that are potentially associated with drought stress. Results from this additional analysis further confirm that MON 87460 is not compositionally different from conventional corn. All compositional analyses, therefore, support the conclusion that MON 87460 is not different from conventional corn when grown under a broad range of environmental conditions.

*Phenotypic and Agronomic Characteristics and Environmental Interactions*

The plant characterization and environmental interactions assessment of MON 87460 demonstrates that it poses no increased plant pest potential, including weediness potential. Data were collected to evaluate the likelihood of altered plant pest potential based on requirements of USDA-APHIS. The phenotypic, agronomic, and environmental interactions data were evaluated from a basis of familiarity and were comprised of a combination of field, greenhouse, and laboratory studies. Phenotypic and agronomic characteristics of MON 87460 were evaluated relative to an appropriate control under a broad range of environmental conditions to assess plant pest potential and potential environmental impact. These assessments included evaluations among 14 plant growth and development characteristics, five seed germination parameters, two pollen characteristics, observations for plant-insect and plant-disease interactions, abiotic stressors, volunteer potential, and survival outside cultivation. Each of the measured characteristics is useful to assess familiarity and plant pest potential of MON 87460 compared to conventional corn. In addition, certain characteristics can be used to assess weediness potential, including seed germination and dormancy (hard seed), pre-harvest seed loss characteristics (lodging and ear drop), and the potential to volunteer in cultivated areas or survive outside cultivation.

The phenotypic field studies were conducted during 2006 and 2007 in the major corn production regions of the U.S. and Chile. Six field studies totaling 31 sites were established using the three water management regimes described above: (1) well-watered (17 sites); (2) well-watered and water-limited treatments (9 sites); and (3) water managed according to typical local agronomic practices and water conditions (5 sites).

Results from the combined-site analyses within each of the six studies detected only four instances of a phenotypic difference between MON 87460 and the control. For the well-watered regime, two separate studies totaling 17 sites were established in the U.S. during 2006 (8 sites) and 2007 (9 sites). In the combined-site analyses of these data no differences were detected between MON 87460 and the control in the 2007 study. In the 2006 study, an increase in root lodged plants was detected for MON 87460 compared to

the control (5.6 vs. 1.5, respectively). For the well-watered and water-limited regime, three different studies totaling nine field sites (six sites included in combined-site analyses) were established in Chile (3 sites in 2006/2007) and the U.S. (3 sites in 2007 from two separate studies). In the Chile 2006/2007 study, no phenotypic differences were detected with the exception of the expected increase in yield for MON 87460 compared to the control under water-limited conditions (114.5 vs. 86.7 bushels/acre, respectively). In the U.S. 2007 Study-1, no phenotypic differences were detected in either the well-watered or water-limited treatments. In the U.S. 2007 Study-2, stay green rating was lower (more green tissue) for MON 87460 compared to the control in both the well-watered (5.8 vs. 6.7) and water-limited treatments (6.3 vs. 8.3), respectively. For the typical agronomic practices regime, one study with five sites was established in the U.S. during 2006 and no differences between MON 87460 and the control were detected in this study. In summary, the phenotypic data support the conclusion that MON 87460 possesses no characteristics that would confer increased plant pest potential, including weediness potential, or result in adverse environmental impacts compared to conventional corn. With the exception of the expected differences in yield under water-limited conditions, the magnitude of the detected differences was small, the differences did not represent a trend in the data across studies and years, and the mean values of MON 87460 were within the range of values observed for the commercial references.

No biologically meaningful differences were detected in the germination and dormancy of seed from MON 87460. In particular, the absence of hard seed supports a conclusion of no increased weediness potential of MON 87460 compared to conventional corn for germination and dormancy characteristics. No differences were detected in pollen morphology or viability between MON 87460 and the control. Results from the environmental interactions assessments also support the conclusion that MON 87460 has no increased susceptibility or tolerance to specific diseases, arthropods, or abiotic stressors, with the exception of drought. Finally, MON 87460 was not altered in its ability to volunteer in cultivated fields or survive in areas not managed for agricultural production compared to conventional corn.

*Non-Target Organisms and Threatened or Endangered Species*

The environmental assessment of MON 87460 demonstrates that it poses negligible risk to non-target organisms and will not affect threatened or endangered species under the conditions of use. The assessment took into consideration the familiarity with CSPB and NPTII protein modes of action and their expression levels in MON 87460. Although CSPB is not known to exert any effects on pest and non-pest organisms, studies were conducted to examine the potential effects of MON 87460 on biotic stressors that may affect corn. Studies demonstrate a lack of any effects observed in various species exposed to MON 87460. Additional data and information presented demonstrate that MON 87460 is not different from conventional corn with respect to persistence, invasiveness, or gene flow.

MON 87460 is unlikely to outcross with sexually compatible species in the U.S. Corn and annual teosinte (*Zea mays* subsp. *mexicana*) are genetically compatible, wind-pollinated, and may hybridize when in close proximity to each other, e.g., in areas of

Mexico and Guatemala. However, teosinte is not present in the U.S. other than as an occasional botanical garden specimen and a few small feral populations of *Zea mexicana* in Florida, Alabama, and Maryland and *Zea perennis* in South Carolina. Differences in factors such as flowering time, geographical separation, and development factors make natural crosses in the U.S. highly unlikely. In contrast with corn and teosinte which easily hybridize under certain conditions, special techniques are required to hybridize corn and *Tripsacum* and the open literature indicates that the offspring of the cross show varying levels of sterility. The species *Tripsacum floridanum*, found in extreme southern Florida, has been categorized as a threatened species by the state of Florida and is listed on the USDA's Natural Resources Conservation Service Database. However, given the level of difficulty for natural hybridization between species of *Tripsacum* and *Zea*, and the occurrence of *T. floridanum* primarily in both highly urbanized and non-agricultural, swampy areas of the state, it is very unlikely there would be any impact on this species due to the introduction of MON 87460.

*Corn Agronomic Practices*

Finally, an assessment of current corn agronomic practices confirmed that the introduction of MON 87460 is no more likely to impact land use, cultivation practices, or the management of weeds, diseases, and insects than the use of conventionally bred drought tolerant corn. The introduction and rapid adoption of biotechnology-derived corn products in the past decade have had no significant impact on land use or cropland acreage in the U.S. The total crop area in the U.S. has remained relatively steady as is the case for field corn acreage. Cumulative impacts to seed production, crop rotation and tillage practices, or weed, disease, and insect management practices are not expected from the introduction of MON 87460. Conventionally bred, drought tolerant hybrids have been planted in the U.S. for decades without any documentation of such effects. Because MON 87460 reduces yield loss under water-limited conditions, it is foreseeable that continued improvements in drought tolerance for corn, including the introduction of MON 87460, may result in the cumulative benefit of decreasing water demands for irrigation in the Great Plains. This trend is already underway due to the continued improvements in conventionally bred drought tolerant corn, and the introduction of MON 87460 is not anticipated to have a significant impact. Thus, MON 87460 is environmentally benign and beneficial to corn growers in areas suitable for commercial corn production, but prone to frequent drought stress.

*Conclusion*

Based on the data and information presented in this petition, it is concluded that MON 87460 is unlikely to be a plant pest. The adoption of MON 87460 may increase economic and environmental benefits, primarily in the Western Dryland region of the Great Plains, due to the protection of corn yield under water-limited conditions, but is not expected to have a significant environmental impact. Therefore, Monsanto Company requests a determination from APHIS that MON 87460 and any progeny derived from crosses between MON 87460 and other commercial corn be granted non-regulated status under 7 CFR Part 340.

## Table of Contents

## List of Tables

## List of Figures

## Abbreviations, Acronyms, and Definitions

Note: Standard abbreviations, e.g., units of measure, are used according to the format described in 'Instructions to Authors' in the *Journal of Biological Chemistry*.

| | |
|---|---|
| 1× LB | Laemmli Buffer [62.5mM Tris-HCl, 5% (v/v) 2-mercaptoethanol, 2% (w/v) sodium dodecyl sulfate, 0.005% (w/v) bromophenol blue, 10% (v/v) glycerol, pH 6.8] |
| 5× LB | Five times concentrated 1× LB |
| 6-FAM | 6-carboxyfluorescein |
| *35S* | Promoter and leader from the Cauliflower mosaic virus (CaMV) 35S RNA |
| AA | Amino acid |
| AACC | American Association of Cereal Chemists |
| *aadA* | Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7 |
| AD8 | Allergen, gliadin, and glutenin protein sequence database |
| ADF | Acid detergent fiber |
| AEC buffer | Buffer solution of 20 mM Tris-HCl, pH 7.0 |
| ALLERGEN-SEARCH | Computer program for the search against known allergens |
| ALLPEPTIDES | Protein sequence database comprised of NRAA and SwissProt databases |
| AOAC | Association of Official Analytical Chemists |
| AOCS | American Oil Chemists Society |
| AOSA | Association of Official Seed Analysts |
| AOSCA | Association of Official Seed Certifying Agencies |
| APHIS | Animal and Plant Health Inspection Service |
| APS | Analytical protein standard |
| ATP | Adenosine triphosphate |
| B | Border |
| *Blp* I | Restriction enzyme isolated from *Xanthomonas badrii* |
| bp | Base pair |
| BSA | Bovine serum albumin |
| *B. subtilis* | *Bacillus subtilis* |
| bu/ac | Bushels per acre |

| | |
|---|---|
| bw | Body weight |
| CBI | Confidential business information |
| CFIA | Canadian Food Inspection Agency |
| CFR | Code of Federal Regulations |
| CI | Confidence interval |
| CIMMYT | International Maize and Wheat Improvement Center |
| CRP | Conservation Reserve Program |
| CS | Coding sequence |
| CSD | Cold shock domain |
| CSP | Cold shock protein |
| *cspB* | Coding sequence for CSPB from *B. subtilis* |
| CSPB | Cold shock protein B from *B. subtilis* |
| CTAB | Cetyltrimethylammonium bromide |
| DAP | Days after planting |
| DAT | Days after treatment |
| dATP | Deoxyadenosine triphosphate |
| dCTP | Deoxycytidine triphosphate |
| DLP | Dual labeled probe |
| DNA | Deoxyribonucleic acid |
| dNTP | Deoxyribonucleotide triphosphate, a generic term referring to the four deoxyribonucleotides: dATP, dCTP, dGTP (deoxyguanosine triphosphate) and dTTP (deoxythymidine triphosphate) |
| dT | Deoxythymidine |
| DW or dw | Dry weight |
| DWCF | Dry weight conversion factor |
| dwt | Dry weight of tissue |
| EC | Electrical conductivity |
| *E. coli* | *Escherichia coli* |
| *Eco*O109 I | Restriction enzyme isolated from *E. coli* |
| *EcoR* V | Restriction enzyme isolated from *E. coli* |
| ECL | Enhanced chemiluminescence |
| EDTA | Ethylenediaminetetraacetic acid |
| EFSA | European Food Safety Authority |
| ELISA | Enzyme-linked immunosorbent assay |
| EPA | Environmental Protection Agency |

| | |
|---|---|
| F15 | Phenylalanine 15 |
| F27 | Phenylalanine 27 |
| F30 | Phenylalanine 30 |
| FA | Fatty acid |
| FASTA | Algorithm used to find local high scoring alignments between a pair of protein or nucleotide sequences |
| FDA | Food and Drug Administration |
| FONSI | Finding of No Significant Impact |
| FW or fw | Fresh weight |
| fwt | Fresh weight of tissue |
| GRAS | Generally recognized as safe |
| H29 | Histidine 29 |
| HC | Health Canada |
| HI | Harvest index |
| HMM | Health Ministry of Mexico |
| *Hin*d III | Restriction enzyme isolated from *Haemophilus influenzae* |
| HPLC | High-performance liquid chromatography |
| HRP | Horseradish peroxidase |
| I | Intron |
| ILSI | International Life Sciences Institute |
| ILSI CCD | International Life Sciences Institute Crop Composition Database |
| I-*Ract1* | Intron from the rice actin gene |
| kb | kilobase |
| L | Leader |
| L2V | Amino acid change in MON 87460–produced CSPB that substitutes lysine in position two to valine |
| Left Border | DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA |
| LOD | Limit of detection |
| LOQ | Limit of quantitation |
| *loxP* | Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase |
| MAFF | Ministry of Agriculture, Forestry and Fisheries (Japan) |
| MALDI-TOF | Matrix Assisted Laser Desorption Ionization - Time of Flight |
| MH+ | Protonated mass ion |

| | |
|---|---|
| MHLW | Ministry of Health, Labor, and Welfare (Japan) |
| MOE | Margin of exposure |
| MON 863 | A Monsanto corn product, producing the insecticidal *Bacillus thuringiensis* Cry3Bb1 protein and NPTII protein |
| MON 87460 | A Monsanto corn product, and the subject of this application, which produces CSPB and NPTII proteins |
| MON 89034 | A Monsanto corn product, producing the insecticidal *Bacillus thuringiensis* Cry1A.105 and Cry2Ab2 proteins |
| mRNA | Messenger RNA |
| MS | Mass spectrometry |
| MT/ha | Metric tons per hectare |
| MWCO | Molecular weight cut-off |
| MW | Molecular weight |
| n | Number of observations |
| N/A | Not applicable |
| NA | Not available |
| NCGA | National Corn Grower's Association |
| NDF | Neutral detergent fiber |
| NEPA | National Environmental Policy Act |
| NFDM | Non-fat dry milk |
| NK603 | A Monsanto corn product producing the glyphosate-tolerant CP4 EPSPS protein |
| NOEL | No observable effect level |
| NOP | National Organic Program |
| *Not* | Restriction enzyme isolated from *Nocardia otitidis* |
| *nptII* | Coding sequence of neomycin phosphotransferase II gene that confers resistance to neomycin and kanamycin |
| NPTII | Neomycin phosphotransferase II |
| NTO | Non-target organism |
| OECD | Organization for Economic Co-operation and Development |
| OFPA | Organic Foods Production Act |
| OR | Origin of replication |
| *ori-pBR322* | Origin of replication from pBR322 for maintenance of plasmid in *E. coli* |
| *ori V* | Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2 |

| | |
|---|---|
| OSL | Overseason leaf |
| OSR | Overseason root |
| OSWP | Overseason whole plant |
| P | Promoter |
| PAR | Photosynthetically active radiation |
| PBS | Phosphate buffered saline |
| PBST | Phosphate buffered saline containing Tween-20 |
| PCR | Polymerase chain reaction |
| PDB | Protein data bank |
| ppm | Parts per million |
| PRESS | Predicted residual sums of squares |
| PTH | Phenylthiohydantoin |
| PVDF | Polyvinylidene difluoride |
| PVP | Polyvinyl pyrrolidone |
| PV-ZMAP595 | Plasmid vector used to develop MON 87460 |
| *Ract1* | The rice actin gene |
| Right Border | DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA |
| RNA | Ribonucleic acid |
| RNP | Ribonucleoprotein |
| RK2 | Broad host range bacterial plasmid |
| *rop* | Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* |
| ROP | Repressor of primer protein |
| SAS® | Originally an acronym for Statistical Analysis System, now an integrated system of software products provided by the SAS Institute, Inc. headquartered in Cary, North Carolina, USA |
| SD | Standard deviation |
| SDS-PAGE | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| S.E. | Standard error |
| SGF | Simulated gastric fluid |
| SIF | Simulated intestinal fluid |
| SOP | Standard operating procedure |
| sp. | Species |
| SPAD | Single photon avalanche diode |

| | |
|---|---|
| ss | Single stranded |
| subsp. | Subspecies |
| *Taq* | *Thermus aquaticus*, a thermophilic bacterium |
| T | Terminator (where used as a prefix to a gene sequence) |
| TCA | Trichloroacetic acid |
| T/C/R | Test/Control/Reference materials |
| T-DNA | Transfer DNA |
| TDF | Total dietary fiber |
| TE buffer | Tris-EDTA buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA, pH 8.0, 1M NaCl) |
| TFA | Trifluoroacetic acid |
| TMB | 3,3',5,5'-Tetramethylbenzidene |
| Tn5 | Prokaryotic *E. coli* transposon from which the nptII coding sequence is derived. |
| T-nos | 3' nontranslated transcript termination sequence of the nopaline synthase gene from *Agrobacterium tumefaciens* |
| Tris | Tris(hydroxymethyl)aminomethane |
| T-*tr7* | 3' nontranslated sequence of the transcript 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation |
| TSSP | Tissue-specific site pool |
| Tween-20 | Polyoxyethylenesorbitan monolaurate |
| USDA | United States Department of Agriculture |
| USDA-APHIS | United States Department of Agriculture – Animal and Plant Health Inspection Service |
| USDA-ERS | United States Department of Agriculture – Economic Research Service |
| USDA-NASS | United States Department of Agriculture – National Agricultural Statistics Service |
| U.S. EPA | United States Environmental Protection Agency |
| UV | Ultraviolet |
| v/v | Volume to volume ratio |
| w/v | Weight to volume ratio |
| Xba | Restriction enzyme isolated from *Xanthomonas badrii* |

# I. Rationale for the Development of MON 87460

## I.A. Basis for the Request for a Determination of Nonregulated Status under 7 CFR Part 340.6

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772) and the Plant Quarantine Act (7 U.S.C. § 151-167), to prevent the introduction and dissemination of plant pests into the United States. The APHIS regulation 7 CFR Part 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

## I.B. Product Concept and Benefits of MON 87460

### I.B.1. Product Concept

Monsanto has developed MON 87460 that reduces yield loss under water-limited conditions compared to conventional corn. Under well-watered conditions, grain yield for MON 87460 is not different from conventional corn. Like conventional corn, MON 87460 is still subject to yield loss under water-limited conditions, particularly during flowering and grainfill periods when corn yield potential is most sensitive to stress, by disrupting kernel development (Claassen and Shaw, 1970; Boyer and Westgate, 2004). Under severe water deficit, corn grain yield for MON 87460, as well as conventional corn, can be reduced to zero.

Knowing that stress response proteins allow organisms to adapt to and survive adverse environments, Monsanto scientists hypothesized that inserting a stress response protein into plants could impart a desirable phenotype. As part of a broad screening effort, the gene encoding CSPB from the soil bacterium *B. subtilis* was inserted into corn using *Agrobacterium*-mediated transformation. Transformed plants were tested for their ability to yield more grain than control plants under water-limited conditions. The CSPB protein expressed in MON 87460 exhibits key behaviors and properties that are similar to what is reported for bacterial cold shock proteins and cold shock domain-containing proteins in plants.

### I.B.2. Product Benefits

MON 87460 is expected to provide significant value to corn producers and consumers. Corn is the largest crop grown in the U.S. in terms of acreage planted and net value (USDA-NASS, 2007). Limited water availability is the single most important factor that reduces global crop yields. In North America alone, it is estimated that 40% of annual crop losses are due to sub-optimal water availability (Boyer, 1982). In both temperate and tropical regions suitable for commercial corn production globally, the average annual corn yield loss attributable to moderate water deficits is approximately 15% (Barker et al., 2005). During periods of severe drought, these losses can be much higher and can potentially result in complete crop failure.

Improved yields under water-limited conditions will help to ensure a stable grain supply, even in years with low rainfall. Growers are expected to adopt MON 87460 in regions suitable for corn production that are most prone to frequent drought stress. In the U.S., the major area of adoption is likely to be the Western Dryland region of the Corn Belt. This region has also been defined as the Great Plains and includes portions of ten states (Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas and Wyoming) contributing 26% of the national corn production (Riebsame, 1990). Annual precipitation in this region averages between 12 to 20 inches (30 to 50 cm) (http://prism.oregonstate.edu). Other corn producing geographies with similar conditions, such as parts of Africa, Europe, and Latin America, may also benefit from this trait.

The adoption of MON 87460 is expected to provide benefits due to reduced yield loss under water-limited conditions. While much progress has been made to improve corn yield in water-limited environments through breeding, selection, and agronomic management practices, there remains potential for additional improvement. Higher corn yield per acre may help conserve the total number of acres needed to meet the needs for food, feed, and biofuel uses or produce more corn grain on the same number of acres already used for corn production. Positive impacts on yield and improved yield stability will provide value to producers, consumers, and the environment.

Efficacy of MON 87460 can be demonstrated by directly comparing its grain yield to a conventional control under water-limited conditions. A detailed assessment of the agronomic and phenotypic characterization of MON 87460, including yield, is presented in Section VIII. A sub-set of these data, including field trials conducted under water-limited conditions, are useful to demonstrate efficacy of MON 87460.

Because MON 87460 reduces yield loss under water-limited conditions, field studies were designed to evaluate the environmental safety of MON 87460 across a broad range of soil moisture and environmental conditions relevant to where commercial production would be expected. Field studies that are relevant to demonstrate the efficacy of MON 87460 were established using two different experimental designs and water management regimes: (1) well-watered only, or (2) both well-watered and water-limited treatments established in the same field arranged in either a strip- or split-plot design.

Field studies managed to impose only well-watered conditions were established at 17 sites in the U.S. during 2006 and 2007. The well-watered treatment was intended to provide optimal grain yield and allowed an evaluation of MON 87460 in the absence of trait bias, with no differences in yield expected between MON 87460 and the control. The water treatment in these studies required available soil moisture to be maintained at $\geq$50% of field capacity for the duration of the study to avoid drought stress conditions. Water was provided by natural rainfall and supplemental irrigation as needed.

Field studies managed to impose both well-watered and water-limited conditions were established at nine sites in the U.S. and Chile during 2006 and 2007. The water-limited

treatment allowed an evaluation of MON 87460 under conditions where it is expected to provide a yield benefit compared to the control. The well-watered treatment in these studies required available soil moisture to be maintained at ≥50% of field capacity for the duration of the study to avoid drought stress conditions and provide optimal grain yield. The water-limited treatment was managed the same as the well-watered treatment, with the exception that available soil moisture be reduced to <50% of field capacity to impose a moderate drought stress during the late vegetative through early grain fill growth stages (~V10 – R3) when corn yield potential is most sensitive to stress (Claassen and Shaw, 1970; Boyer and Westgate, 2004).

For the nine field sites established with both well-watered and water-limited treatments, criteria were established to identify sites that were managed appropriately to impose the defined treatment levels (Section VIII.C). Specifically, for a site to be considered water-limited, commercial reference hybrids in the water-limited plots had to exhibit a minimum 15% reduction in yield compared to the same reference hybrids planted in the well-watered plots. The 15% yield loss criteria represents a meaningful stress level, as moderate water deficits result in approximately a 15% yield loss annually for corn grown in both temperate and tropical regions (Barker et al., 2005). Reductions in plant height, ear height, and days to 50% silking are also phenotypic indicators of moisture deficit stress in corn (Campos et al., 2006); therefore, these characteristics were assessed to confirm the inclusion of sites that met the 15% yield reduction requirement. Due to untimely rainfall during the imposed water-limitation treatments and complications in water management techniques, only six of the nine established sites met the inclusion criteria of having both well-watered and water-limited treatments for plant characterization assessments requiring both treatments (Section VIII.C, Table VIII-3).

The efficacy of MON 87460 under water-limited conditions is demonstrated by plotting the yield advantage of MON 87460 versus the relative stress level imposed among the nine field sites (Figure I-1). Yield advantage of MON 87460 was calculated as the average yield of MON 87460 compared to the control within each site. Relative stress level is represented by the yield loss of the commercial references grown under water-limited conditions compared to well-watered treatments. Yield loss of references was calculated as the average yield of the references in the well-watered treatment compared to the average yield under the water-limited treatment within each site. The presented data represent the nine sites that were established with both well-watered and water-limited treatments (Section VIII.C, Table VIII-3). As expected, the yield advantage for MON 87460 tended to increase with higher levels of water deficit stress, but there was large variation. The three data points in the lower left portion of Figure I-1 represent the three sites that did not meet the inclusion criteria (i.e., QUI site in Chile 2006/2007 and the KS and NE sites in U.S. 2007 Study-2) (Table VIII-3). Yield loss of the references at these three sites did not meet the required minimum 15% reduction due to the imposed water-limitation treatment, indicating the appropriate water stress level was not established. Consistent with the data for MON 87460 grown under well-watered field conditions, MON 87460 did not provide a yield advantage compared to the control at these three sites when water stress was minimal. As described previously (Section I.B.1), MON 87460 is still subject to yield loss under water-limited conditions, and under severe

water deficit, yield can be reduced to zero. Intuitively, as stress levels (i.e., yield loss of the references) increase beyond the range presented in Figure I-1, the yield advantage of MON 87460 compared to the control should be expected to decrease and be eliminated as the grain yield for both MON 87460 and the control falls to zero.

A comparison of the average percent yield advantage of MON 87460 compared to the control under both field trial designs also demonstrates the efficacy of MON 87460 under water-limited conditions (Figure I-2). Percent yield advantage values were calculated as the average yield of MON 87460 versus the control, averaged across all sites for each treatment. Reference yield values presented at the top of the graph represent the average yield of the commercial references averaged across all sites for each treatment. The well-watered and water-limited treatments include only the six sites that met the required inclusion criteria (Section VIII.C, Table VIII-3). In the well-watered only experimental design, yield potential was 179 bushels per acre (bu/ac), calculated as the average yield of the references across all 17 sites. In the strip- and split-plot experimental designs, yield potentials were 207 and 144 bu/ac for the well-watered and water-limited treatments, respectively. As expected, MON 87460 provided a yield benefit compared to the control under water-limited conditions and similar yield to the control under well-watered conditions. Yield advantage values for MON 87460 in the water-limited treatment were highly variable, but are representative of the natural variation expected for corn grown under sub-optimal soil moisture conditions.

It is known that corn hybrids are variable in their response to drought stress (Campos et al., 2006). Breeders have been selecting for improved drought tolerance in conventional corn through traditional breeding and selection methods for decades. The variation in yield response observed among MON 87460, the control, and commercial reference hybrids in the field studies discussed above is within normal levels expected for conventional corn grown under sub-optimal soil moisture environments. Therefore, the data from these field studies are relevant to the safety assessment of MON 87460. In the water-limited sites, the data are also relevant to demonstrate the efficacy of MON 87460

Taken together, these data support that MON 87460 produces yields similar to the control under well-watered conditions and provides a yield advantage (i.e., reduced yield loss) under water-limited conditions. This yield advantage occurs over a range of water-limited conditions but will decrease as water deficit stress becomes too severe. Among the broad range of geographical and environmental conditions suited for commercial corn production, it is expected that MON 87460 will produce a range of responses based on the timing, severity, and duration of water-limited conditions experienced during the growing season. On average, under water-limited conditions, MON 87460 hybrids are expected to provide a 6% or greater yield advantage compared to commercial hybrids.

Monsanto and other seed companies commonly evaluate their commercial hybrids under a range of environmental conditions each year and assign drought tolerance ratings as a performance characteristic for each hybrid (http://www.asgrowanddekalb.com/seedresourceguide/search/seeds). Commercial hybrids of MON 87460 will be evaluated for drought tolerance in a manner consistent with the breeding and selection of other

commercial corn hybrids. As per standard process for all commercial products, both conventional and biotechnology-derived, Monsanto will conduct yield trials under relevant conditions to ensure that hybrids released for sale meet appropriate yield performance standards across several evaluation and selection criteria.

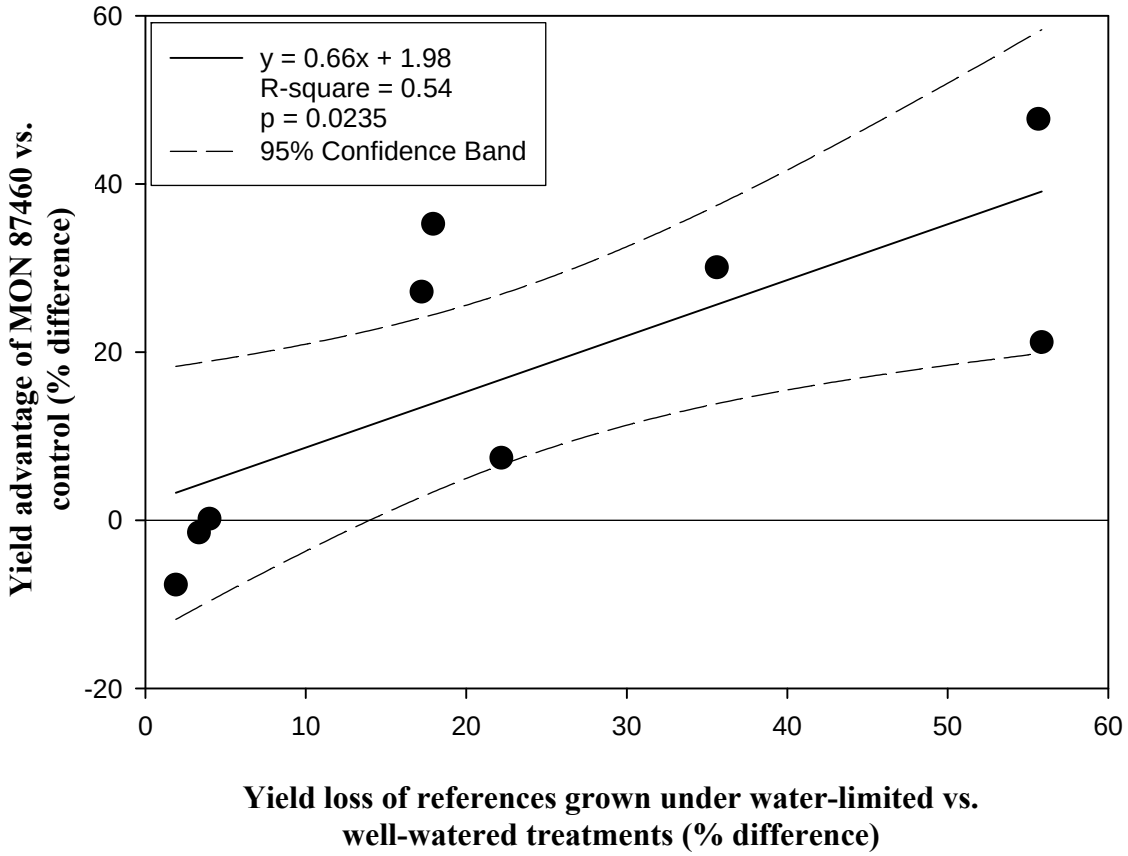



**Figure I-1. Percent Yield Advantage for MON 87460 Compared to the Control against Percent Yield Loss of the References in U.S. and Chilean Field Studies during 2006 and 2007**

Yield advantage of MON 87460 was calculated as the average yield of MON 87460 compared to the control within each site. Yield loss of references was calculated as the average yield of the reference materials in the well-watered treatment compared to the average yield under the water-limited treatment within each site. Data presented represent the nine sites that were established with both well-watered and water-limited treatments (Section VIII.C, Table VIII-3).

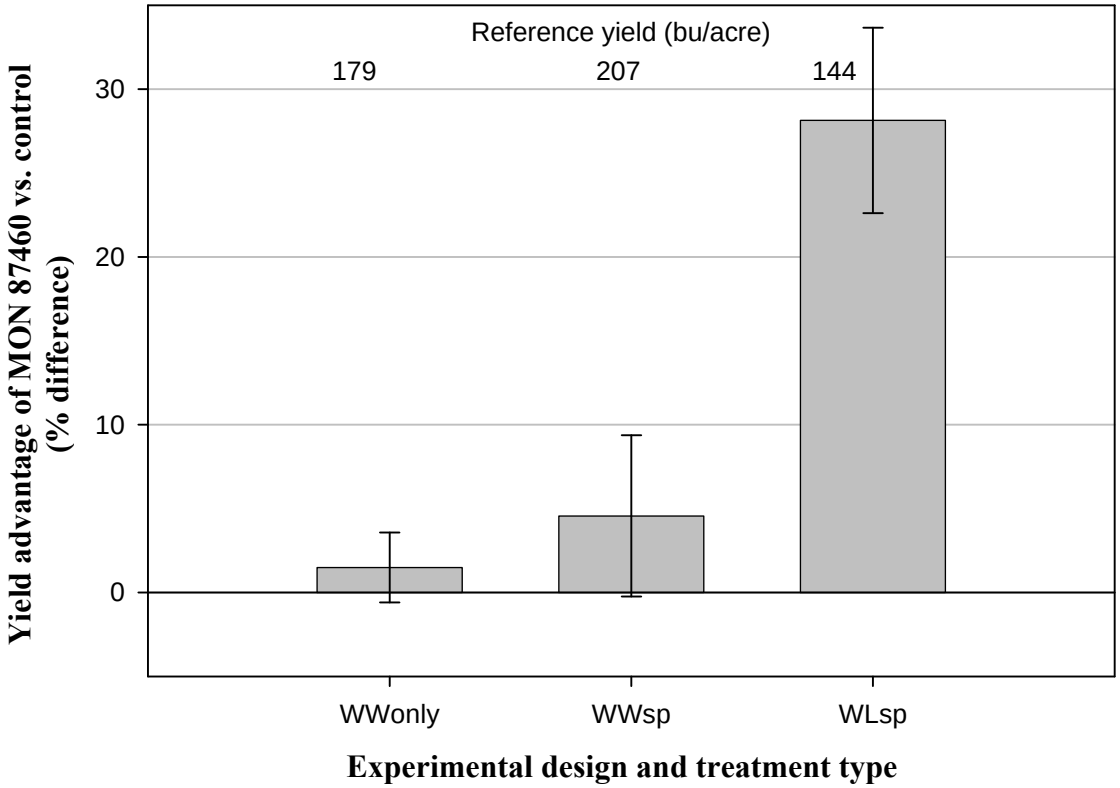



**Figure I-2. Percent Yield Advantage for MON 87460 Compared to the Control in U.S. and Chilean Field Studies during 2006 and 2007**

Experimental design and treatment type: WWonly = well-watered only design (n = 17 sites); WWsp = well-watered treatment in either a strip- or split-plot design (n = 6 sites); WLsp = water-limited treatment in either a strip- or split-plot design (n = 6 sites); Error bars represent standard error.

## I.C. Intended Function of the Genetic Modification

MON 87460 was chosen for development based on its yield advantage under water-limited conditions compared to the control and absence of negative pleiotropic effects on plant performance. The insertion of the *cspB* gene in MON 87460 confers tolerance to water-limited conditions that would otherwise negatively impact yield. The *npt*II gene was inserted to facilitate selection of plants containing *cspB* during early product development. The presence of NPTII does not pose any safety concerns (EFSA, 2007; FDA 1994 and 1998; Flavell et al., 1992; Fuchs et al., 1993a & 1993b; and Nap et al., 1992).

## I.D. Mode of Action of the Drought Tolerance Trait

*Cold shock proteins confer environmental stress tolerance in bacteria.*

Efficacy in MON 87460 is derived by expression of the inserted *Bacillus subtilis* CSPB protein. The CSPB protein in MON 87460 belongs to the CSP family, which has been extensively studied in bacteria. Early investigations of bacterial responses to cold-induced stress led to the discovery of CSPs, a group of small proteins that contain a highly conserved RNA-binding sequence identified as a cold shock domain (CSD).

In bacteria, a variety of environmental stresses are known to disrupt normal cell physiology, in part due to the production of RNA secondary structures which leads to a reduction in protein synthesis. Under environmental stress, CSD-containing proteins have been shown to bind many types of RNA (Cristofari and Darlix, 2002), leading to sustainable translation, maintenance of mRNA levels, and improved cellular function. While some members of the bacterial CSP family accumulate strictly in response to temperature shift (Etchegaray et al., 1996), others, including the *B. subtilis* CSPB protein, are also involved in maintaining normal cellular functions at both optimal temperatures (Graumann et al., 1997) and under nutrient limitation (Anderson et al., 2006). In actively transcribing *B. subtilis* cells, CSPB is localized around the nucleoid, co-localizing with the ribosomes (Mascarenhas et al., 2001; Weber et al., 2001). In stationary-phase cells CSPB is distributed throughout the cell, indicating that specific localization of CSPB depends on cell development stage (Weber et al., 2001). It has been suggested that accumulation of the CSPB protein in *B. subtilis* cells can be triggered under several stress conditions that share a common signal such as inactivation of ribosomes (Schindler et al., 1999; Graumann et al., 1997).

*CSD-containing proteins also confer environmental stress tolerance in plants.*

Similar to bacteria, CSD-containing proteins in plants also bind RNA, unfold RNA secondary structures caused by environmental stress, and help maintain cellular functions under stress. These plant CSD proteins share a high level of similarity to the bacterial CSPs and have been shown to share *in vitro* and *in vivo* functions with bacterial CSPs (Karlson and Imai, 2003; Kim et al., 2007; Nakaminami et al., 2005 and 2006; Chaikam and Karlson, 2008; Fusaro et al., 2007). Plant CSD proteins have been reported to respond to abiotic stresses in *Arabidopsis* (Fusaro et al., 2007), wheat (Karlson et al., 2002), and rice (Chaikam and Karlson, 2008), and to play an important role in various aspects of plant development (Fusaro et al., 2007; Chaikam and Karlson, 2008). Direct relationships between the ability of CSD-containing proteins to bind RNA and/or ssDNA and stress tolerance have been established (Nakaminami et al., 2006; Castiglioni et al., 2008) and results of *in vitro* experiments show that plant CSD proteins can bind RNA, synthetic mRNA, and ssDNA (Sasaki et al., 2007). The apparent absence of binding sequence specificity indicates that plant CSD proteins could be involved in a more general response to stress by binding RNAs and, therefore, helping cells to maintain cellular functions following the stress. CSD proteins from rice and *Arabidopsis* have been shown to be highly expressed in apical meristems, ovules, embryos, and seeds (Fusaro et al., 2007; Chaikam and Karlson, 2008) and, therefore, could potentially affect growth rate, flowering time, and seed development. The CSD proteins have been localized both in the cytoplasm and the nuclei (Sasaki et al., 2007; Fusaro et al., 2007)

indicating that these proteins can potentially be involved in multiple steps of RNA metabolism including localization, translation and stability.

*CSPB protein expressed in MON 87460 has a similar structure and function to other CSD-containing proteins.*

As with bacterial and other plant CSD-containing proteins, the CSPB protein from *B. subtilis*, which is expressed in MON 87460, binds RNA, unfolds RNA secondary structures, and accumulates in actively growing tissues. Data from *in vitro* and *in vivo* experiments indicate that CSPB preferentially binds plant RNA, but not dsDNA (Figures I.3 - 5, 6). CSPB was also effective in unfolding secondary RNA structures *in vitro,* while variants of the CSPB protein (CSPB_F30R and CSPB_F30A) with impaired RNA binding functions were unable to bind and unfold RNA (Table I-1). *In vitro* co-immunoprecipitation experiments with CSPB, CSPB_F30A and RNA from MON 87460 further confirm that CSPB interacts with RNA while a variant lacking a functional RNA binding site will not interact with RNA *in vitro* (Figure I-3). Finally, gel shift experiments demonstrate that CSPB is capable of binding both ribosomal RNA (rRNA) and messenger RNA (mRNA) (Figures I-4 and I-5). To demonstrate the *in vivo* interaction between the CSPB protein and RNA in corn plants expressing CSPB, a CSPB/RNA complex was co-immunoprecipitated from leaf tissue, confirming that CSPB interacts with corn RNA *in vivo* (Figure I-6).

In MON 87460, the expression of the CSPB protein is under control of the rice actin promoter which enables constitutive expression of the protein and decouples expression of CSPB protein from the cold shock response in bacteria. Using a CSPB specific-Enzyme-Linked Immunosorbent Assay (ELISA), the pattern of CSPB accumulation in MON 87460 leaf tissue, seedlings and developing reproductive tissues was evaluated (Figures I-7 – I-9). CSPB accumulation is highest in rapidly growing areas of the leaf and seedlings and tends to decline as the tissue matures. CSPB concentrations tend to increase over time in developing ears and decline over time in silks. Likewise, CSPB concentrations increase over time in immature tassels and either remain the same or decline in pollen. Sub-cellular localization of the CSPB protein was evaluated by immunohistochemistry. In MON 87460 coleoptiles, CSPB was distributed between cytoplasm and nucleus (Figure I-10). Similar sub-cellular localization of the CSPB protein was previously observed in corn protoplasts (Castiglioni et al., 2008).

Taken together, the data on RNA binding, CSPB accumulation and CSPB localization in MON 87460 are consistent with the pattern of RNA binding, accumulation, localization, and functions described for plant CSD-containing proteins (Fusaro et al., 2007; Sasaki et al., 2007; Chaikam and Karlson, 2008).

*CSPB protein expression improves yield and vegetative productivity under water-limited conditions.*

Water limitation during the growing season can diminish corn productivity and yield, particularly during flowering and grainfill periods when corn yield potential is most sensitive to stress by disrupting fertilization and kernel development (Claassen and Shaw, 1970; Boyer and Westgate, 2004; Campos et al., 2006).

Using a high through-put biotechnology approach, Castiglioni et al. (2008) demonstrated that bacterial CSPs can confer improved stress adaptation to multiple plant species. CSPB-containing events were evaluated in water-limited field trials in environments that received no rainfall during the 10 to 14 days immediately prior to flowering. The water-limited treatment resulted in an average reduction in growth rates to 50% of the well-watered rate. Using an across-event analysis, the CSPB-containing events demonstrated increases in leaf extension rates relative to the controls, improvements in chlorophyll content and improvements in photosynthetic rates. These measures of vegetative performance indicated that the CSPB protein has a positive impact on overall plant productivity and, therefore, yield potential. When plants were grown under well-watered conditions in both the greenhouse and field, no appreciable difference between CSPB-expressing lines and the control were detected.

In field trials under water-limited conditions, MON 87460 demonstrated improvements in yield and yield components through trends toward increased yield (16.5%), kernels per ear (13.1%), and kernel weight (3.9%) (Table I-2). Similar results were observed in a subsequent study, where differences were detected in yield (9.3%), kernels per ear (8.5%) and a trend toward increased kernel weight (2.5%) (Table I-3). Results from these studies demonstrate that the major component contributing to the improved yield of MON 87460 under water-limited conditions is the increased number of kernels per ear, which is consistent with the current understanding of the effect of drought stress on corn yield potential (Westgate et al., 2004; Campos et al., 2006; Welcker et al., 2007).

In a greenhouse study conducted under water-limited conditions, young MON 87460 plants showed a trend toward increased leaf growth rate relative to the control plants and overall better vegetative performance as measured by photosynthetic rate, carbon fixation, and stomatal conductance (Figures I-11 – I-13, respectively). The improved vegetative productivity is believed to be correlated to the high accumulation of CSPB in the rapidly growing tissues (Figures I-14 – I-21). Leaf growth and improved vegetative performance provide the physiological capacity necessary for the development of reproductive organs such as silks and pollen especially under drought stress. In turn, improvements in plant physiological capacity leads to increased numbers of kernels per ear and overall improved yield potential (Andrade et al., 2002; Bruce et al., 2002; Campos et al., 2006; Welcker et al., 2007; Fuad-Hassan et al., 2008; Chenu et al., 2008).

*CSPB protein expression does not affect common drought tolerant responses in corn plants*

Common mechanisms of plant response to drought stress are not altered in transgenic CSPB-containing corn plants. Greenhouse experiments were conducted with CSPB events that are genetically and phenotypically similar to MON 87460 to evaluate if these characteristics had been altered when compared with controls. The plants containing CSPB protein show broadly the same relative water content (Table I-4), leaf water potential (Figure I-22), and leaf osmotic potential (Figure I-23) as the control plant. CSPB-containing plants accumulate similar levels of abscisic acid (ABA) (Figure I-24) and osmotically active solutes (sucrose, fructose, glucose, choline, proline, glycine

betaine) as observed in controls (Figures I-25 - 28). Results from these experiments suggest that common mechanisms of plant response to drought stress are not altered in transgenic CSPB-containing corn plants.

*CSPB protein function: Conclusion*

In summary, expression of the CSPB protein in MON 87460 results in reduced yield loss under drought conditions when compared to conventional corn grown under identical conditions. The major component contributing to the improved yield of MON 87460 under water-limited conditions is the increased number of kernels per plant, which is consistent with the current understanding of the effect of drought stress on corn yield potential. CSD-containing proteins moderate stress responses in bacteria and plants, primarily through stabilization of RNA. Like endogenous CSD proteins found in plants, the CSPB protein in MON 87460 interacts with RNA and accumulates and localizes to rapidly growing tissues and in developing reproductive organs, thereby helping to maintain normal cellular function in those tissues critical to yield. Under water-limited conditions, there is a trend toward improved growth rate, photosynthetic rate, carbon fixation, and stomatal conductance for MON 87460 compared to the control, but accumulation of ABA and osmotically active solutes are not altered in transgenic CSPB-containing corn plants.

**Table I-1. *In vitro* Melting Activity of CSPB and CSPB Variants**

| Protein | Specific Activity (pmoles opened DLP[1]/µg CSPB) |
|---|---|
| CSPB | 0.905 ± 0.033 |
| CSP_F30R | <LOD[2] |
| CSPB_F30A | <LOD |

[1]DLP – Dual-Labeled Probe
[2]LOD – Limit of Detection

The melting activities of CSPB and the different variants were measured for all proteins using 3 µg of protein for each replicate. The activity represents the average of three replicates. In this assay, a hairpin-shaped (stem-loop) molecular beacon is labeled with a fluorophore at the 5'end and quencher at the 3'-terminus. Due to the close proximity of the fluorescent tag and quencher in the hairpin conformation, the fluorescence is efficiently quenched. When a CSPB protein "melts" the hairpin conformation, the fluorescent tag and quencher are spatially separated which permits fluorescence. CSPB is cold shock protein B. CSPB_F30A contains alanine instead of phenylalanine in position 30 and CSPB_F30R contains an arginine instead of phenylalanine in the same position. CSPB, CSPB_F30A and CSPB_F30R were produced in *E. coli*.

**Table I-2. Yield Component and Physiology Data from a Water-Limited Kansas Field Trial in 2003**

| Endpoint | MON 87460 Mean (S.E.)[1] | Control Mean (S.E.) | Difference, MON 87460 minus control | Diff (%) | p-value |
|---|---|---|---|---|---|
| **Yield[2] (bu/ac)[3]** | 80.0 (10.9) | 68.7 (10.9) | 11.3 | 16.4 | 0.153 |
| **Kernels per ear[4]** | 289 (36.9) | 256 (36.9) | 33 | 12.9 | 0.233 |
| **200 kernel weight (g)[5]** | 72.6 (2.7) | 69.9 (2.7) | 2.7 | 3.9 | 0.283 |
| **Ears per plot[6]** | 33.8 (1.3) | 33.5 (1.3) | 0.3 | 0.9 | 0.834 |
| **Stomatal conductance ($mmol/m^2/s$)** | 262.7 (20.78) | 235.8 (20.78) | 26.9 | 11.4 | 0.064 |
| **Photosynthetic rate ($\mu mol\ CO_2/m^2/s$)[7]** | 37.2 (2.24) | 34.1 (2.24) | 3.1 | 9.1 | 0.066 |
| **Transpiration rate ($mmol/m^2/s$)** | 6.1 (0.35) | 5.8 (0.35) | 0.3 | 5.2 | 0.126 |
| **Leaf extension rate (cm/5 d)[8]** | 21.2 (1.79) | 17.4 (1.79) | 3.8 | 21.8 | 0.008 |

[1]S.E. – standard error

[2]Yield data were normalized to 15.5% moisture.

[3]bu/ac – bushels per acre

[4]Kernel per ear measurements were collected from a subsample out of each plot.

[5]Kernel weights were taken from 200 kernel samples from a subsample of ears.

[6]The number of harvestable ears was counted from each of the plots at the end of the season.

MON 87460 and the control were planted in two row plots, 34 plants per row, at a density of 32,000 plants per acre. Twelve paired-plot replicates were planted in a randomized block design.

[7]Photosynthesis measurements were made using a PP Systems Ciras-1 Portable Photosynthesis System.

[8]Photosynthesis and leaf extension rate measurements were collected on six plants each of MON 87460 and the control.

**Table I-3. Yield Component Data from a Well-Watered and Water-Limited California Field Trial in 2007**

| Endpoint | MON 87460 Mean (S.E.)[1] | Control Mean (S.E.) | Difference, MON 87460 minus control | Diff (%) | p-value |
|---|---|---|---|---|---|
| **Well-watered yield (bu/ac)**[2, 3] | 297 (4.08) | 295 (3.98) | 2 | 0.7 | 0.568 |
| **Water-limited yield (bu/ac)** | 206 (12.7) | 188 (12.7) | 18 | 9.6 | 0.038 |
| **Water-limited kernels per ear**[4] | 483.1 (20.50) | 445.2 (20.50) | 37.9 | 8.5 | 0.004 |
| **Water-limited 50 kernel weight (g)**[5] | 17.4 (0.4) | 16.9 (0.4) | 0.5 | 3.0 | 0.068 |
| **Water-limited leaf extension rate (cm/8 d)**[6] | 27.8 (1.20) | 24.7 (1.20) | 3.1 | 12.6 | 0.034 |
| **Water-limited plant height (cm)**[6] | 220 (1.77) | 215 (1.73) | 5 | 2.3 | 0.046 |
| **Water-limited plant biomass increase (g/8 d)**[6] | 24.0 (1.62) | 22.0 (1.70) | 2.0 | 9.1 | 0.376 |

[1]S.E. – standard error

[2]Yield data were normalized to 15.5% moisture.

[3]bu/ac – bushels per acre

[4]Kernel per ear measurements were collected from a subsample out of each plot.

[5]Kernel weights were taken from 50 kernel samples from a subsample of ears.

[6]Leaf extension, plant height and plant biomass measurements were collected from three plants in each of the ten water-limited replicates.

The well-watered treatment contained five replicates. The water-limited treatment contained 10 replicates. Each replicate consisted of four six-row plots with 20.5 foot rows planted with 50 kernels per row.

**Table I-4. Average Relative Water Content of Transgenic CSPB Plants and the Control Grown under Water-Limited Conditions in the Greenhouse**

| Entry | Ave RWC (%) | Std Dev RWC |
|---|---|---|
| Control | 78 | 4.9 |
| ZM_M38245 | 82 | 5.7 |
| ZM_M38705 | 77 | 5.7 |
| ZM_M38727 | 75 | 5.0 |
| ZM_M38835[1] | 75 | 5.0 |
| ZM_M38840 | 76 | 4.8 |
| ZM_M38862 | 80 | 5.3 |

[1]Entry ZM_M38835 is MON 87460

Other entries contain the same construct as MON 87460.

All plants were subjected to a 12 day period of moderate stress based on pot weight.

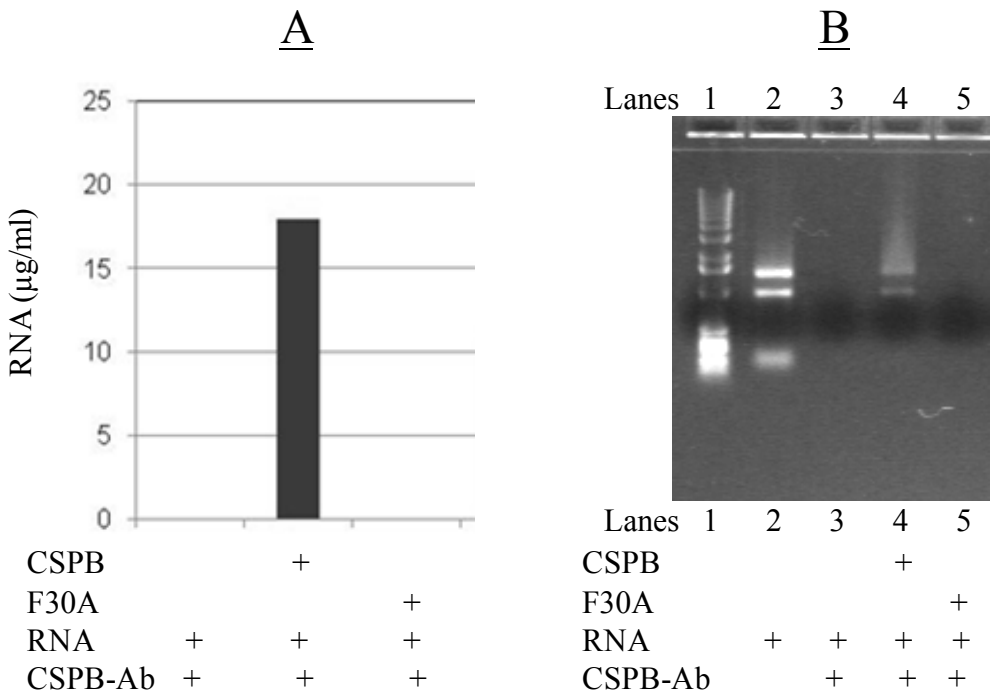
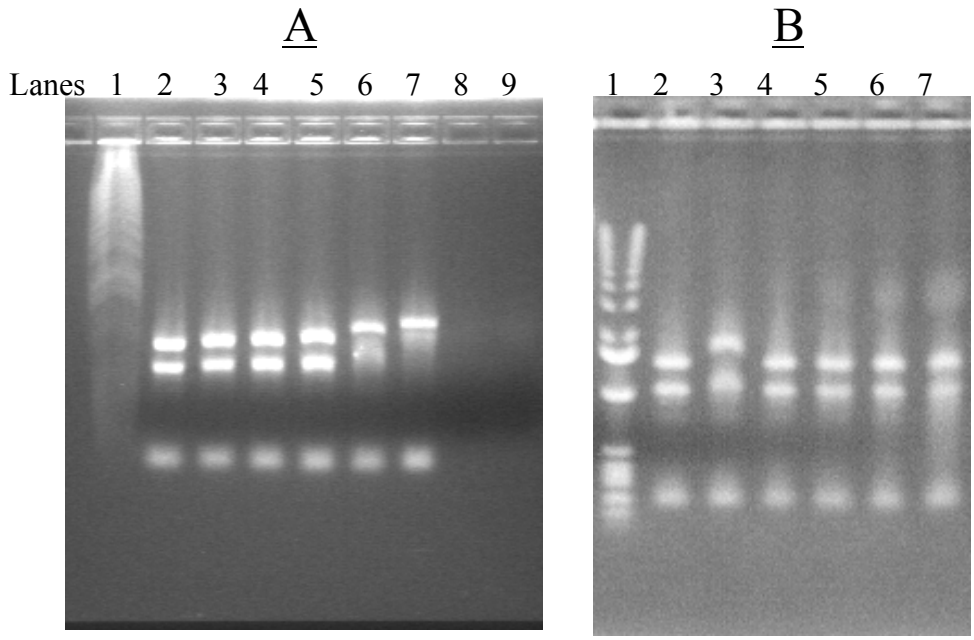



**Figure I-3. Co-Immunoprecipitation of RNA from MON 87460 using CSPB and the CSPB_F30A Variant**

(A) Total RNA (2.5 µg) isolated from MON 87460 leaf tissue was incubated with CSPB (5 µg) or the CSPB_F30A variant (F30A) (5 µg). CSPB was immunoprecipitated with an affinity purified anti-CSPB antibody (30 µg). Any RNA that co-precipitated was quantified using fluorescent probes. Immunoprecipitation conditions are described in the table below the graph. CSPB and CSPB_F30A were produced in *E. coli*.

(B) Aliquots of total RNA from MON 87460 were mixed with CSPB (Lane 4) or the CSPB_F30A variant (F30A) (Lane 5). The CSPB-RNA complex, if present, was immunoprecipitated with an affinity purified anti-CSPB antibody (CSPB-Ab). Samples were mixed with ethidium bromide and run on a 1% agarose gel. Lane 1 contains a 1 kb molecular weight ladder. Lane 2 contains MON 87460 RNA alone in the absence of immunoprecipitation as a positive control. Lane 3 is the immunoprecipitate of MON 87460 RNA and CSPB-Ab and demonstrates that CSPB-Ab with RNA alone produces no signal. CSPB and CSPB_F30A were produced in *E. coli*. Results confirm that CSPB binds detectable amounts of RNA but CSPB_F30A does not.




| Lanes | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| CSPB(µg) | 0 | 5 | 10 | 20 | 50 | 100 | 50 | 100 | |
| BSA (µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

| Lanes | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| CSPB(µg) | | 0 | 50 | 0 | 0 | 0 | 0 |
| BSA (µg) | | 0 | 0 | 25 | 50 | 100 | 200 |

**Figure I-4. Gel Shift Analysis of Total RNA Isolated from MON 87460**

(A) Aliquots of total RNA (2.5 µg) purified from MON 87460 leaf tissue were incubated with different amounts of CSPB, mixed with ethidium bromide and run on an agarose gel. A shift in the RNA banding pattern is seen with increasing concentrations of CSPB. Lanes 8 and 9 do not contain RNA, demonstrating no signal was observed for CSPB alone. (B) BSA was used as a control and mixed with total RNA (2.5 µg) purified from MON 87460. No shifts in the banding pattern are observed for increasing amounts of BSA. Lane 1 in each gel contains a 1Kb molecular weight ladder. The amount of protein added to each reaction is described in the table below each image.

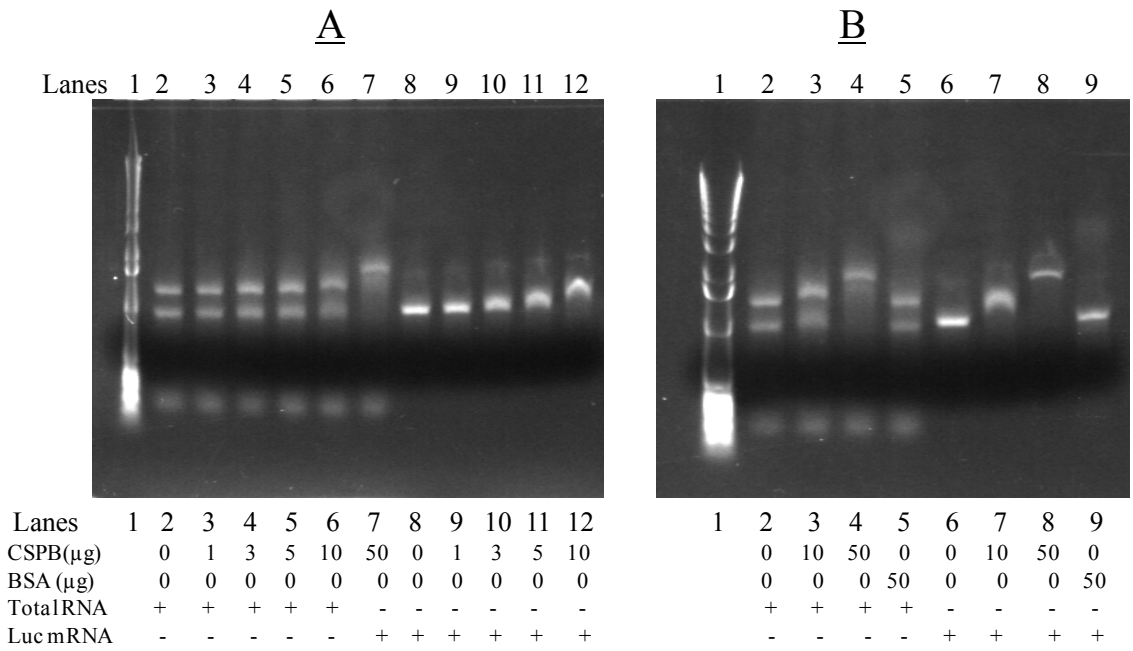



**Figure I-5. Gel Shift Analysis of rRNA and Luciferase mRNA**

(A) Aliquots of total RNA (0.5 µg) purified from MON 87460 leaf tissue and *in vitro* transcribed Luciferase mRNA (Luc mRNA, 0.5 µg) were incubated with increasing amounts of CSPB and mixed with ethidium bromide. The shifting in the banding of the RNA was observed on 10% agarose gels under UV light. (B) BSA was used as a control and mixed with total RNA (0.5 µg) purified from MON 87460 and Luciferase mRNA (Luc mRNA, 0.5 µg) and compared to shifts produced by CSPB. No shifts are observed for increasing amounts of BSA. Lane 1 in each gel contains a 1Kb molecular weight ladder.

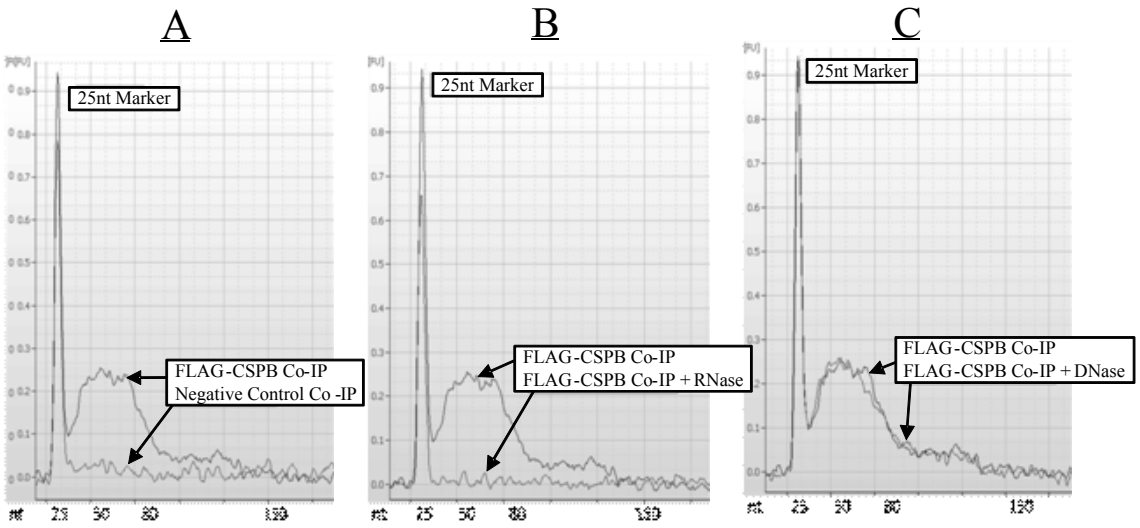



**Figure I-6. Co-Immunoprecipitation of CSPB-FLAG:RNA Complexes from Leaf Tissue**

Leaf tissue from corn plants expressing a FLAG-tagged version of CSPB was ground and extracted. The FLAG-CSPB:RNA complexes were immunoprecipitated using anti-FLAG antibodies and analyzed on an Agilent Bioanalyzer. The results show the nucleic acid precipitated compared to a conventional leaf (A) and the digestion of the Co-IP sample with RNAse (B) and DNAse(C). The 25nt Marker shows where a 25 nucleotide (nt) sequence would elute. Results confirm that CSPB binds RNA in vitro but not DNA.

CSPB-FLAG corn plants are transgenic events containing the CSPB coding region with an additional sequence which adds 24 nucleotides to the 3' end resulting in an eight peptide C-terminal addition, called FLAG, to the translated CSPB protein. This eight peptide group allows the FLAG-tagged protein to be co-immunoprecipitated with a commercial kit (Sigma, St. Louis, MO). The CSPB-FLAG corn expression construct contained the same promoter and terminator as those used to drive CSPB expression in MON 87460 (i.e., rice actin promoter and Tr7 terminator). The event used for the Co-IP experiments had a single copy transgene insertion and it expressed the expected protein as confirmed by western blot and MALDI-TOF Mass Spectrometry. Plants were kept well watered and samples were taken 20 days after planting by cutting the plant at the leaf collar of the V4 or V5 leaf, removing the leaf and harvesting the basal 1/8th of the next three youngest leaves from test and control plants.

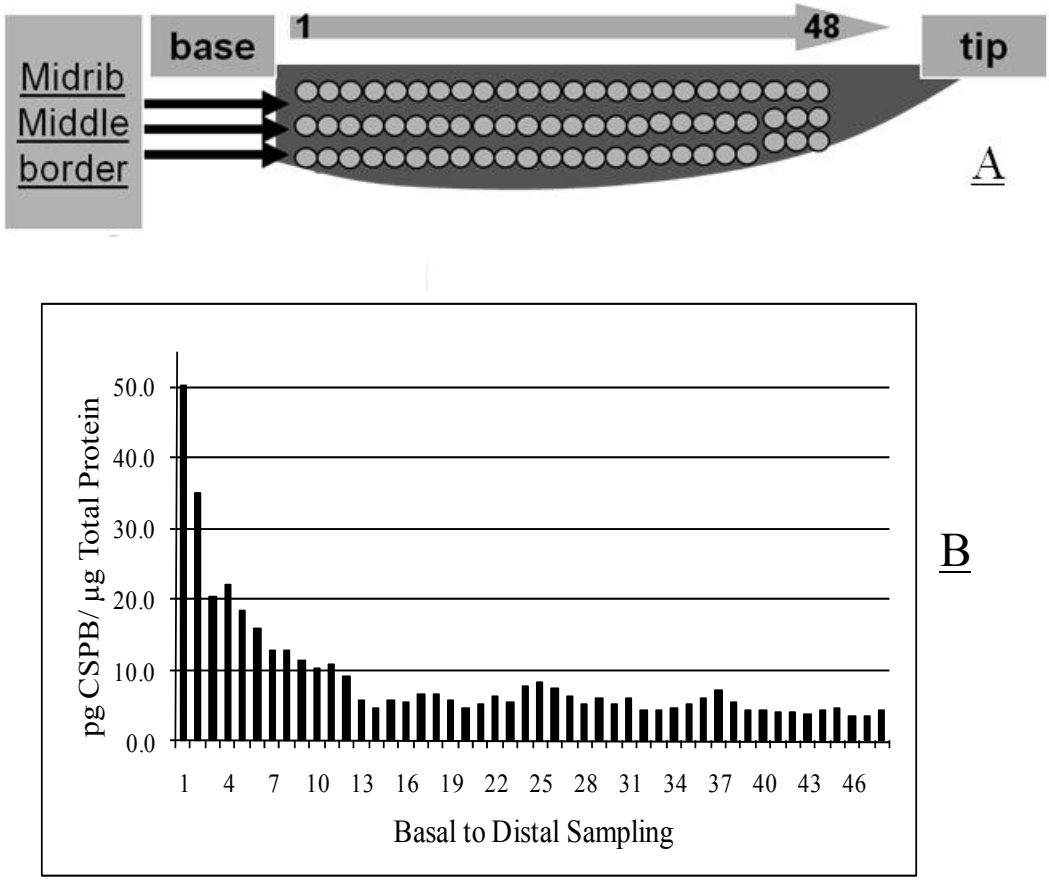



**Figure I-7. Differential Accumulation of CSPB across Leaves of MON 87460**

CSPB levels in different V10 leaf sections of corn MON 87460 sampled according to the leaf diagram (A) were determined using a validated CSPB-specific ELISA (B). The values represent the means of three leaf sections from two different V10 leaves. The sample locations from leaf base to leaf tip shown in panel A correspond with the numbers in the x-axis of panel B. The basal, rapidly growing, portion of the leaf contained a significantly higher level of CSPB than the rest of the leaf segments, with the distal portion of the leaf having the lowest concentrations of CSPB.

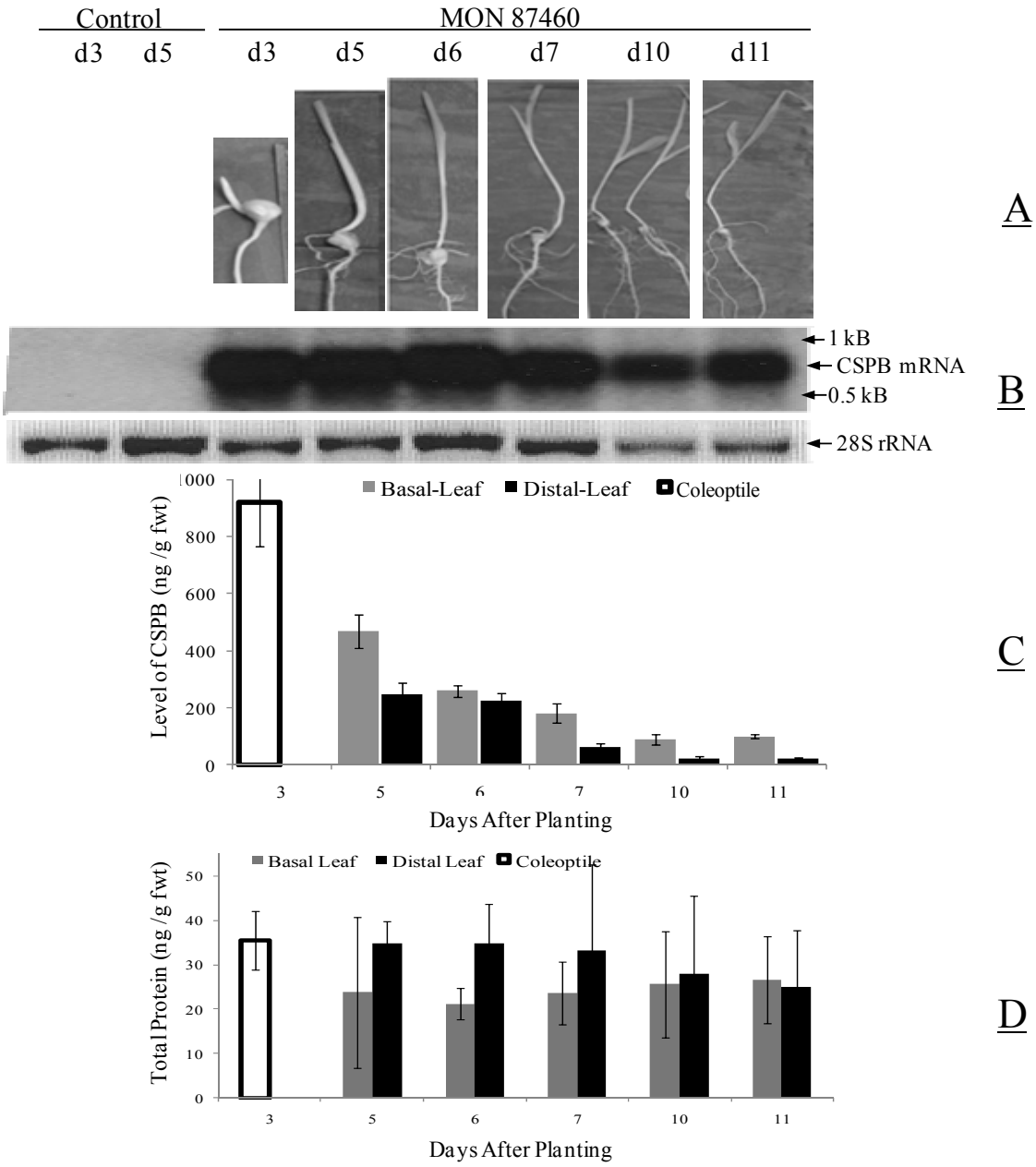



**Figure I-8. Expression and Accumulation of CSPB Protein in Germinating MON 87460 Seedlings**

Plants harvested were dissected into basal and distal leaf (A). The expression of the CSPB mRNA was determined by northern blot using a CSPB-specific probe. The total rRNA was used as a control for the amount of RNA loaded on the gel (B). The level of CSPB (C) and total protein (D) in both tissue types were determined from tissue homogenates using a validated ELISA and a colorimetric assay, respectively.

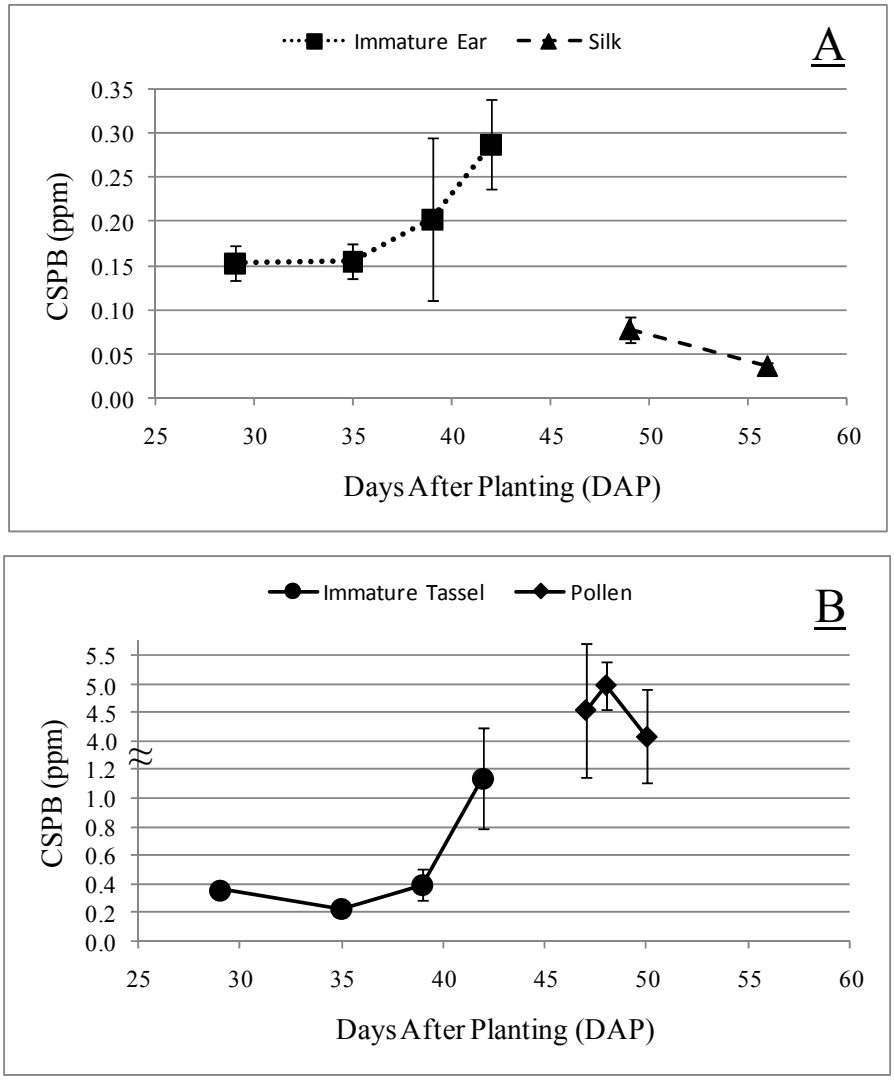



**Figure I-9. Expression of CSPB in Tissues of MON 87460 Grown in a Greenhouse**

MON 87460 plants were grown under well-watered conditions in a greenhouse and tissues were sampled on different days after planting. CSPB levels were determined by a validated ELISA and are based on fresh weight of the tissue. (A) Immature ears and silks (B) Immature tassels and pollen. CSPB levels increased in developing ears and tassels. Highest CSPB levels were detected in pollen whereas silks had the lowest levels.

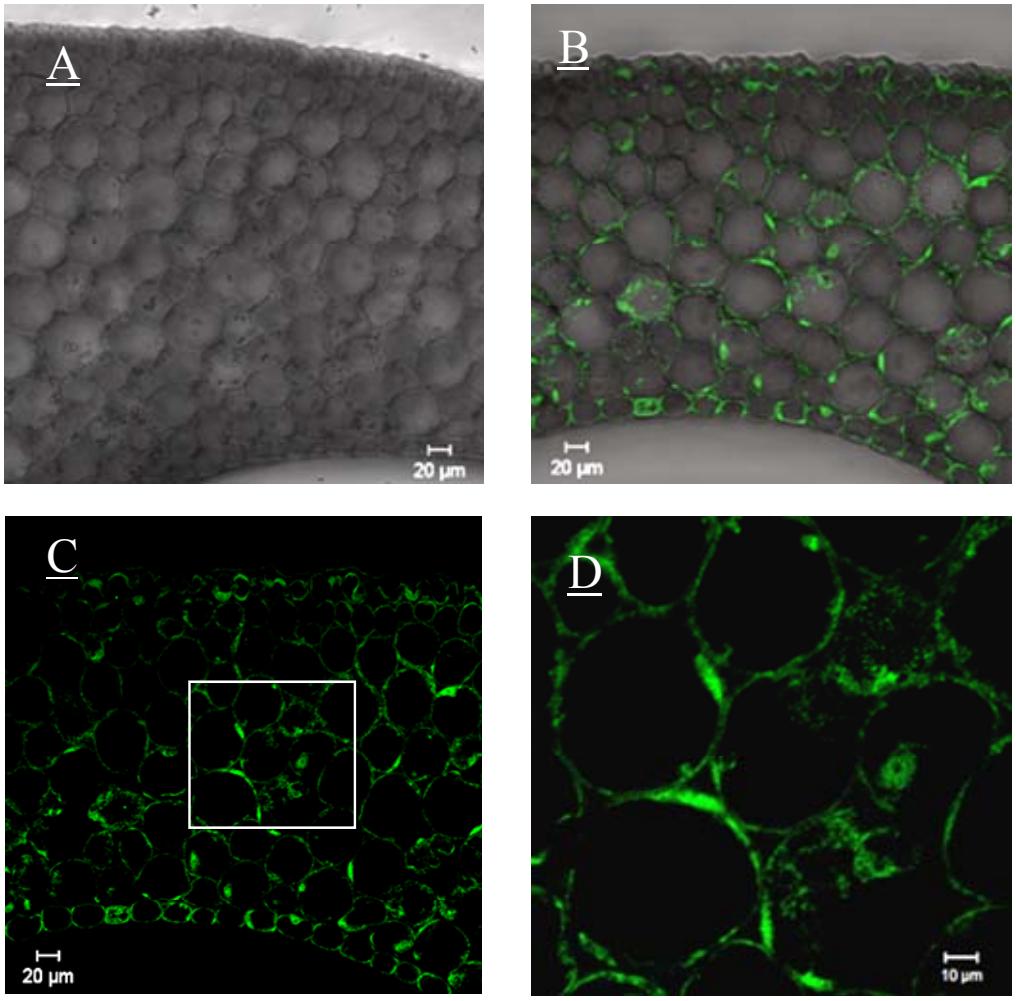



**Figure I-10. Immunohistochemical Localization of CSPB in Plant Tissue**

Shoot (coleoptiles) sections from 3-day post-germination plants were incubated with affinity purified goat anti-CSPB. The CSPB antibody was detected using a fluorescently labeled small fragment secondary antibody. The control tissue (A) had no specific fluorescent signal, while in MON 87460 (B) the fluorescent signal can be clearly seen; both images are overlays with bright light to view the cells at 20X magnification. Observation of just the fluorescent signal under 20X (C) and 60X (D) magnification shows that the CSPB is localized in the nucleus and cytoplasm.

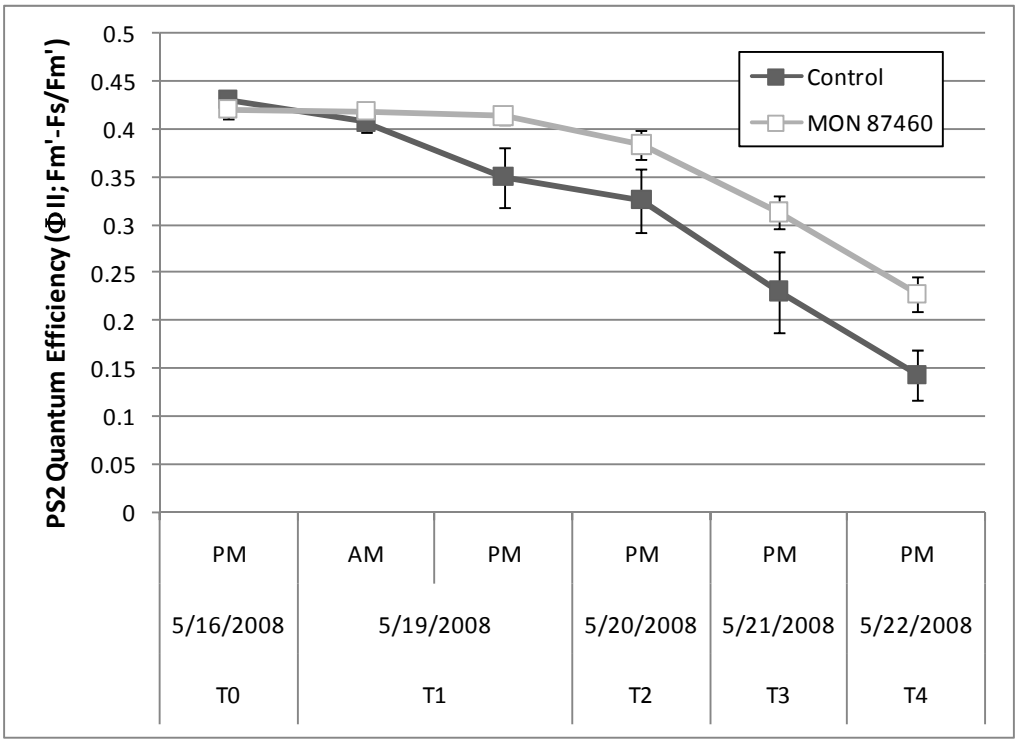



**Figure I-11. Quantum Efficiency of Photosystem II-Mediated Electron Transfer, or ΦII, for MON 87460 and the Control over Time**

Measurements of the maximum fluorescence quantum yield of photosystem II (PS2) mediated electron transfer ($\Phi_{II}$) were obtained with an OS30P chlorophyll fluorometer as a measure of photosystem II activity. Readings were made on leaves that were at the top of the canopy and whose surface was visibly exposed to illumination. Results were obtained from six paired replicate plants of MON 87460 and the control that were subjected to six days of water-limited conditions based on pot weight beginning at the V5 growth stage.

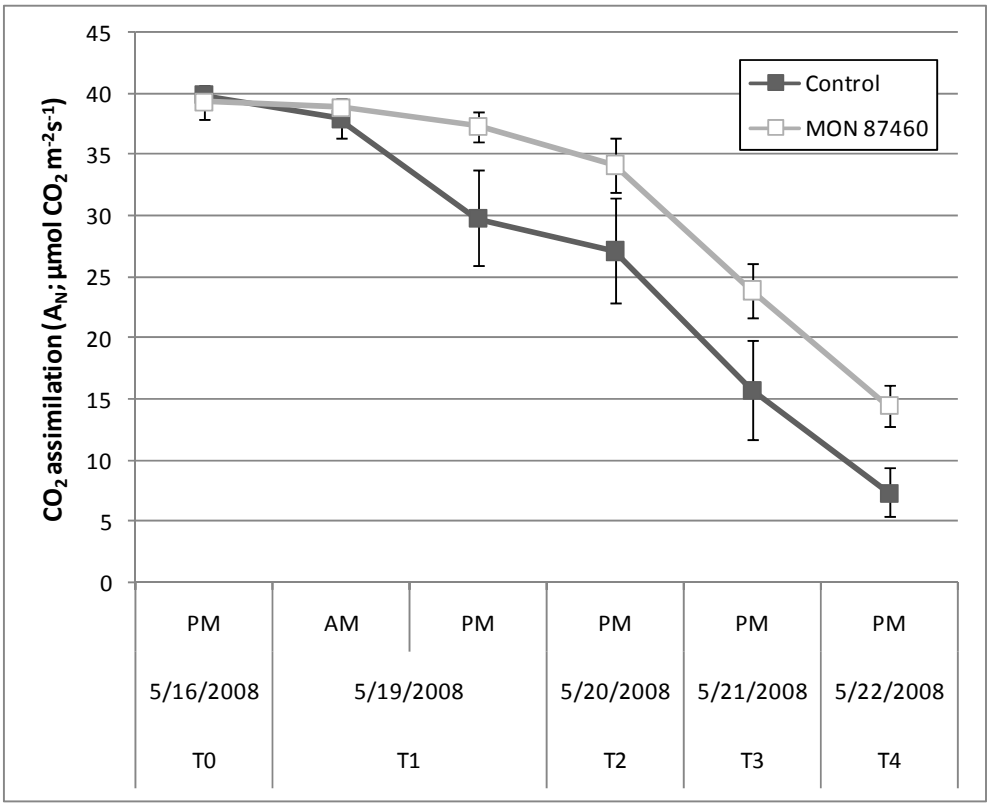



**Figure I-12. $CO_2$ Assimilation over Time for MON 87460 and the Control**

Measurements of $CO_2$ assimilation ($A_N$) were obtained using a LICOR 6400 instrument. Measurements were made on small sections of mature V5 leaves midway between the base and the tip of the leaf that had reached steady-state in the measurement chamber. Gas exchange parameters were obtained under steady-state illumination. Results were obtained from six paired replicate plants of MON 87460 and the control that were subjected to six days of water-limited conditions based on pot weight beginning at the V5 growth stage.

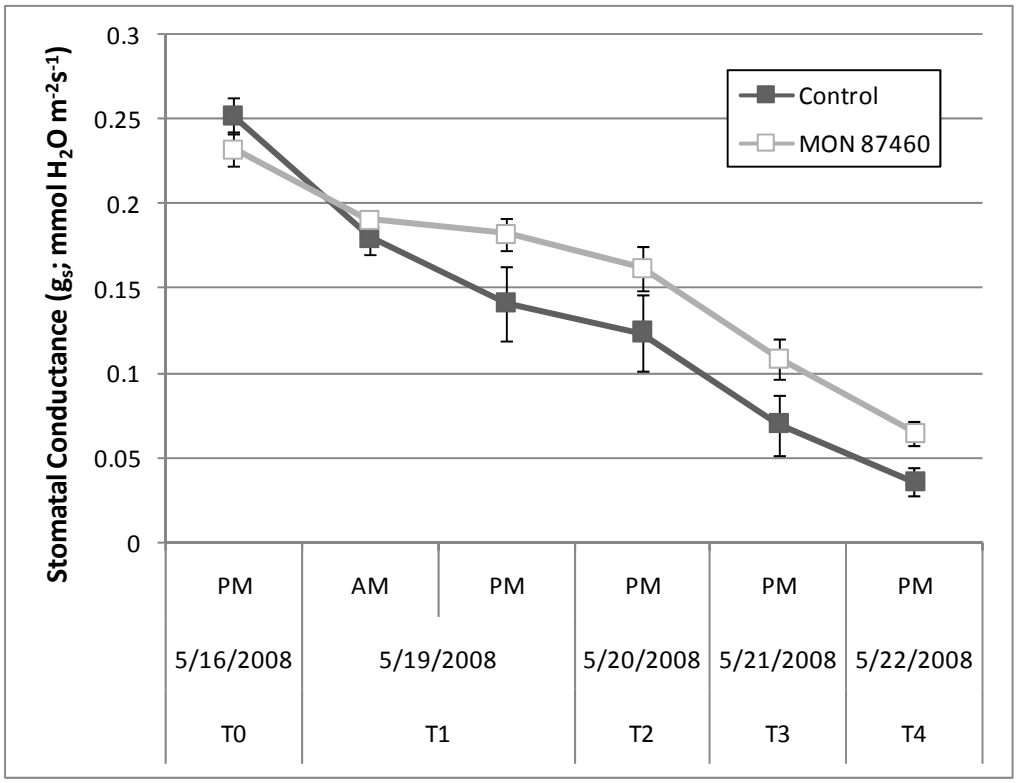



**Figure I-13. Stomatal Conductance over Time for MON 87460 and the Control**

Measurements of stomatal conductance ($g_s$) were obtained using a LICOR 6400 instrument. Measurements were made on small sections of mature V5 leaves midway between the base and the tip of the leaf that had reached steady-state in the measurement chamber. Gas exchange parameters were obtained under steady-state illumination. Results were obtained from six paired replicate plants of MON 87460 and the control that were subjected to six days of water-limited conditions based on pot weight beginning at the V5 growth stage.

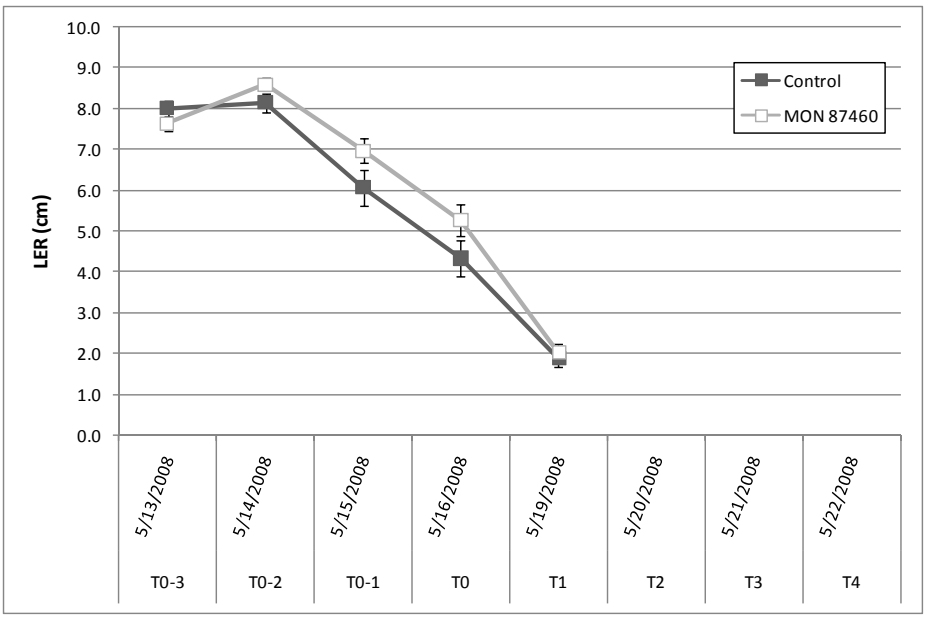



**Figure I-14. Leaf 5 Daily LER Pre-Drought (T0-3 to T0) and Post-Drought Initiation (T1-T4)**

T1 leaf extension rate (LER) is an average based on three-day height difference between T1 and T0. T0-1, -2 & -3 are all well-watered measurements prior to treatment initiation at T0. Leaves were measured from the soil to the tip of the leaf. Fifteen plants each of MON 87460 and the control were measured. Water-limited conditions were imposed after the last watering on the morning of May 16 based on pot weight. For leaf 5, measurements were terminated after emergence of the ligule (May 19) because little growth accumulates after that stage.

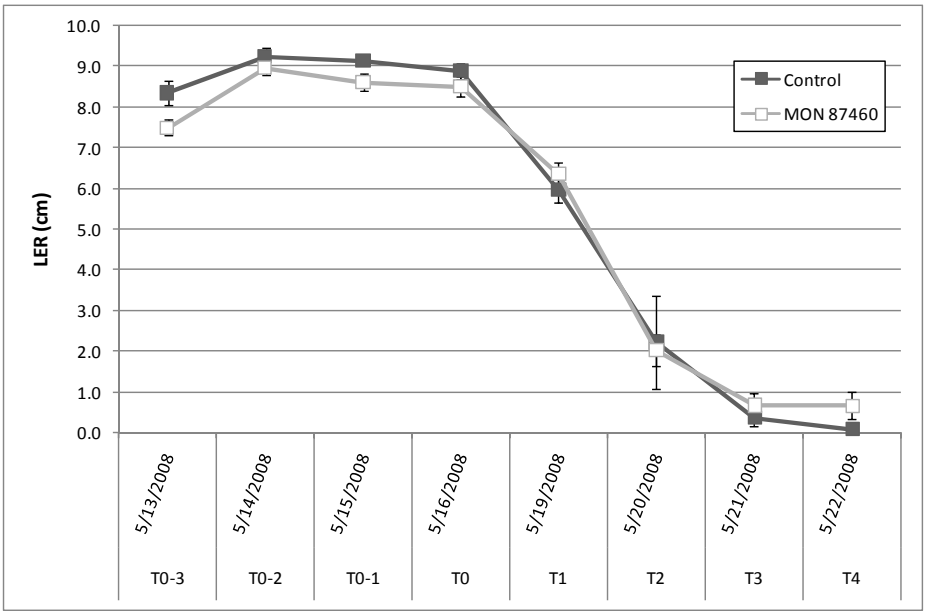



**Figure I-15. Leaf 6 Daily LER Pre-Drought (T0-3 to T0) and Post-Drought Initiation (T1-T4)**

T1 LER is an average based on three-day height difference between T1 and T0. T0-1, -2 & -3 are all well-watered measurements prior to treatment initiation at T0. Leaves were measured from the soil to the tip of the leaf. Fifteen plants each of MON 87460 and the control were measured. Water-limited conditions were imposed after the last watering on the morning of May 16 based on pot weight.

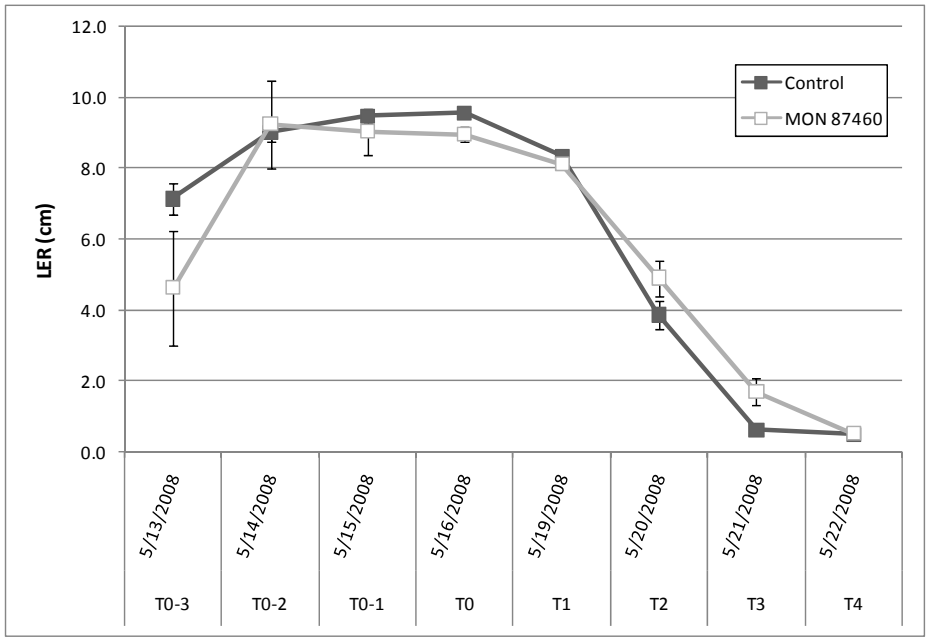



**Figure I-16. Leaf 7 Daily LER Pre-Drought (T0-3 to T0) and Post-Drought Initiation (T1-T4)**

T1 LER is an average based on three-day height difference between T1 and T0. T0-1, -2 & -3 are all well-watered measurements prior to treatment initiation at T0. Leaves were measured from the soil to the tip of the leaf. Fifteen plants each of MON 87460 and the control were measured. Water-limited conditions were imposed after the last watering on the morning of May 16 based on pot weight.

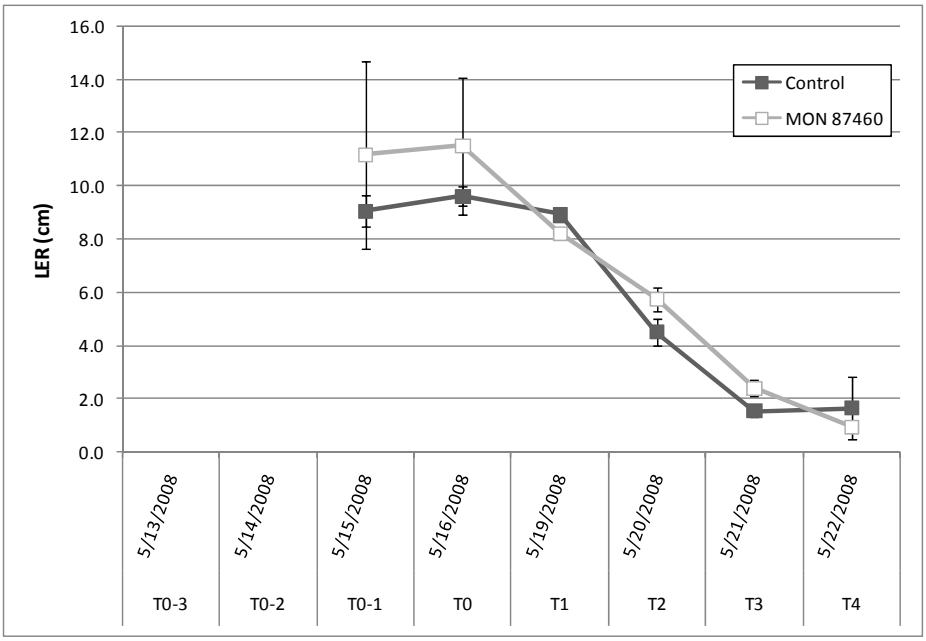



**Figure I-17 Leaf 8 Daily LER Pre-Drought (T0-3 to T0) and Post-Drought Initiation (T1-T4)**

T1 LER is an average based on three-day height difference between T1 and T0. T0-1 is a well-watered measurement prior to treatment initiation at T0. Leaves were measured from the soil to the tip of the leaf. Fifteen plants each of MON 87460 and the control were measured. Water-limited conditions were imposed after the last watering on the morning of May 16 based on pot weight.

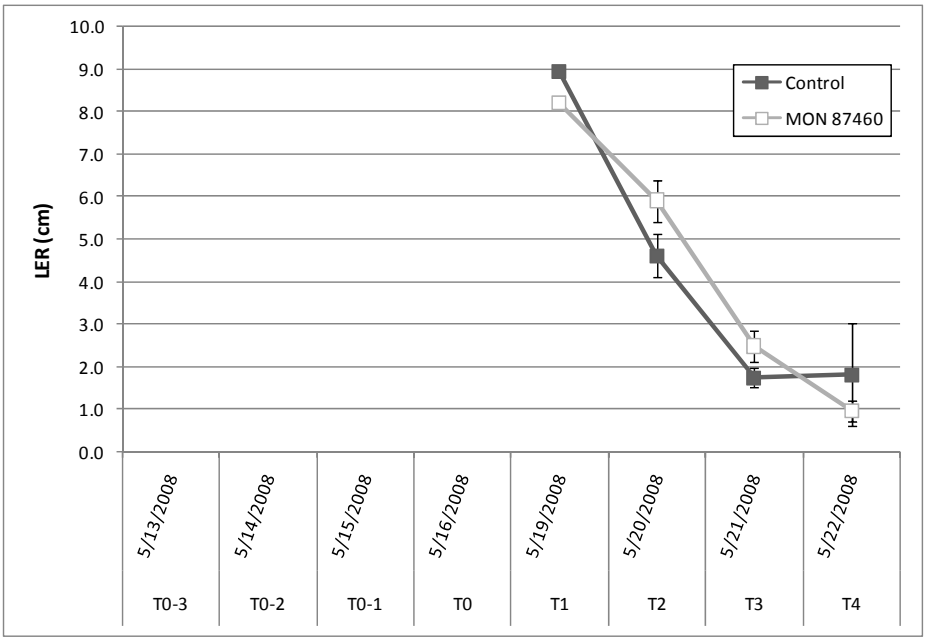



**Figure I-18. Leaf 9 Daily LER during the Drought Progression (T1-T4)**

T1 LER is an average based on three-day height difference between T1 and T0. The water-limited treatment initiated at T0. Leaves were measured from the soil to the tip of the leaf. Fifteen plants each of MON 87460 and the control were measured. Water-limited conditions were imposed after the last watering on the morning of May 16 based on pot weight.

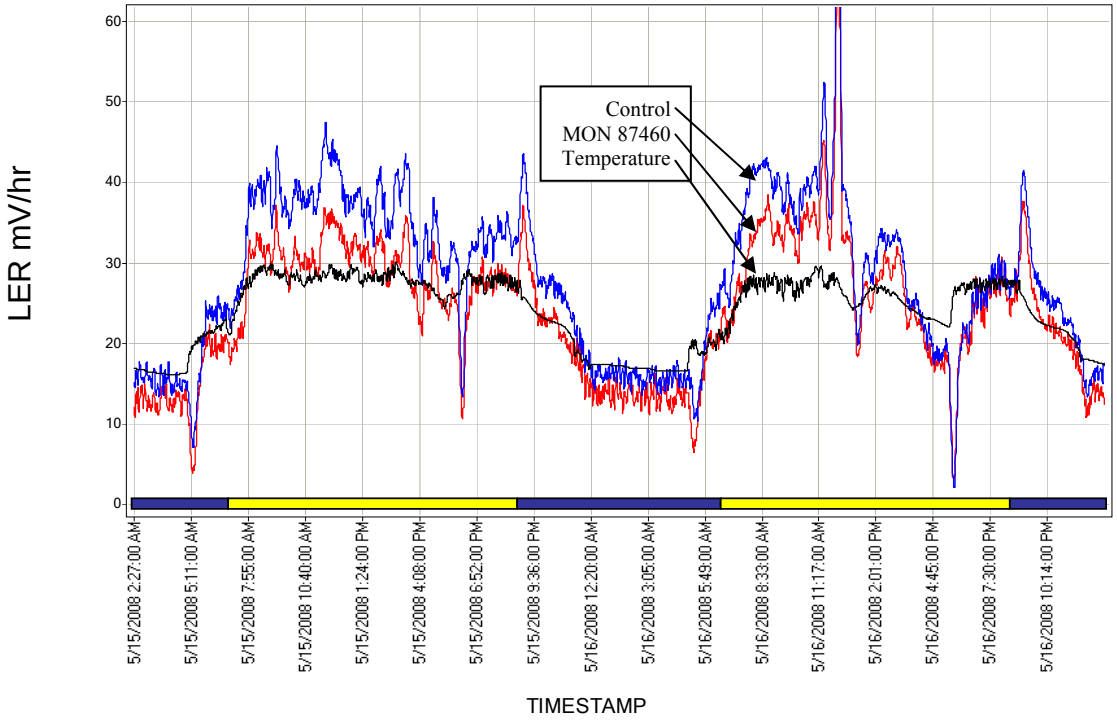



**Figure I-19. Automated LER Data and Plant Temperature for an Approximately 48 Hour Growth Period during Well-Watered Conditions**

Blue line is the control, the red line is MON 87460 and the black line is temperature, as indicated by the arrows.

LER measurements were made using a draw wire sensor potentiometer mounted above the plants. The device continuously outputs a range of voltage linearly dependent on the retraction length of each wire. 1 mV/hr = 0.1556 mm/hr. Voltage was sampled every 5 seconds, averaged and logged every minute. Four plants each of MON 87460 and the control were measured. Well-watered conditions were based on pot weight.

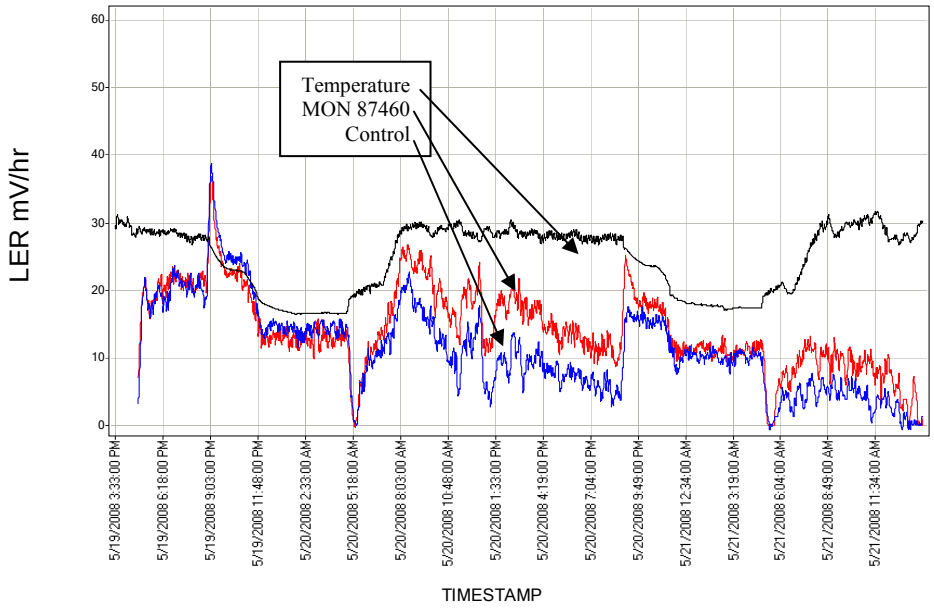



**Figure I-20. Automated LER Data and Plant Temperature for an Approximately 48 Hour Growth Period during Water-Limited Conditions**

Blue line is the control, the red line is MON 87460 and the black line is temperature, as indicated by the arrows.

LER measurements were made using a draw wire sensor potentiometer mounted above the plants. The device continuously outputs a range of voltage linearly dependent on the retraction length of each wire. 1 mV/hr = 0.1556 mm/hr. Voltage was sampled every 5 seconds, averaged and logged every minute. Four plants each of MON 87460 and the control were measured. Water-limited conditions were based on pot weight.

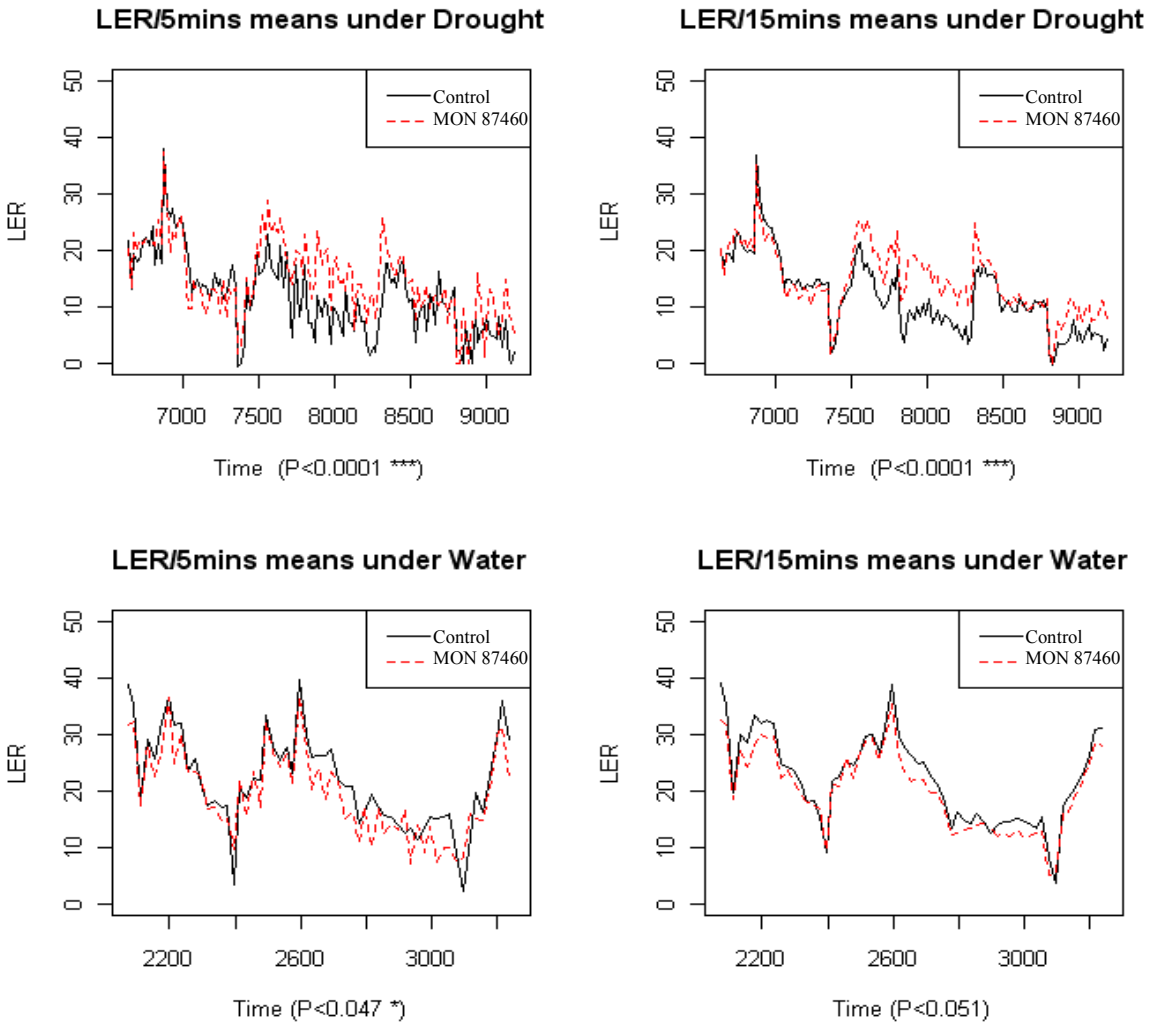



**Figure I-21. Automated LER Data under Well-Watered and Water-Limited Conditions for 5- and 15-min Windows**

LER measurements were made using a draw wire sensor potentiometer mounted above the plants. The device continuously outputs a range of voltage linearly dependent on the retraction length of each wire. 1 mV/hr = 0.1556 mm/hr. Voltage was sampled every 5 seconds, averaged and logged every minute. LER was calculated as the difference of mV output over a 5-minute period or a 15-minute period. Four plants each of MON 87460 and the control were measured. Water treatments were based on pot weight.

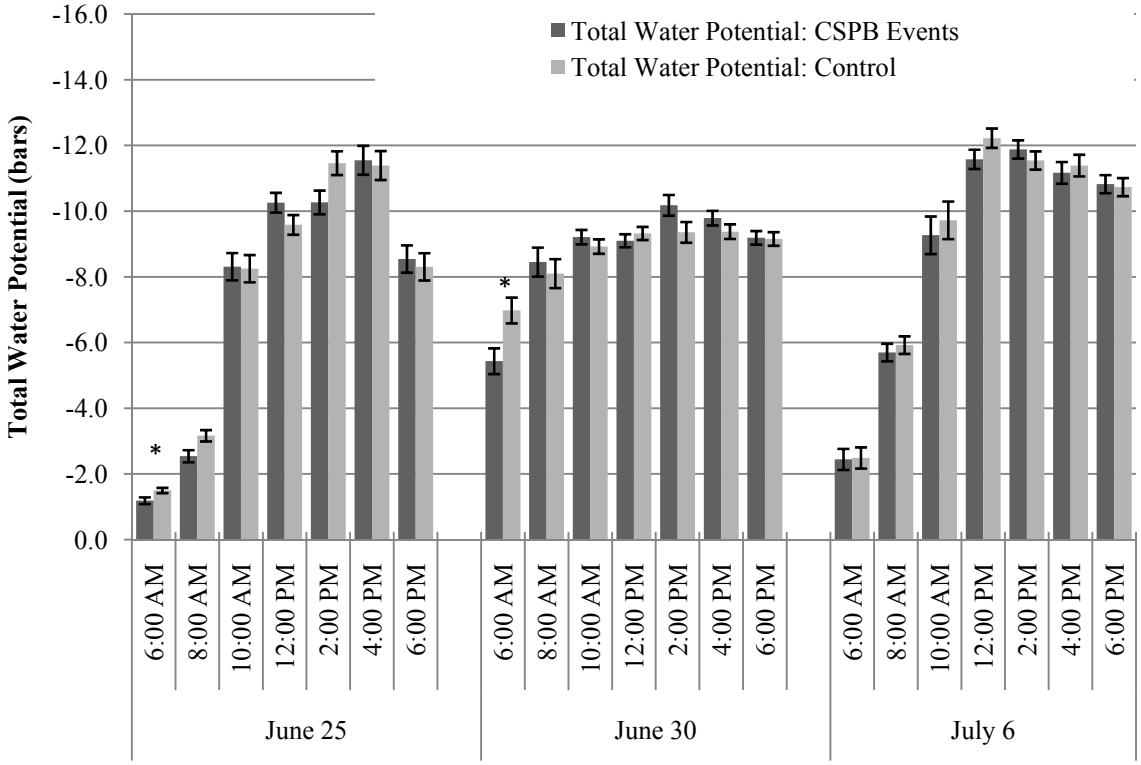



**Figure I-22. Water Potential of Transgenic CSPB Plants and the Control throughout the Duration of Stress Imposition**

The study was conducted using three CSPB-containing events and a control. All events contained the same construct as MON 87460. Entries were planted in two row plots with three replications per experiment. Plots were irrigated weekly to the V8 stage and then water was withheld until the R2 developmental stage. Physiological parameters were assessed on three separate days during the water-limitation treatment cycle initiating at the onset of drought, before visible wilting. Water potential measurements were taken on three plants per plot every two hours between 6 a.m. and 6 p.m. An asterisk indicates a statistically significant difference at a p value of less than 0.100 using an analysis of variance.

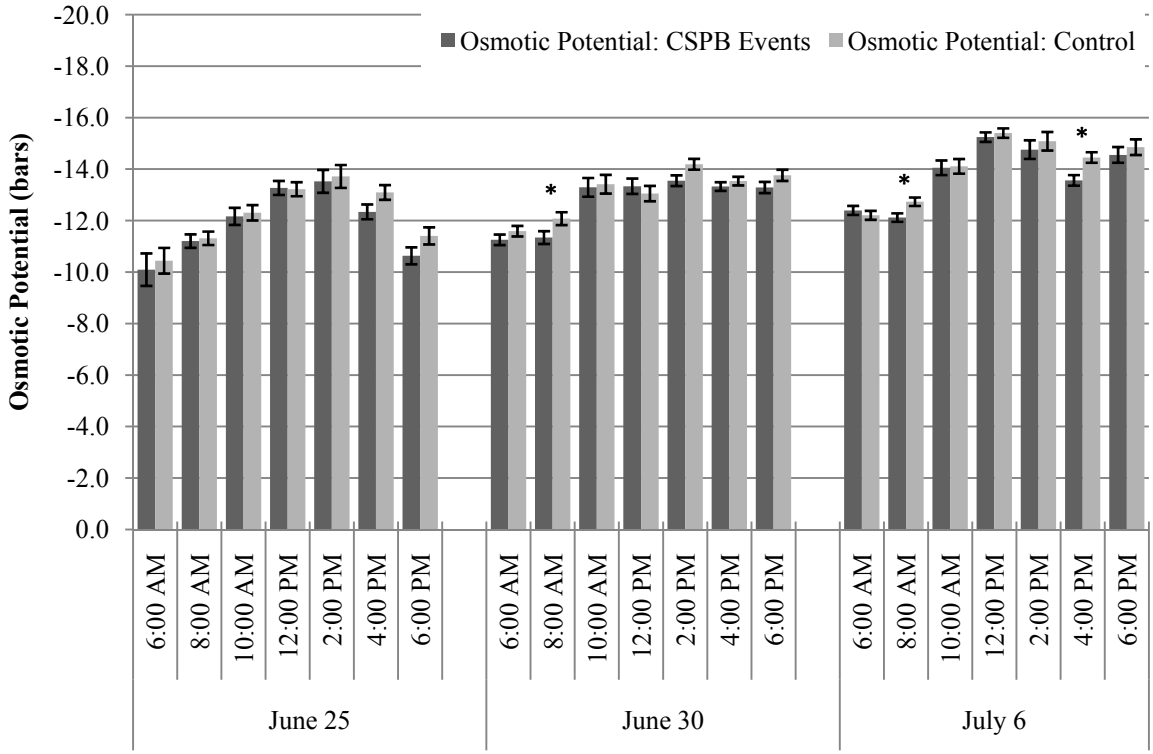



**Figure I-23. Osmotic Potential of Transgenic CSPB Plants and the Control throughout the Duration of Stress Imposition**

The study was conducted using three CSPB-containing events and a control. All events contained the same construct as MON 87460. Entries were planted in two row plots with three replications per experiment. Plots were irrigated weekly to the V8 stage and then water was withheld until the R2 developmental stage. Physiological parameters were assessed on three separate days during the water-limitation treatment cycle initiating at the onset of drought, before visible wilting. Osmotic potential measurements were taken on three plants per plot every two hours between 6 a.m. and 6 p.m. An asterisk indicates a statistically significant difference at a p value of less than 0.100 using an analysis of variance.

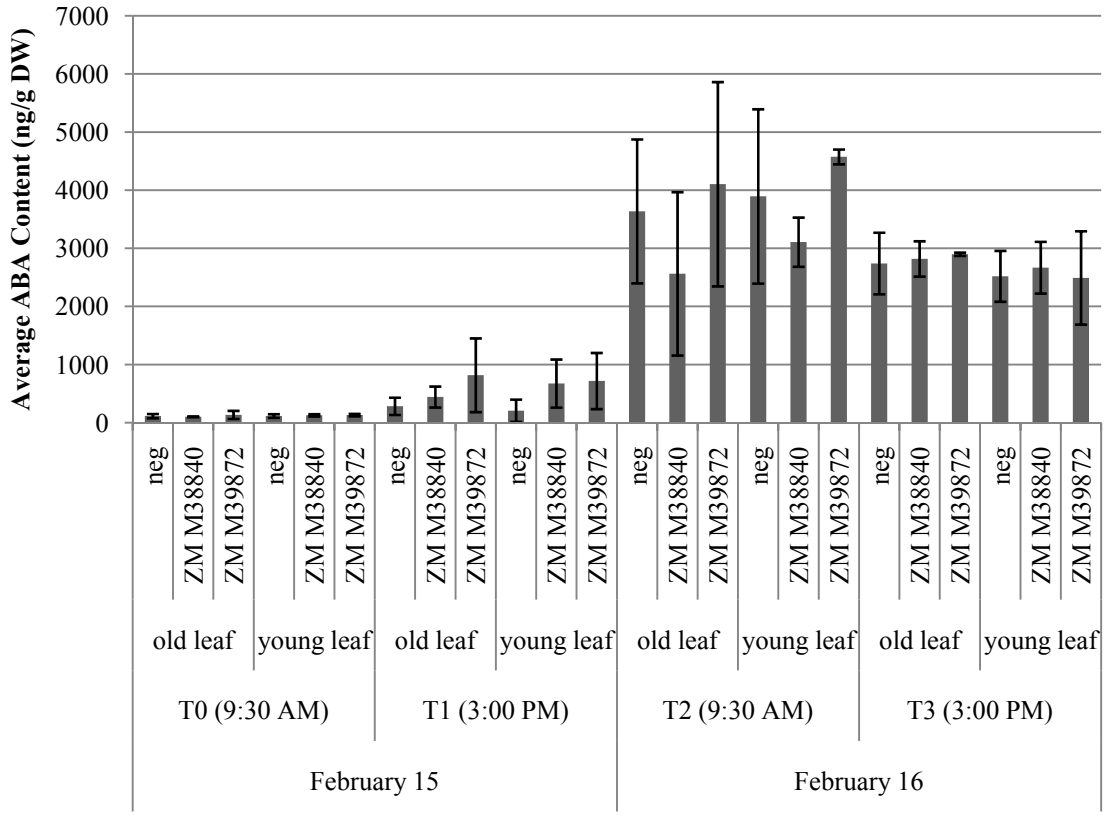



**Figure I-24. Average Abscisic Acid Content in Leaves from Transgenic CSPB Plants and the Control Taken over the Course of Two Days under Water-Limited Conditions**

The study was conducted using three CSPB-containing events (identified with codes beginning 'ZM') and a control. All events contained the same construct as MON 87460. All plants were grown for approximately five weeks prior to the experiment. The water-limited treatment was established based on pot weight. Samples were taken at four time-points over the course of two days, tracking with the onset of stress. One gram leaf samples were taken from 12 control plants and six replicates for each of the two transgenic entries at each time-point. For each plant, two samples were taken from two different leaves (young leaves and old leaves).

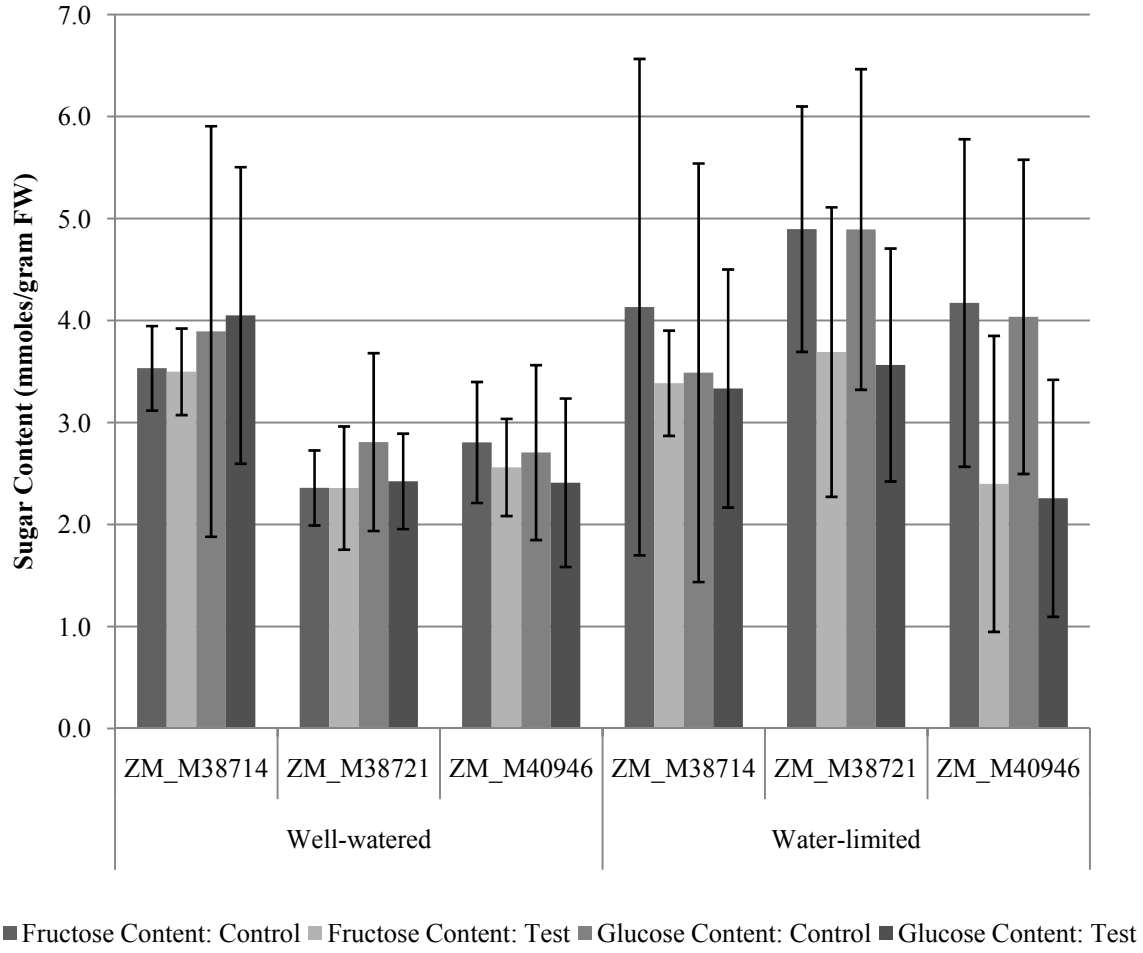



**Figure I-25. Leaf Fructose and Glucose Content in Transgenic CSPB Plants and the Control**

The study was conducted using three CSPB-containing events (identified with codes beginning 'ZM') and a control. All events contained the same construct as MON 87460. Plants were produced under well-watered and water-limited conditions in a greenhouse.

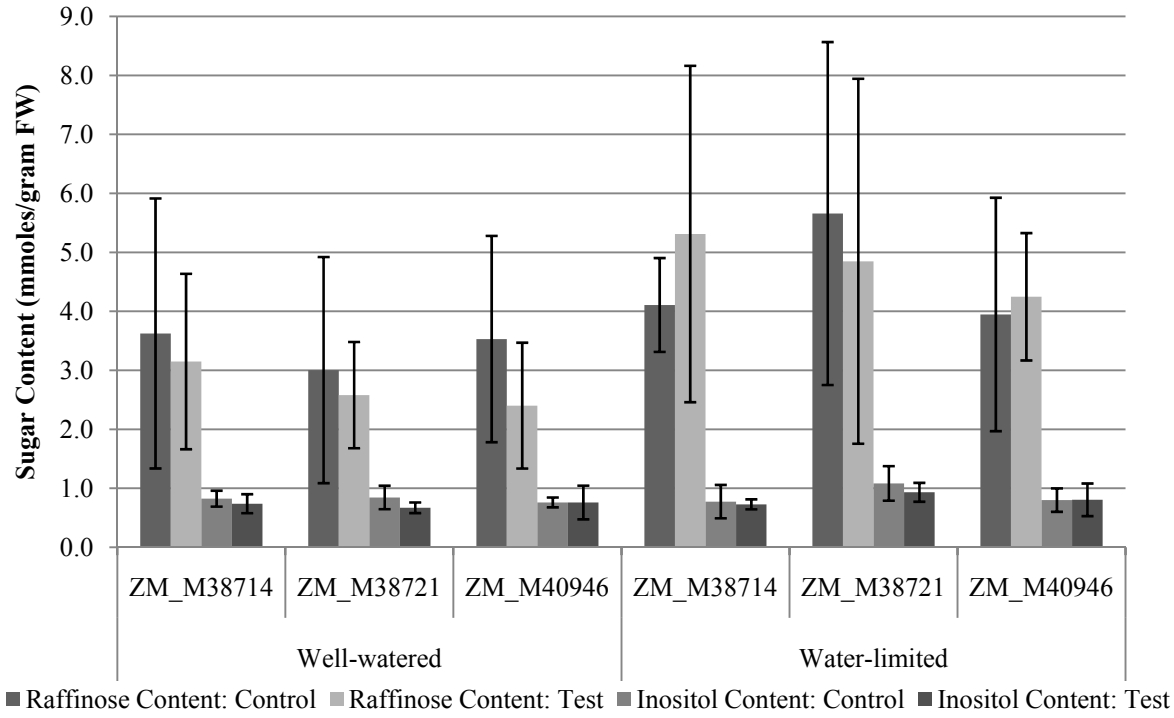



**Figure I-26. Leaf Raffinose and Inositol Content in Transgenic CSPB Plants and the Control**

The study was conducted using three CSPB-containing events (identified with codes beginning 'ZM') and a control. All events contained the same construct as MON 87460. Plants were produced under well-watered and water-limited conditions in a greenhouse.

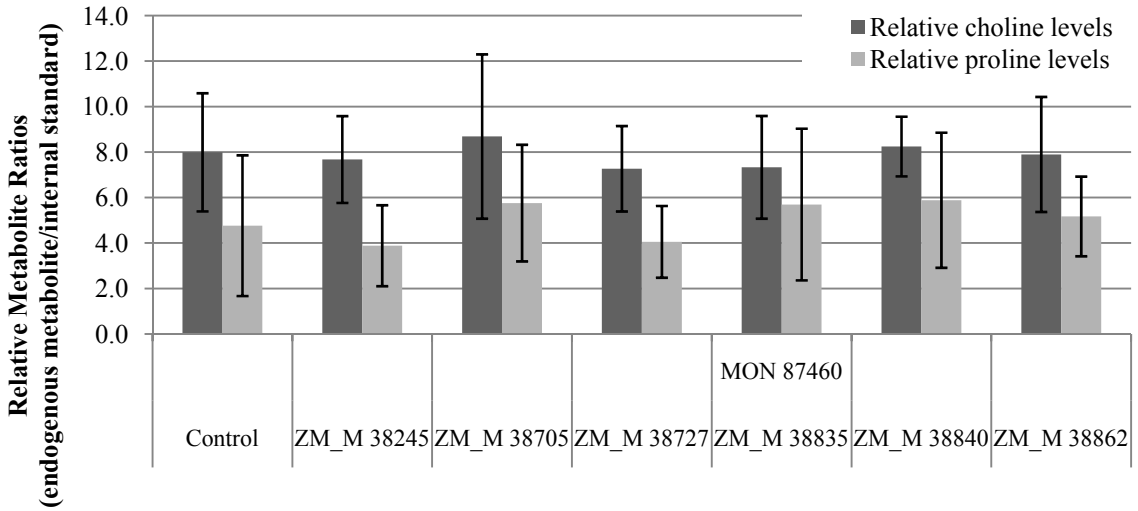



**Figure I-27. Choline and Proline of Transgenic CSPB Plants and the Control throughout the Duration of Stress Imposition**

The study was conducted using MON 87460, five other events (identified with codes beginning 'ZM') containing the same construct as MON 87460 and a control. All plants were subjected to a 12 day period of moderate stress based on pot weight in the greenhouse. Six leaf punches from 12 plants per entry were collected 12 days after the initiation of stress. Samples were analyzed for choline and proline. Prior to sample extraction, deuterated homologues (d9 choline and d7 proline) were added as internal standards to control for extraction efficiency. Data are reported as the ratio of the analyte recovered relative to the recovery of the corresponding internal standard.

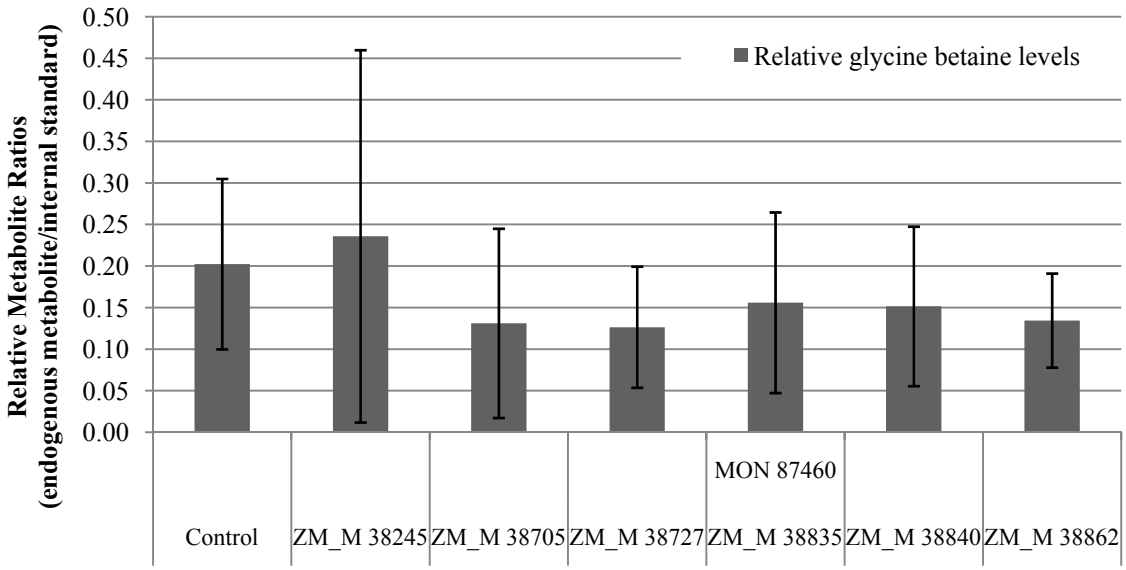



**Figure I-28. Relative Glycine Betaine Levels of Transgenic CSPB Plants and the Control throughout the Duration of Stress Imposition**

The study was conducted using MON 87460, five other events (identified with codes beginning 'ZM') containing the same construct as MON 87460 and a control. All plants were subjected to a 12 day period of moderate stress based on pot weight in the greenhouse. Six leaf punches from 12 plants per entry were collected 12 days after the initiation of stress. Samples were analyzed for glycine betaine. Prior to sample extraction, the deuterated homologue (d9 glycine betaine) was added as an internal standard to control for extraction efficiency. Data are reported as the ratio of the analyte recovered relative to the recovery of the internal standard.

## I.E. Adoption, Estimated Use, and Global Trade Flows of MON 87460

As described previously (Section I.B.2), growers are expected to adopt MON 87460 in regions suitable for corn production that are most prone to frequent drought stress. In the U.S., the major area of adoption is likely to be the Western Dryland region of the Corn Belt known as the Great Plains, which contributes 25% of the U.S. corn production (Riebsame, 1990). Other corn producing geographies with similar conditions, such as parts of Africa, Europe, and Latin America, may also benefit from this technology. The successful adoption of MON 87460 is expected to increase economic and environmental benefits to producers and consumers due to the protection of corn yields under water-limited conditions. Increased corn yield per acre will help conserve the total number of acres needed to meet the needs for food, feed, and biofuel uses. It is expected that MON 87460 will enter commerce as part of the normal trade flows of yellow corn and it will be utilized in the same manner as any other yellow corn.

## I.F. Submissions to Other Regulatory Agencies

### I.F.1. Submission to the FDA

MON 87460 falls within the scope of the Food and Drug Administration's (FDA) policy statement concerning regulation of products derived from new plant varieties, including those produced through genetic engineering. Monsanto has voluntarily initiated and will complete a consultation process with FDA prior to commercial distribution of this product. A safety and nutritional assessment of food and feed derived from MON 87460 was submitted to the FDA dated December 19, 2008.

### I.F.2. Submissions to Foreign Government Agencies

Regulatory submissions for import and production approvals will be made to countries that import U.S. corn grain and have regulatory approval processes in place. These will include submissions to a number of foreign government regulatory agencies, including Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF) and the Ministry of Health, Labor and Welfare (MHLW); the Canadian Food Inspection Agency (CFIA) and Health Canada; the Health Ministry of Mexico (HMM); the European Food Safety Authority (EFSA), and the regulatory authorities in many other countries. As appropriate, notifications of import will be made to importing countries that do not have a formal approval process.

## I.G. References


Anderson, J.R., D. Mukherjee, K. Muthukumaraswamy, K.C.M. Moraes, C.J. Wilusz, and J. Wilusz. 2006. Sequence-specific RNA binding mediated by the RNase PH domain of components of the exosome. RNA-a Publication of the RNA Society. 12:1810-1816.

Andrade, F. H., L. Echarte, R. Rizzalli, A. Della Maggiora, and M. Casanovas. 2002. Kernel number prediction in maize under nitrogen or water stress. Crop Science. 42:1173-1179.

Barker, T., H. Campos, M. Cooper, D. Dolan, G. Edmeades, J. Habben, J. Schlusser, D. Wright, and C. Zinselmeier. 2005. Improving Drought Tolerance in Maize. Pages 173-253 in Plant Breeding Reviews. Vol 25, J. Janick, (ed.) John Wiley and Sons, Inc., Hoboken, New Jersey.

Boyer, J.S. 1982. Plant productivity and environment. Science. 218:443-448.

Boyer, J. S. and M. E. Westgate. 2004. Grain yields with limited water. Journal of Experimental Botany. 55:2385-2394.

Bruce, W.B., G.O. Edmeades, T.C. Barker. 2002. Molecular and physiological approaches to maize improvement for drought tolerance. Journal of Experimental Botany. 53:13-25.

Campos, H., M. Cooper, G.O. Edmeades, C. Loffler, J.R. Schussler, and M. Ibanez. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. Maydica. 51:369-381.

Castiglioni, P., D. Warner, R.J. Bensen, D.C. Anstrom, J. Harrison, M. Stoecker, M. Abad, G. Kumar, S. Salvador, R. D'Ordine, S. Navarro, S. Back, M. Fernandes, J. Targolli, S. Dasgupta, C. Bonin, M.H. Luethy, and J.E. Heard. 2008. Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiology. 147:446-455.

Chaikam, V., and Karlson, D. 2008. Functional characterization of two cold shock domain proteins from *Oryza sativa*. Plant Cell and Environment. 31: 995-1006.

Chenu K, Chapman SC, Hammer GL, McLean G, Salah HB, Tardieu F. 2008. Short-term responses of leaf growth rate to water deficit scale up to whole-plant and crop levels: an integrated modeling approach in maize. Plant Cell and Environment. 31: 378-391.

Claassen, M. M. and R. H. Shaw. 1970. Water deficit effects on corn. II. Grain components. Agronomy Journal. 62: 652-655.

Cristofari, G., and J.L. Darlix. 2002. The ubiquitous nature of RNA chaperone proteins. Progress in Nucleic Acid Research and Molecular Biology. Vol 72 72:223-268.

EFSA. 2007. Statement of the Scientific Panel on Genetically Modified Organisms on the safe use of the *npt*II antibiotic resistance marker gene in genetically modified plants. European Food Safety Authority. Brussels, Belgium http://www.efsa.europa.eu.

Etchegaray, J.P., Jones, P.G., and Inouye, M. 1996. Differential thermoregulation of two highly homologous cold shock genes, *cspA* and *cspB*, of *Escherichia coli*. Genes to Cells. 1:171-178.

FDA. 1994. Secondary direct food additives permitted in food for human consumption: food additives permitted in feed and drinking water of animals: aminoglycoside 3'-phosphotransferase II; final rule. Federal Register.

FDA. 1998. U.S. Food and Drug Administration. Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants (Draft Guidance released September 4, 1998).

Flavell, R.B., Dart, E., Fuchs, R.L., and Fraley, R.T. 1992. Selectable marker genes: safe for plants? BioTechnology. New York. 10:141-144.

Fuad-Hassan, A., F. Tardieu, O. Turc. 2008. Drought-induced changes in anthesis-silking interval are related to silk expansion: a spatio-temporal growth analysis in maize plants subjected to soil water deficit. Plant Cell and Environment. 31:1349-1360.

Fuchs, R.L., R.A. Heeren, M.E. Gustafson, G.J. Rogan, D.E. Bartnicki, R.M. Leimgruber, R.F. Finn, A. Hershman, and S.A. Berberich. 1993a. Purification and characterization of microbially expressed neomycin phosphotransferase II (NPTII) protein and its equivalence to the plant expressed protein. BioTechnology. 11:1537-1542.

Fuchs, R.L., J.E. Ream, B.G. Hammond, M.W. Naylor, R.M. Leimgruberand, and S.A. Berberich. 1993b. Safety assessment of the neomycin phosphotransferase II (NPTII). BioTechnology. New York. 11:1543-1547.

Fusaro, A.F., Bocca, S.N, Ramos, R.L.B., Barroca, R.M., Magioli, C., Jorge, V.C., Coutinho, T.C., Rangel-Lima, C.M., De Rycke, R., Inze, D., Engler, G., and Sachetto-Martins, G. 2007. AtGRP2, a Cold-induced Nucleo-cytoplasmic RNA-binding Protein, has a role in flower and seed development. Planta. 225:1339-1351

Graumann, P., T.M. Wendrich, M.H.W. Weber, K. Schroder, and M.A. Marahiel. 1997. A family of cold shock proteins in *Bacillus subtilis* is essential for cellular growth and for efficient protein synthesis at optimal and low temperatures. Molecular Microbiology. 25:741-756.

Karlson, D., and Imai, R. 2003. Conservation of the cold shock domain protein family in plants. Plant Physiology. 131:12-15

Karlson, D., K. Nakaminami, T. Toyomasu, and R. Imai. 2002. A cold-regulated nucleic acid-binding protein of winter wheat shares a domain with bacterial cold shock proteins. Journal of Biological Chemistry. 277:35248-35256.

Kim, J.Y., S.J. Park, B.S. Jang, C.H. Jung, S.J. Ahn, C.H. Goh, K. Cho, O. Han, and H.S. Kang. 2007. Functional characterization of a glycine-rich RNA-binding protein 2 in Arabidopsis thaliana under abiotic stress conditions. Plant Journal. 50:439-451.

Mascarenhas, J., Weber, M.H., and Graumann, P.L. 2001. Specific polar localization of ribosomes in Bacillus subtilis depends on active transcription. EMBO Reports. 2:685-689.

Nakaminami, K., Sasaki, K., Kajita, S., Takeda, H., Karlson, D., Ohgi, K., and Imai, R. 2005. Heat stable ssDNA/RNA-binding activity of a wheat cold shock domain protein. FEBS Letters. 579: 4887-4891.

Nakaminami, K., Karlson, D.T., and Imai, R. 2006. Functional conservation of cold shock domains in bacteria and higher plants. Proceedings of the National Academy of Sciences USA. 103:10122-10127

Nap, J.P., J. Bijvoet, and W.J. Stiekema. 1992. Biosafety of kanamycin-resistant transgenic plants: an overview. Transgenic Research. 1:239-249.

Riebsame, William E. 1990. The United States Great Plains. Ch. 34, p. 561-575 in B.L. Turner II et. al., The Earth as Transformed by Human Action. Cambridge University Press, NY.

Sasaki, K., M.H. Kim, and R. Imai. 2007. Arabidopsis COLD SHOCK DOMAIN PROTEIN2 is a RNA chaperone that is regulated by cold and developmental signals. Biochemical and Biophysical Research Communications. 364:633-638.

Schindler, T., P.L. Graumann, D. Perl, S.F. Ma, F.X. Schmid, and M.A. Marahiel. 1999. The family of cold shock proteins of *Bacillus subtilis* - stability and dynamics *in vitro* and *in vivo*. Journal of Biological Chemistry. 274:3407-3413.

USDA-NASS. 2007. Online database www.nass.usda.gov at the United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS). Accessed June 30, 2008.

Weber M.H.W., Volkov, A., Fricke, I., Marahiel, M.A. and Graumann, P.L. 2001. Localization of cold shock proteins to cytosolic spaces surrounding nucleoids in *Bacillus subtilis* depends on active transcription. Journal of Bacteriology. 183:6435-6443.

Welcker, C., B. Boussuge, C. Bencivenni, J-M. Ribaut and F. Tardieu. 2007. Are source and sink strengths genetically linked in maize plants subjected to water deficit? A QTL study of the responses of leaf growth and of Anthesis-Silking Interval to water deficit. Journal of Experimental Botany. 58:339 - 349.

Westgate, M.E., M.E. Otegui, and F.H. Andrade. 2004. Physiology of the Corn Plant. in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

## II. The History, Biology, and Global Use of Corn

This section summarizes the history, biology, and global use of corn based on: (1) the consensus document on the biology of corn (*Zea mays* L.) developed as part of the Organization for Economic Co-operation and Development (OECD, 2003a); (2) a summary prepared by the Biotechnology Regulatory Services of the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS, 2009); (3) information provided in the USDA petition for MON 89034 (Monsanto Company petition No. 06-CR-166U); and (4) other published literature.

### II.A. Introduction

Corn is a versatile crop that provides food, feed, and fuel to a global economy. Recently, a surge in demand for corn has been created by growing economies in the developing world and its use as an alternative fuel source in the developed world. These demands are exceeding production, leading to diminished grain reserves worldwide. In addition, climate change may have variable impacts on crop yields, potentially creating further supply disruptions. The combination of these factors places a premium on corn yield stability in sub-optimal environments.

Drought stress is the major cause of yield reduction in corn and its effects have far reaching global socio-economic implications. In North America alone, it is estimated that 40% of annual crop losses are due to sub-optimal water availability (Boyer, 1982). Consequently, increasing drought tolerance in corn is a major goal of agricultural breeding and biotechnology. Advances in breeding and agronomic practices have made significant contributions to increasing corn yield potential and drought tolerance. Biotechnology provides additional tools that can be used in combination with breeding and agronomic practices to improve productivity.

Monsanto has developed drought tolerant corn MON 87460 that provides reduced yield loss under water-limited conditions compared to conventional corn. Efficacy in MON 87460 is derived by expression of the inserted *Bacillus subtilis* cold shock protein B (CSPB). Understanding the applications, benefits, and function of MON 87460 in meeting the global demands on corn production requires an understanding of corn's origins and history, its biology and cultivation, and its importance to global trade. A summary of the history, taxonomy, and biology of corn, including gene flow between cultivated corn and its wild relatives, and global use of corn is presented below.

### II.B. History of Corn

From its likely origin as a wild grass, corn has undergone continuous breeding, modification, and selection for properties that suit the needs of consumers and growers who wish to produce corn grain. Corn originated in the highlands of Mexico 7,000 to 10,000 years ago. European contact introduced corn to the rest of the world and has allowed it to become an essential crop for food, feed, and fuel (Goodman, 1988).

One of the most striking differences between cultivated corn and its wild relatives is the obvious emphasis that millennia of breeding and selection efforts have placed on grain

yield. All modern corn hybrids produce multi-rowed ears containing hundreds of kernels. Corn's closest wild relative, teosinte, produces a cluster of spikes, each with one or two rows of seeds. The transformation of spikes into a single enclosed ear is one of the key achievements in the development of corn as a crop (Wilkes, 2004). Since the earliest cultivation efforts in Mexico until the early 20th century, corn existed primarily in the form of open pollinated varieties. The discovery of corn hybridization led to the development of modern-day dent corn, better adaptation to previously adverse environments, and significant yield increases (Duvick et al., 2004).

In the 1940s, when hybrid corn began to predominate, average U.S. yields were 30 – 40 bushels/acre (bu/ac, 2.2 metric tons/hectare (MT/ha)) (Duvick et al., 2004). By 2000, average U.S. yields were over 130 bu/ac (8.6 MT/ha). Advances in breeding and the widespread availability of fertilizers and pesticides made significant contributions to these advancements (Troyer, 2004). Biotechnology provides additional tools to improve productivity by reducing the inputs needed to control weeds and insects (Hicks and Thomison, 2004; Kaeppler, 2004).

In 2007, herbicide tolerance and insect resistance traits were grown on 73% of U.S. corn acres. Combined trait products, with combinations of herbicide tolerance and insect resistance, were the largest category at 28% of U.S. corn acres (NCGA, 2008). As a complement to these existing traits, MON 87460 provides yield stability under water-limited conditions that otherwise limit plant performance. Increasing demand for corn in the food, feed, and fuel sectors places a premium on technologies that stabilize yield and allow more corn to be produced on existing acres.

### II.C. Taxonomy and Phylogenetics

Several hypotheses exist on the origin of corn but the preponderance of evidence supports the hypothesis that corn descended from teosinte (Galinat, 1988). The teosinte genome is similar to corn; teosinte easily crosses with corn, and teosinte has several morphological traits similar to corn. Teosinte has a more weedy appearance and more tillers than modern corn hybrids. The one major distinguishing difference between corn and teosinte is the female inflorescence, or ear. Modern corn hybrids have one to three lateral branches that terminate in an ear with 8 to 24 rows of kernels that are enclosed in modified leaves or husks. Teosinte also has lateral branches, but they terminate in two-rowed spikes of perhaps 12 fruit cases, with each fruit case having one seed enclosed by an indurated glume (Goodman and Brown, 1988).

Corn (*Zea mays* L.) is a member of the tribe Maydae, which is included in the subfamily Panicoideae of the grass family Gramineae. Table II-1 summarizes the taxonomic classification of corn and its close relatives.

**Table II-1. Taxonomic Classification of Corn and its Close Relatives**

Family - *Gramineae*
Subfamily - *Panicoideae*
Tribe - *Maydae*

**Western Hemisphere:**

I. Genus - *Zea*
A. Subgenus - *Luxuriantes*
1. *Zea luxurians* (2n = 20)
2. *Zea perennis* (2n = 40)
3. *Zea diploperennis* (2n = 20)
B. Subgenus - *Zea*
1. *Zea mays* (2n = 20) (**corn)**
Subspecies
1. *Z. mays parviglumis* (2n = 20)
2. *Z. mays huehuetenangensis* (2n = 20)
3. *Z. mays mexicana* (Schrad.) (2n = 20) (**teosinte**)

II. Genus – *Tripsacum*
A. Section – *Tripsacum*
Species
1. *T. andersomii* (2n = 64)
2. *T. australe* (2n = 36)
Varieties
a) *T. australe* var. *australe*
b) *T. australe* var. *hirstum*
3. *T. bravum* (2n = 36, 72)
4. *T. cundinamarce* (2n = 36)
5. *T. dactyloides* (2n = 72)
Varieties
a) *T. dactyloides* var. *hispidum*
b) *T. dactyloides* var. *dactyloides*
c) *T. dactyloides* var. *meridonale*
d) *T. dactyloides* var. *mexicanum*
6. *T. floridanum* (2n = 36)
7. *T. intermedium* (2n = 72
8. *T. manisuroides* (2n = 72)
9. *T. latifolium* (2n = 36)
10. *T. percuvianum* (2n = 72, 90, 108)
11. *T. zopilotense* (2n = 36, 72)

B. Section – *Fasciculata*
Species
1. *T. jalapense* (2n = 72)
2. *T. lanceolatum* (2n = 72)
3. *T. fasciculatum* (2n = 36)
4. *T. maizar* (2n = 36, 72)
5. *T. pilosum* (2n = 72)
Varieties
a) *T. pilosum* var. *guatemalense*
b) *T. pilosum* var. *pilosum*

**Asia:**

I. Genera—
*Chionachne* (2n = 20)
*Coix* (2n = 10, 20)
*Polytoca* (2n = 20)
*Schlerachne* (2n = 20)
*Trilobachne* (2n = 20)

Tribe—*Andropogoneae*
I. Genus - *Manisuris*

The genera included in the tribe Maydae include *Zea* and *Tripsacum* in the Western Hemisphere, and *Coix*, *Polytoca*, *Chionachne*, *Schlerachne*, and *Trilobachne* in Asia. Although some researchers have implicated the Asian genera in the origin of corn, the evidence for them is not as extensive and convincing as for the genera located in the Western Hemisphere.

The genus *Zea* includes two sub-genera: *Luxuriantes* and *Zea*. Corn (*Zea mays* L.) is a separate species within the subgenus *Zea,* along with three subspecies. All species within the genus *Zea*, except corn, are different species of teosinte. Until recently, the teosinte species were included in the genus *Euchlaena* rather than the genus *Zea*.

## II.D. Biology of Corn and Environmental Effects on its Development

### II.D.1. Genetics

Corn is genetically one of the best developed and best characterized of the higher plants. Because of the separation of male and female inflorescence, number of seeds produced on the female inflorescence, ease in handling (growing and hand pollinating), nature of the chromosomes, and low basic chromosome number (n = 10), corn has been accessible for study at all levels of genetics.

Corn was one of the first crop species studied in genetics laboratories to obtain a basic understanding of mitosis, meiosis, chromosome segregation, linkage and effects of crossing-over, and transposable elements. Because of the importance of corn in the U.S. and world economies, and the genetic information obtained since 1900, corn has continued to receive extensive study in modern genetics laboratories.

A key concept in breeding is quantitative trait loci (QTLs). QTLs are regions of chromosomes that are known to play a role in imparting physiological traits of interest and represent a significant area of advancement in modern crop breeding. Identifying QTLs of interest and combining them into a single line of germplasm can produce progeny with increased yield potential and tolerance to multiple stresses (Takeda and Matsuoka, 2008). Corn has QTLs related to flowering synchrony under drought stress on chromosomes 1, 2, 5, 6, 8, and 10. Physiological traits associated with tolerance of other stresses are also controlled by multiple QTLs, indicating that stress responses are multigenic traits rather than the effect of a single gene or pathway (Chinnusamy et al., 2005). A key challenge for relying on QTLs to impart abiotic stress tolerance is the difficulty in imposing targeted stresses under field conditions to select for desirable phenotypes (Takeda and Matsuoka, 2008). In addition, selecting for a particular trait through QTLs or other approaches, even when the gene has been identified, does not necessarily mean the exact function of the gene is known (Takeda and Matsuoka, 2008).

### II.D.2. Growth and Development

Corn development is measured in vegetative (V) and reproductive (R) stages (Figure II-1). The V number corresponds to the number of leaves with a visible collar or ligule. Leaves initiate at the growing point, or meristem, which in corn and other grasses is at the base of the plant. At V5, ear shoot formation is complete and all leaves have initiated.

Until V6, the growing point of the plant is below ground. Beginning at V10, corn plants accumulate a reserve of nutrients and dry weight to support reproductive development and grain production. V stages proceed through VT (tassel emergence or anthesis) and the R stages begin at silking (R1). R2 and R3 represent early to mid grainfill while R4 and R5 represent mid to late grainfill. R6 is physiological maturity (Hanway, 1966).

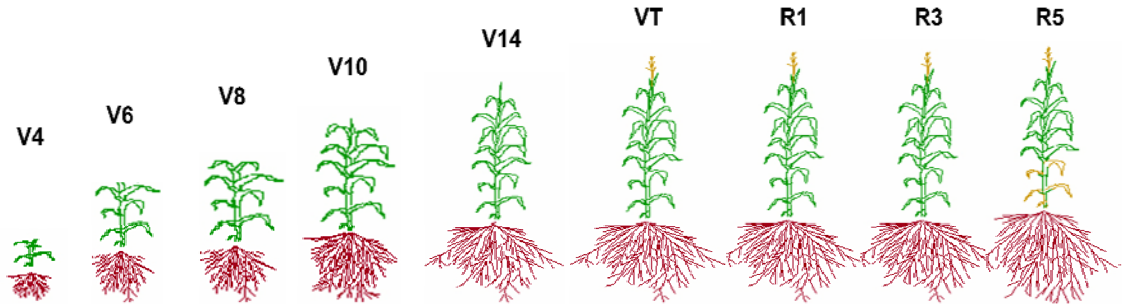



**Figure II-1. Growth Stages of Corn from Early Vegetative (V4) through Late Grainfill (R5)**

Corn yield is driven by both kernel number and kernel weight. Kernel number is a function of how many ovules are fertilized and supported through maturity and is the primary determinant of overall yield (Westgate et al., 2004). Kernel weight is a function of the amount of dry matter available to support kernel development and the amount of water available during grainfill. Harvest index (HI) is the ratio of grain biomass to shoot biomass and is used to quantify the relationship between sink tissue and source tissue. In modern hybrids HI is approximately 0.5 (Westgate et al., 2004). The amount of shoot biomass available at the end of the season to support kernel development is a function of the amount of photosynthetically active radiation (PAR) the plant is able to intercept. This in turn is a function of the amount of leaf area exposed to sunlight, which is determined by the rate of leaf expansion. Sufficiently severe, adverse conditions during any growth stage can limit plant growth and therefore limit yield (Westgate et al., 2004).

### II.D.3. Environmental Effects on Growth and Development

Corn has been adapted to a wide geographical range, but its growth, development, and yield potential are still closely related to environmental conditions, including temperature, nutrients, and water. Soil temperatures must be above 10 °C for corn seed to germinate. Corn cannot survive temperatures below 0 °C for more than six to eight hours after the growing point is above ground, which occurs at V6. Likewise, soil moisture must be sufficient for germination to occur. Once plants emerge, nutrient supplies, particularly nitrogen must be sufficient to allow rapid growth. Water limitation during these growth stages can impair growth, but the effects are often reversible (Barker et al., 2005). Until V6, cold soil temperatures will adversely effect corn growth and development. As canopy closure begins in the V6 growth stage, high plant density (more

than 30,000 plants per acre) can limit PAR interception, reducing growth rate (Westgate et al., 2004) and limiting the supply of photosynthate. Shading caused by high planting densities also reduces the amount of available photosynthate, reducing the ability of a plant to support developing kernels (Setter et al., 2001). As corn reaches reproductive stages (VT-R1), periods of extended high temperatures can reduce pollen viability (Schoper et al., 1987) and yield.

Among all of the environmental stressors that affect corn development and yield, water availability is by far the most important. In North America alone, it is estimated that 40% of annual crop losses are due to sub-optimal water availability (Boyer, 1982). As a C4 plant, corn is inherently more water use efficient and transpires only half as much water as C3 plants (Taiz, 1991), e.g., rice, soybean, and cotton. Despite this key advantage, water limitation still adversely impacts corn growth, development, and yield, particularly during reproductive growth stages. In general, water limitation causes plants to lose turgor pressure and stomata close to limit water loss. Water limitation also causes corn leaves to roll, or curl upon themselves, reducing surface area, decreasing photosynthesis and, ultimately, growth. Water limitation manifests itself visually in corn as reduced plant height and yield. Limiting water during later reproductive growth stages results in scorching and leaf firing (Hsiao, 1973; Westgate et al., 2004).

The flowering growth stages (VT – R1) are a particularly vulnerable period for corn plant development and the resulting yields. Four days of visible wilting in the growth stages just prior to VT can reduce corn yields by up to 25 percent. Four days of visible wilting between the final V stages and R2 can reduce yield by 50 percent (McWilliams, 2002). An important mechanism by which drought reduces corn yield is by preventing synchronous flowering. Optimal yields depend upon silking (R1) occurring within a day of anthesis (VT). Synchrony is measured as the anthesis-silking interval (ASI). As ASI increases, silks emerge too late to intercept pollen and yields decrease dramatically. As silks are dependent upon a sufficient supply of water to extrude at the correct time, water limitation is a primary cause of large ASI values (Westgate et al., 2004). Silks are more sensitive to low water potential than leaves or roots, low water potential being associated with poor solute accumulation and an inability to retain turgor (Maiti and Wesche-Ebeling, 1998). Failure to intercept pollen is not the sole cause of poor kernel number, however. Abortion of fertilized kernels may also be a significant factor in yield loss under water limitation (Westgate et al., 2004). Water limitation during late vegetative stages through flowering will reduce kernel number and increase the number of plants without ears (barrenness). Water limitation during grain filling will result in smaller kernels (Campos et al., 2006). Kernels will not accumulate dry matter once their moisture level falls below 30%.

Breeders have sought to improve drought tolerance in corn for decades. Breeding under water-limited conditions has led to improved germplasm that is able to produce better yields than non-drought tolerant hybrids. The primary mechanism for improvement was by selection for synchronous flowering. Breeders conducted this selection simply by self-pollinating plants under drought stress. Those plants that were silking while pollen was being shed could be selfed, in effect selecting for plants that were able to maintain

normal function under drought stress. A retrospective study of advances in drought tolerance over 50 years found that the greatest breeding yield gains were obtained under flowering stress at slightly more than 0.1 MT/ha/year. These breeding advances also improved yields under optimal conditions, although sensitivity to mid- to late-grainfill stress was also increased (Campos et al., 2006).

### II.D.4. Cultivation

Corn is currently grown on over 150 million hectares globally. In 2007-2008 the U.S., China, Brazil, the European Union, and Mexico were the top five producers (USDA-FAS, 2008). Crop inputs play a significant role in determining yield and will vary based on climate and soil type. Water is a key input with 50-60 cm of water needed under temperate conditions. Corn rapidly depletes nitrogen and phosphorous and these essential nutrients must be replenished with each crop. Insect pressure and competition from weeds are also central concerns in corn management and must be managed to minimize yield losses (Olson and Sander, 1988). As with crop inputs, the appropriate choice of hybrid is essential for optimal yield and will vary with planting location.

Hybridization is a fundamental concept used in global corn breeding and production. Corn was originally developed as an open-pollinated (cross-fertilizing) crop species, and until the beginning of the 20$^{th}$ century, only open-pollinated corn varieties were grown. The fundamental concepts for development of hybrid corn were defined by 1920 (Sprague, 1946). Continuous selfing of individuals for multiple generations results in pure (inbred) lines within which every plant has similar traits. In these inbred lines, heterozygous loci are eliminated by inbreeding to homozygous loci of either one of the two alleles at each locus. The fixation of alleles in pure lines causes a general reduction in vigor and productivity. Crossing two inbred lines restores vigor (heterosis). Selecting for inbred lines and crosses that possess desirable traits forms the basis of modern corn breeding. Recent techniques such as marker-assisted breeding have reduced the time and number of generations required to produce pure inbreds (Yousef and Juvik, 2001).

Since 1996, biotechnology has provided growers with an array of traits to further improve yields by reducing competition from weeds and insects. Herbicide tolerance and insect resistance traits increase productivity while decreasing inputs (Brookes and Barfoot, 2008; Hicks and Thomison, 2004; Kaeppler, 2004). Globally, between 1996 and 2006, herbicide tolerant corn allowed a 3.9% reduction in the use of herbicide active ingredients and insect resistant corn allowed a 5.0% reduction in the use of insecticide active ingredients. Notably, over this same period, an additional $1.1 billion and an additional $3.6 billion of global farm income can be attributed to the adoption of herbicide tolerant and insect resistant corn, respectively. Improved yields are responsible for 43% of these economic gains (Brookes and Barfoot, 2008).

Rotation with other crops is typically advantageous for corn yield. Rotation benefits corn production by allowing alternate weed, insect and disease management strategies and improving soil structure (Hicks and Thomison, 2004). In the U.S. Corn Belt, soybean is a common rotational crop although sorghum is also used. In the Western Dryland region, wheat, sorghum and soybean may be planted in rotation with corn. Wheat-fallow

rotation is also used to store rainfall during the fallow season. Recent research has shown that wheat-fallow rotations may actually reduce the amount of soil moisture available to wheat crops because fallow season management requires herbicides or tillage, the cost of which can exceed profits from the subsequent wheat crop. In order to maximize the return on any stored water, a more intensive crop such as corn, sorghum or millet should be grown in rotation with wheat (Croissant et al., 1998).

### II.D.5. Potential Gene Transfer

#### II.D.5.1. Outcrossing with Wild Zea and Tripsacum Species

Gene flow between corn and its closest relative, teosinte (*Z. mays mexicana* Schrad.) is specific to Mexico and Central America. In the Central Plateau and Valley of Mexico, corn can grow sympatrically with teosinte providing the opportunity for hybridization (Wilkes, 1967; Sanchez et al., 1998). The genetic exchange between corn and teosinte is dependant on (1) the spatial isolation of the two species, (2) the seasonal isolation of the two species and (3) the fitness of the hybrids combined with the types of selection operating in the teosinte populations. Wilkes (1967) suggested the presence of hybrids under natural conditions in Central Plateau and Valley of Mexico as an unequivocal indication of cross compatibility between corn and teosinte. Baltazar et al. (2005) and Ellstrand et al. (2007) presented further evidence of hybridization between corn and teosinte. Hybrid and open-pollinated corn ears produced a mean of 8 and 11 seeds per ear, respectively, when hand-pollinated with teosinte pollen, which is approximately 1–2% of the ovules normally produced on a hybrid corn ear (Baltazar et al., 2005). Hybridization in the other direction is difficult but teosinte ears can produce up to 0.2–0.3 seeds per ear when pollinated with corn pollen (Baltazar et al., 2005; Ellstrand et al., 2007).

With the exception of *Tripsacum floridanum*, it is difficult to cross *Tripsacum* with corn, and the offspring of the cross show varying levels of sterility. *Tripsacum*-corn hybrids have not been observed in the field, and *Tripsacum*-teosinte hybrids have not been produced (Wilkes, 1972).

#### II.D.5.2. Outcrossing with Cultivated Zea Varieties

Gene flow in corn is closely associated with the biology of the staminate and pistillate inflorescences. Corn is a cross- and wind-pollinated crop that produces pollen in copious quantities. A hybrid tassel of normal size can produce up to 25 million pollen grains (Kiesselbach, 1999). Corn pollen has a mean diameter of approximately 100-106 microns (Rodriguez et al., 2006). Dispersal of corn pollen is determined by a diversity of environmental and physical factors. Wind direction, turbulence and velocity are directly linked to pollen movement (Jones and Brooks, 1950; Di-Giovanni and Kevan, 1991). Other factors such as pollen density, air density and viscosity, pollen sedimentation velocity, and pollen radius seem to influence pollen transport and deposition (Paterniani and Stort, 1974; Di-Giovanni et al., 1995; Aylor, 2002). Once in the atmosphere, pollen grains must remain viable long enough to be able to reach a viable silk to complete the pollination process. On average, corn pollen loses 100% viability after two hours of atmospheric exposure (Luna et al., 2001; Aylor, 2003).

### II.D.5.3. Survival Capacity and Weediness of Corn

Modern-day corn cannot survive outside of cultivation (Gould, 1968). Volunteer corn is not typically observed growing in fence rows, ditches, and roadsides as a weed. Although corn from the previous crop year can overwinter and germinate the following year, it cannot persist as a weed. The appearance of corn in soybean fields following the corn crop from the previous year is a common occurrence. Measures often are taken to eliminate either the plants with a hoe or to use herbicides to kill the corn plants in soybean fields, but the plants that remain and produce seed usually do not persist in the following years.

It is difficult for corn to survive as a weed because of past selection in the evolution of corn. In contrast with weedy plants, corn has a polystichous female inflorescence (or ear) on a stiff central spike (or cob) enclosed with husks (modified leaves). Consequently, seed dispersal of individual kernels does not occur naturally because of the structure of the ears of corn. Individual kernels of corn, however, can be distributed during grain harvest and transportation to storage facilities. In neither instance (natural or mechanical harvesting) does corn become a weed. Corn cannot survive without human assistance and is not capable of surviving as a weed.

### II.D.6. Natural Habitat of Corn and Significant Ecological Interactions

As corn is the product of domestication it does not have a natural habitat. Corn's closest wild relative, teosinte, is native to Mexico and Guatemala. Teosinte prefers areas that are seasonally dry and receive summer rain (Gonzalez and Corral, 1997; Wilkes, 1972).

Water requirements for corn growth vary over the season, reaching a peak at VT. Prior to VT, corn requires approximately 0.1 inches of water per day (0.25 cm/day). From VT to R3, corn requires approximately 0.35 inches per day (0.9 cm/day) or about 7 inches (18 cm) total. (McWilliams, 2002). While modern-day corn hybrids have been developed through breeding and biotechnology to increase drought tolerance, this basic water requirement has not been surpassed and will not allow corn to be grown or persist outside of current corn growing regions

Corn is the target of a variety of microbial pathogens and insect pests. Rusts, smuts, leaf blights, and stalk rots are among the more common diseases of microbial origin. Their prevalence and economic importance vary from country to country (Smith and White, 1988). Fungi such as *Aspergillus* sp. and *Fusarium* sp. produce mycotoxins that can adversely impact humans and livestock that consume contaminated grain. Insect damage and abiotic stresses such as drought can exacerbate fungal infections (Dowd, 2001).

The primary insect pests of corn belong to the orders Lepidoptera and Coleoptera. Lepidopterans feed on leaves and stalks as larvae and ears as adults. Examples include the European corn borer (*Ostrinia nubialis*), the Asian corn borer (*O. furnacalis*), the spotted stem borer (*Chillo partellus*), fall army worm (*Spodoptera frugiperda*), and members of the genus *Diatrea*. Coleopterans feed on roots and stalks as larvae and silks, pollen, and leaves as adults. Examples include the northern corn rootworm (*Diabrotica*

*barberi*), the western corn rootworm (*D. vergifera vergifera*), and the southern corn rootworm (*D. undecimpunctata*) (Dicke and Guthrie, 1988).

### II.D.7. Corn as a Test System in this Petition

The transformation for MON 87460 was conducted with LH59, an inbred corn line. A single transformed plant was then self-crossed to increase seed supplies. A homozygous inbred line was developed though self-crossing and selection, and then used to produce other lines used for product testing, regulatory studies, and commercial production.

## II.E. Global Use of Corn

Corn is used globally for food, feed, and fuel, and in recent years the demand for corn has increased, leading to higher prices for raw grain and its derivatives (NCGA, 2008). OECD-FAO's joint 2008-2017 Agricultural Outlook forecasted that corn prices will remain 40-60% higher in the next decade than they have been for the last decade. The report also concluded that increased yields on existing agricultural land will be more important to improving commodity supplies than bringing new land into cultivation. In developing countries, economic growth, changing diets, and growing populations are driving added demand. In developed countries, fuel uses are the largest source of new demand. These factors along with diminished stocks and climate change will lead to variability in agricultural product supply and possibly result in price spikes (OECD-FAO, 2008).

Food uses include sweet corn, popcorn, and processed field corn, which are all hybrids of *Zea mays*. Of the corn used for food and industrial uses, the majority is processed by wet milling to produce starch and sweetener products (e.g., high fructose corn syrup) for use in foodstuffs. Non-food products such as industrial starches, corn gluten feed and corn gluten meal are also manufactured through the wet mill process (May, 1987; Watson, 1988). The primary products derived from the dry milling process are corn meal, corn flour, and ethanol.

Because of its high starch content, corn is used as a valuable energy source in animal feed for domestic livestock, such as cattle, pigs and poultry. This starch content is also amenable to fermentation, providing ethanol for use as fuel. Whole corn is usually ground and mixed with a high-protein feed compound and with vitamin and mineral supplements to balance the ration according to the nutritional requirements of the animals being fed (Leath and Hill, 1987). Corn is also used for processing and the production of derivatives, which have a wide range of food, feed and industrial applications. Some of the processed fractions are used for animal feed, such as corn gluten, a resource that is rich in corn protein. Corn is also used for the production of feed additives. Ethanol production from the dry mill process provides dried distiller's grain solubles (DDGS) which are another source of animal feed (RFA, 2008).

### II.E.1. Historical Uses of Corn and its Major Processed Products

Corn and its processed products have been in use for at least the last 7,000 years. The earliest uses were likely as flour or dough. This continues to be the primary use in

developing countries throughout Latin America and Africa. In industrialized countries and developing Asian countries, the primary use for corn grain is animal feed although ethanol production has been rising steadily (NCGA, 2008; OECD, 2003b; OECD-FAO, 2008).

### II.E.2. Global Cultivation and Trade Flows for Corn and its Major Processed Products

From 2003 to 2007, worldwide corn grain production averaged 702 million metric tons (MMT) per year. During this same period, the top corn grain producers were the U.S., China, the European Union (EU), Brazil, and Mexico, accounting for 74% of average annual global corn production. Also during this period, corn production trended upwards from 627 MMT in 2003 to 769 MMT in 2007 (USDA-FAS, 2008). During 2000 to 2005, the latest period for which data are available, silage and forage production averaged 372 MMT and corn oil production averaged 2 MMT per year. (FAOSTAT, 2007).

From 2003 to 2007, the top corn grain exporters were the United States, Argentina, Brazil, China, and Ukraine. Together, these countries accounted for 93% of average annual exports (USDA-FAS, 2008). The major exported processed corn products are gluten feed, flour, oil, bran, and starch. In recent years, annual exports of DDGS from the U.S. have increased significantly from approximately 0.7 MMT to just under 1.8 MMT, second only to gluten feed in terms of processed corn product exports from the U.S. (ProExporter, 2008). Annual exports increased between 2003 and 2007 from 79 MMT to 92 MMT. From 2003 to 2007, the top five grain importers were Japan, Korea, Mexico, EU and Egypt, accounting for 51% of average annual imports (FAOSTAT, 2007).

Between 1997 and 2007, global demand for cereals (coarse grains, wheat and rice) was equal to or greater than production (FAO, 2008). This has led to reduced overall cereal supply with stocks at a 25 year low. Prices of cereals have increased globally, with U.S. corn prices rising 38% between 2007 and 2008. Similarly, wheat and rice prices have doubled in some markets. For corn, the price change is largely the result of increased demand while for the other cereals, particularly wheat, price increases are the result of both increased demand and reduced supply. The reduced wheat supply is the result of persistent drought in wheat production areas such as Australia (FAO, 2008).

In the U.S., demand for corn is driven by the demand for feed and fuel. In 2007 animal feed accounted for over 45% of corn consumption followed by nearly 25% for ethanol, and approximately 10% for food and industrial uses with the remainder being exported (NCGA, 2008). Globally, feed uses account for much of the demand. Global demand for animal feed rose 20% between 2007 and 2008. Expanding economies in China, India and Brazil between 1990 and 2006 have led to greater consumption of meat and dairy products (von Braun, 2007).

Within this context, the impacts of climate change take on a new urgency with respect to corn production. In a 2008 report, the U.S. Climate Change Science Program stated that climate change is already impacting agricultural productivity. The report concluded that

a combination of increased temperature variability, altered rainfall patterns and the resulting increases in drought frequency will magnify yield variability (CCSP, 2008; Hatfield et al., 2008).

### II.F. Conclusions

Corn is a versatile crop that provides food, feed and fuel. This versatility, coupled with growing economies in the developing world and the need to rely on alternative fuel sources in the developed world have led to a surge in demand. These demands are exceeding production, leading to diminished corn grain reserves worldwide. Diminished reserves magnify the impacts of supply disruptions. Climate change will have variable impacts on crop yields, potentially creating supply disruptions. The combination of these factors places a premium on corn yield stability in sub-optimal environments.

### II.G. References


Aylor, D.E. 2002. Settling speed of maize (*Zea mays*) pollen. Journal of Aerosol Science. 33:1601-1607.

Aylor, D.E. 2003. Rate of dehydration of corn (*Zea mays* L.) pollen in the air. Journal of Experimental Botany. 54(391): 2307-2312.

Baltazar, B.M., J.J. Sánchez-Gonzalez, L. De la Cruz-Larios, and J.B. Schoper. 2005. Pollination between maize and teosinte: an important determinant of gene flow in Mexico. Theoretical and Applied Genetics. 110(3): 519-526.

Barker, T., H. Campos, M. Cooper, D. Dolan, G. Edmeades, J. Habben, J. Schlusser, D. Wright, and C. Zinselmeier. 2005. Improving Drought Tolerance in Maize. Pages 173-253 in Plant Breeding Reviews. Vol 25, J. Janick, (ed.) John Wiley and Sons, Inc., Hoboken, New Jersey.

Boyer, J.S. 1982. Plant productivity and environment. Science. 218:443-448.

Brookes, G. and P. Barfoot. 2008. GM Crops: Global Socio-economic and Environmental Impacts 1996-2006. P.G. Economics http://www.pgeconomics.co.uk.

Campos, H., M. Cooper, G.O. Edmeades, C. Loffler, J.R. Schussler, and M. Ibanez. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. Maydica. 51:369-381.

CCSP. 2008. The effects of climate change on agriculture, land resources, water resources, and biodiversity. A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. U.S. Climate Change Science Program and the Subcommittee on Global Change Research., U.S. Environmental Protection Agency, Washington, D.C.

Chinnusamy, V., L. Xiong, and J.K. Zhu. 2005. Use of genetic engineering and molecular biology approaches for crop improvement for stress environments. Pages 47-89 in Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches, M. Ashraf and P.J.C. Harris, (eds.) Food Products Press, New York, New York.

Croissant, R.L., G.A. Peterson, and D.G. Westfall. 1998. Dryland Cropping Systems. Colorado State University http://www.ext.colostate.edu/.

Dicke, F.F., and W.D. Guthrie. 1988. The most important corn insects. Pages 769-880 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, WI.

Di-Giovanni, F. and P.G. Kevan. 1991. Factors affecting pollen dynamics and its importance to pollen contamination: a review. Canadian Journal of Forest Research. 21: 1155-1170.

Di-Giovanni, F., P.G. Kevan, and M.E. Nasr. 1995. The variability in settling velocities of some pollen and spores. Grana. 34: 39-44.

Dowd, P.F. 2001. Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial fields. Journal of Economic Entomology. 94:1067-1074.

Duvick, D.N., J.S.C. Smith, and M. Cooper. 2004. Changes in performance, parentage, and genetic diversity of successful corn hybrids, 1930-2000. in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

Ellstrand, C.N., L.C. Garner, S. Hegde, R. Guadagnuolo, and L. Blancas. 2007. Spontaneous hybridization between maize and teosinte. Journal of Heredity. 98:183-187.

FAO. 2008. Crop Prospects and Food Situation. Global Information and Early Warning System on Food and Agriculture. No. 2, April 2008.

FAOSTAT. 2007. FAOSTAT Database. http://faostat.fao.org [Accessed December 15, 2007].

Galinat, W.C. 1988. The origin of corn. Pages 1-31 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Gonzalez, J., and J. Corral. 1997. Teosinte distribution in Mexico. Proceedings of a forum: gene flow among maize landraces, improved maize, varieties and Teosinte: implications for transgenic maize:18-39.

Goodman, M. M. 1988. The history and evolution of maize. CRC Critical Reviews in Plant Sciences. 7:197-220.

Goodman, M.M., and W.L. Brown. 1988. Races of corn. Pages 33-79 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Gould, F.W. 1968. Grasses and man. Page 382 in Grass Systematics McGraw Hill, New York, New York.

Hanway, J.J. 1966. How a corn plant develops. Special Report. Iowa State university cooperative extension service, Ames, IA 48.

Hatfield, J., K. Boote, P. Fay, L. Hahn, C. Izaurralde, B.A. Kimball, T. Mader, J. Morgan, D. Ort, W. Polley, A. Thomson, and D. Wolfe. 2008. Agriculture. in The Effects of Climate Change on Agriculture, Land Resources, Water Resources, and Biodiversity. A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. U.S. Climate Change Science Program and the Subcommittee on Global Change Research, Washington, D.C.

Hicks, D.R., and P.R. Thomison. 2004. Corn management. in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

Hsiao, T.C. 1973. Plant responses to water stress. Annual Review of Plant Physiology. 24:519-570.

Jones, J.M., and J.S. Brooks. 1950. Effectiveness and distance of border rows in preventing outcrossing in corn. Oklahoma Agricultural Experiment Station Technical Bulletin. No. T-38.

Kaeppler, S. 2004. Biotechnology: new horizons. in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

Kiesselbach, T.A. 1999. The structure and reproduction of corn. 50th Anniversary Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Leath, M.N., and L.D. Hill. 1987. Economics of production, marketing and utilization, Pages 210-219 in Corn: Chemistry and Technology., S.A. Watson and R.E. Ramstad, (eds.) American Association of Cereal Chemists, St. Paul, MN.

Luna, V. S., J.M. Figueroa, B.M. Baltazar, R.L. Gomez, R. Townsend and J.B. Schoper 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Science. 41:1551-1557.

Maiti, R.K. and P. Wesche-Ebeling. 1998. Maize Science. Pages 132-133. Science Publishers, Inc. Enfield, New Hampshire.

May, J.B. 1987. Wet milling: processes and products. Pages 377-397 in Corn: Chemistry and Technology, S.A. Watson and P.E. Ramstad, (eds.) American Association of Cereal Chemists, St. Paul, MN.

McWilliams, D. 2002. Drought Strategies for Corn and Grain Sorghum. New Mexico State University http://www.cahe.nmsu.edu.

NCGA. 2008. The World of Corn 2008. National Corn Growers Association http://www.ncga.com/WorldOfCorn/main/index.asp Accessed May 15, 2008.

OECD. 2003a. Consensus document on the biology of *Zea mays* subsp. *mays* (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology. ENV/JM/MONO(2003)11, Organization for Economic Co-operation and Development, Paris, France http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/NT0000426E/$FILE/JT00147699.PDF Accessed January 9, 2009.

OECD. 2003b. Considerations for the safety assessment of animal feedstuffs derived from genetically modified plants. Series on the Safety of Novel Foods and Feeds. ENV/JM/MONO(2003)10, Organization for Economic Co-operation and Development, Organization for Economic Co-operation and Development, Paris, France http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/env-jm-mono(2003)10 Accessed February 5, 2007.

OECD-FAO. 2008. OECD-FAO Agricultural Outlook 2008-2017. OECD-FAO Paris, France http://www.agri-outlook.org/dataoecd/44/18/40713249.pdf.

Olson, R.A., and D.H. Sander. 1988. Corn Production. Pages 639-686 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, WI.

Paterniani, E. and A.C. Stort. 1974. Effective maize pollen dispersal in the field. Euphytica. 23:129-134.

ProExporter. 2008. PRX Grain Database - May 9, 2008.

RFA. 2008. Industry Resources: Co-Products. Renewable Fuels Association http://www.ethanolrfa.org/industry/resources/coproducts/ Accessed May 15, 2008.

Rodriguez, J.G.F., J.J. Sanchez G. Baltazar, M.B. De la Cruz L., Santacruz-Ruvalcaba, E., Ron, J.P. and Schoper, J.B. 2006. Characterization of floral morphology and synchrony among *Zea* species in Mexico. Maydica. 51: 383-398.

Sanchez, J.J., G., T.A. Kato Y., M. Aguilar san Miguel, J.M. Hernandez C., A. Lopez R., and J.A Ruiz C. 1998. Ditribucion y caracterizacion del *teocintle*. Libro Tecnico No. 2 del Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP).

Schoper, J. B., R.J. Lambert, B.L. Vasilas, and M.E. Westgate. 1987. Plant factors controlling seed set in maize. Plant Physiology. 83:121-125.

Setter, T.L., B.A. Flannigan, and J. Melkonian. 2001. Loss of kernel set due to water deficit and shade in maize: carbohydrate supplies, abscisic acid, and cytokinins. Crop Science. 41:1530-1540.

Smith, D.R., and D.G. White. 1988. Diseases of corn. Pages 687-766 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, WI.

Sprague, G.F. 1946. The experimental basis for hybrid maize. Biology Review. 21:101-120.

Taiz, L. 1991. Water balance of the plant. Pages 97 - 98. in Plant Physiology. The Benjamin/Cummings Publishing Company, Redwood City, California.

Takeda, S., and M. Matsuoka. 2008. Genetic approaches to crop improvement: responding to environmental and population changes. Nature Reviews. 9:444-457.

Troyer, A.F. 2004. Persistent and popular germplasm in seventy centuries of corn evolution in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

USDA-APHIS. 2009. Biotechnology: Additional Guidance on Agronomic Performance Data of Corn. United States Department of Agriculture, Animal and Plant Health Inspection Service. www.aphis.usda.gov/biotechnology/cornguidance.shtml Accessed on January 9, 2009.

USDA-FAS. 2008. FASOnline. United States Department of Agriculture - Foreign Agricultural Service http://www.fas.usda.gov/psdonline/psdQuery.aspx Accessed May 20, 2008.

von Braun, J. 2007. The World Food Situation: New Driving Forces and Required Actions. International Food Policy Research Institute, Washington, D.C.

Watson, S.A. 1988. Corn marketing, processing, and utilization. Pages 881-940 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J. W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Westgate, M.E., M.E. Otegui, and F.H. Andrade. 2004. Physiology of the corn plant in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

Wilkes, H.G. 1967. Teosinte: the closest relative of maize. Bussey Institution Harvard University.

Wilkes, H.G. 1972. Maize and its wild relatives. Science. 177:1071-1077.

Wilkes, G. 2004. Corn, strange and marvelous: but is a definitive origin known? in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

Yousef, G.G., and J.A. Juvik. 2001. Crop breeding, genetics and cytology. Crop Science. 41-645-655.

## III. Description of the Transformation System

### III.A. Transformation System

MON 87460 was developed through *Agrobacterium*-mediated transformation of corn line LH59 embryos and expresses cold shock protein B (CSPB) from *Bacillus subtilis* (Kingdom: Bacteria, Phylum: Firmicutes, Class: Bacilli). The transformation was performed with the binary plasmid vector PV-ZMAP595 (Figure III-1). *Agrobacterium tumefaciens* strain ABI contains a modified Ti plasmid that is incapable of inducing tumor formation due to the deletion of the phytohormone genes originally present in the *Agrobacterium* plasmid (Koncz and Schell, 1986). The vector, PV-ZMAP595, contains both the left and right border sequences flanking the transfer DNA (T-DNA) to facilitate transformation.

The *Agrobacterium*-mediated corn transformation to produce MON 87460 was based on the method described by Armstrong and Phillips (Armstrong and Phillips, 1988). Briefly, freshly isolated immature corn embryos were used for callus initiation. After co-culturing with *Agrobacterium* carrying the transformation vector, the calli were transferred from filter paper to callus initiation medium containing carbenicillin to eliminate *Agrobacterium* and paromomycin to eliminate cells that were not transformed, so that only cells containing the T-DNA survived. The resulting transformed cells were then subcultured several times on a selection medium and regenerated into plants.

The $R_0$ plants generated through the above transformation were self-pollinated, and the subsequent $R_1$ plants were screened for the presence of CSPB protein, tolerance to kanamycin, and homozygosity of the inserted gene. Only the plants that were homozygous for the *cspB* insert and tolerant to kanamycin were advanced for development, and their progenies were subjected to further molecular (Southern blot) and phenotypic assessments. MON 87460 was selected as the lead event based on its reduced yield loss under water-limited conditions, phenotypic characteristics, and molecular profile. Additional studies were conducted with MON 87460 to further characterize the genetic insertion and the expressed protein, and to establish the food, feed, and environmental safety relative to conventional corn. The major steps involving the development of MON 87460 are depicted in Figure III-2.

### III.B. References

Armstrong, C.L. and R.L. Phillips. 1988. Genetic and cytogenetic variation in plants regenerated from organogenic and friable, embryogenic tissue cultures of maize. Crop Science 28:363-369.

Koncz, C. and J. Schell. 1986. The promoter of Tl-DNA gene 5 controls the tissue specific expression of chimeric genes carried by a novel type of Agrobacterium binary vector. Molecular and General Genetics. 204:383-396.

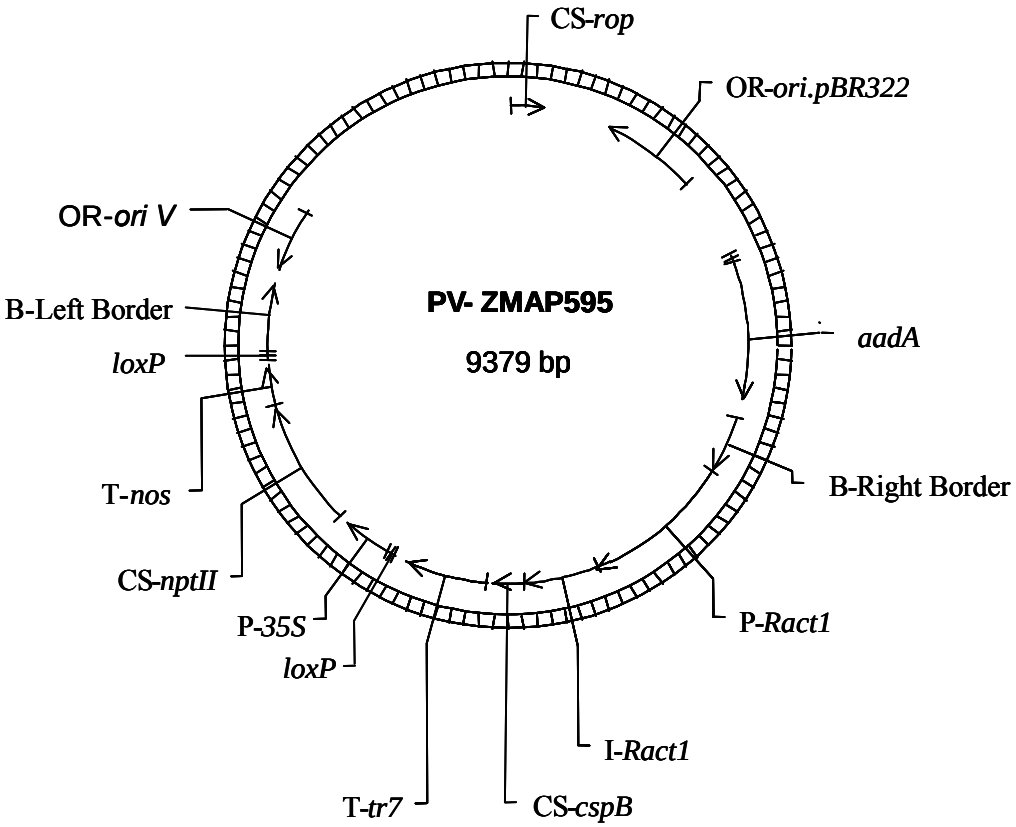



**Figure III-1. Circular Map of Plasmid PV-ZMAP595**

Plasmid PV-ZMAP595 containing the T-DNA used in *Agrobacterium*-mediated transformation to produce MON 87460. Approximate locations of the genetic elements are indicated on the map.

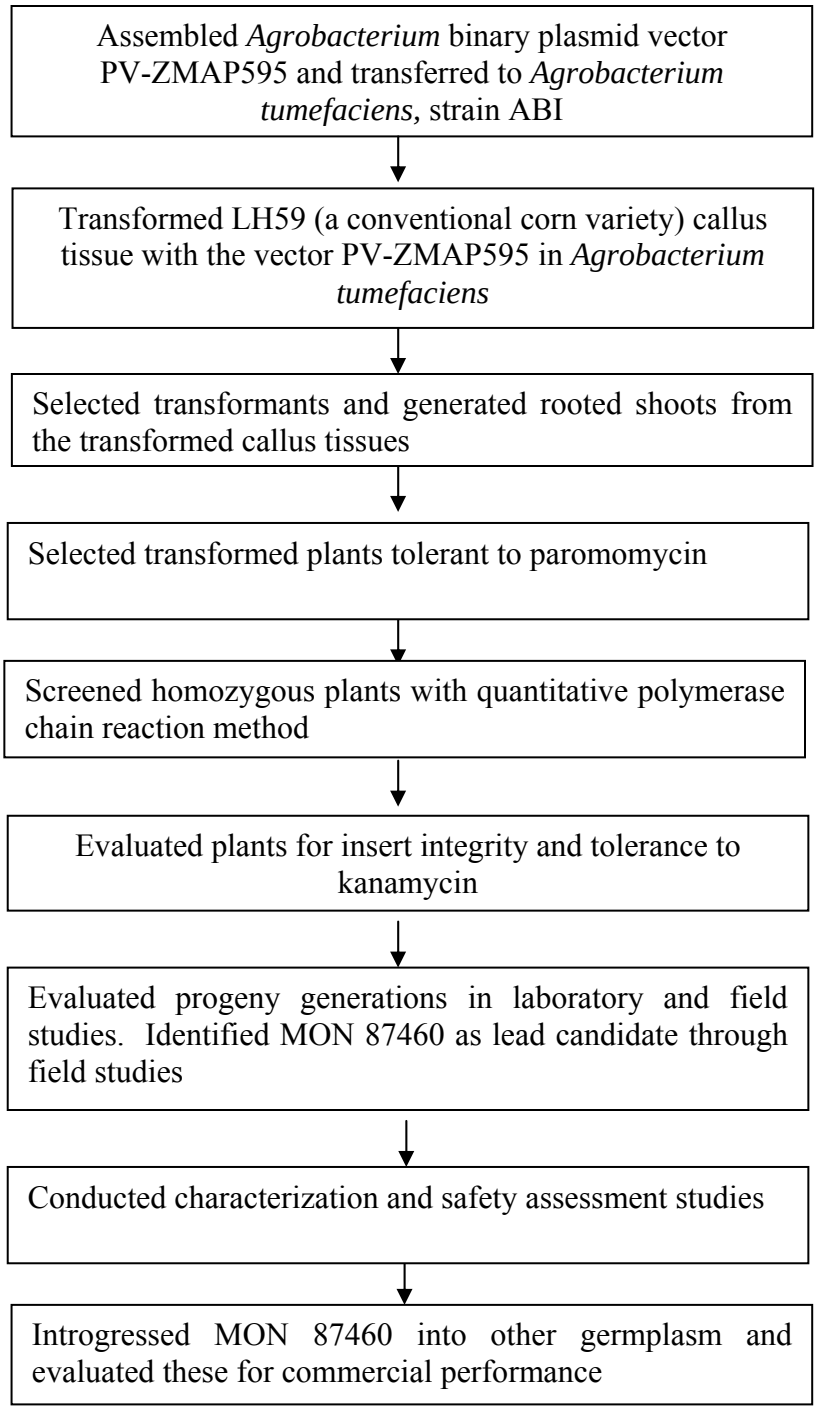



**Figure III-2. Schematic Representation of the Development of MON 87460**

## IV. Donor Genes and Regulatory Sequences

This section describes the donor genes and regulatory sequences used in the development of MON 87460 and the deduced amino acid sequences of the CSPB and NPTII proteins produced in MON 87460. In this section, T-DNA refers to DNA that is transferred to the plant during transformation. An expression cassette is composed of a coding sequence and the regulatory elements necessary for the expression of the coding sequence.

### IV.A. Vector PV-ZMAP595

The PV-ZMAP595 vector used for the transformation of corn embryos to produce MON 87460 is shown in Figure III-1 and its elements described in Table IV-1. This vector is approximately 9.4 kb and contains a single T-DNA delineated by left and right border regions that contains two expression cassettes: a *cspB* gene expression cassette, which contains coding sequence for CSPB from *Bacillus subtilis* and a neomycin phosphotransferase II (*nptII*) expression cassette, which confers resistance to kanamycin. The T-DNA that is expected to incorporate into the corn genome is approximately 4.6 kb and the DNA backbone region that is not incorporated into the corn genome is approximately 4.8 kb.

The *cspB* expression cassette contains the *cspB* coding sequence under the regulation of the *Ract1* promoter and leader, *Ract1* intron, and the *tr7* 3' nontranslated sequence. The *nptII* expression cassette contains the *nptII* coding sequence under the regulation of the *35S* promoter and the *nos* 3' nontranslated sequence.

The backbone region outside of the T-DNA contains two origins of replication for maintenance of plasmid in bacteria (OR-*oriV,* OR-*ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (CS-*rop*). A description of the genetic elements and their prefixes (e.g. P-, L-, I-, TS-, OR-, B-, CS-, and T-) in PV-ZMAP595 is provided in Table IV-1.

### IV.B. T-DNA

#### IV.B.1. The cspB Coding Sequence and CSPB Protein

MON 87460 expresses the CSPB protein, an RNA chaperone protein from *Bacillus subtilis*, which is associated with reduced yield loss under water limitation through the unfolding of RNA secondary structure thereby, facilitating RNA translation (Phadtare et al., 2002). The amino acid sequence of the CSPB protein produced in MON 87460 is identical to the native CSPB protein (Willimsky et al., 1992) produced in *Bacillus subtilis* with the exception of one amino acid change in the second position from leucine to valine, designated as CSPB-L2V. This amino acid change was implemented to facilitate the assembly of the plasmid vector PV-ZMAP595 for plant transformation. The full-length amino acid sequence is shown in Figure IV-1.

**Table IV-1. Summary of Genetic Elements in Plasmid Vector PV-ZMAP595**

| Genetic Element | Location in Plasmid | Function (Reference) |
|---|---|---|
| **Vector Backbone** | | |
| Intervening Sequence | 1 – 52 | Sequence used in DNA cloning |
| **CS[1]-*rop*** | 53 – 244 | Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989) |
| Intervening Sequence | 245 – 671 | Sequence used in DNA cloning |
| **OR[2]-*ori.pBR322*** | 672 – 1260 | Origin of replication from pBR322 for the maintenance of the plasmid in *E. coli* (Sutcliffe, 1978) |
| Intervening Sequence | 1261 – 1790 | Sequence used in DNA cloning |
| ***aadA*** | 1791 – 2679 | Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7 that confers spectinomycin and streptomycin resistance(Fling et al., 1985) (GenBank accession X03043) |
| Intervening Sequence | 2680 – 2815 | Sequence used in DNA cloning |
| **T-DNA** | | |
| **B[3]-Right Border** | 2816 – 3172 | DNA from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982) |
| Intervening Sequence | 3173 – 3204 | Sequence used in DNA cloning |
| **P[4]-*Ract1*** | 3205 – 4128 | Promoter and leader from the rice actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1990) |
| **I[5]-*Ract1*** | 4129 – 4605 | Intron from the rice actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1991) |
| Intervening Sequence | 4606 – 4607 | Sequence used in DNA cloning |
| **CS-*cspB*** | 4608 – 4811 | Codon modified coding sequence of the *cspB* gene from *Bacillus subtilis* encoding CSPB (Willimsky et al., 1992) |
| Intervening Sequence | 4812 – 4841 | Sequence used in DNA cloning |
| **T[6]-*tr7*** | 4842 – 5349 | 3' nontranslated sequence of *transcript* 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation (Dhaese et al., 1983) |
| Intervening Sequence | 5350 – 5423 | Sequence used in DNA cloning |

[1]CS – Coding Sequence; [2]OR – Origin of Replication; [3]B – Border; [4]P – Promoter; [5]I – Intron.

Table IV-1 continues on next page.

**Table IV-1 (cont.). Summary of Genetic Elements in Plasmid Vector PV-ZMAP595**

| | | |
|---|---|---|
| **T-DNA (cont.)** | | |
| **T[6]-*tr7*** | 4842 – 5349 | 3' nontranslated sequence of *transcript* 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation (Dhaese et al., 1983) |
| Intervening Sequence | 5350 – 5423 | Sequence used in DNA cloning |
| ***loxP*** | 5424 – 5457 | Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al., 1992) |
| Intervening Sequence | 5458 – 5483 | Sequence used in DNA cloning |
| **P-*35S*** | 5484 – 5776 | Promoter for the 35S RNA of the Cauliflower Mosaic Virus (Odell et al., 1985) |
| Intervening Sequence | 5777 – 5840 | Sequence used in DNA cloning |
| **CS-*nptII*** | 5841 – 6635 | Coding sequence from *Tn5* (Beck et al., 1982) in *E. coli* encoding neomycin and kanamycin resistance (Fraley et al., 1983) |
| Intervening Sequence | 6636 – 6666 | Sequence used in DNA cloning |
| **T-*nos*** | 6667 – 6919 | 3' nontranslated sequence of the *nopaline synthase* (NOS) gene from *Agrobacterium tumefaciens* which terminates and directs polyadenylation (Bevan et al., 1983) |
| Intervening Sequence | 6920 – 6944 | Sequence used in DNA cloning |
| ***loxP*** | 6945 – 6978 | Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al., 1992) |
| Intervening Sequence | 6979 – 6998 | Sequence used in DNA cloning |
| **B-Left Border** | 6999 – 7440 | DNA from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983) |
| **Vector Backbone** | | |
| Intervening Sequence | 7441 – 7526 | Sequence used in DNA cloning |
| **OR-*ori V*** | 7527 – 7923 | Origin of replication from the broad host range plasmid RK2 for maintenance of the plasmid in *Agrobacterium* (Stalker et al., 1981) |
| Intervening Sequence | 7924 – 9379 | Sequence used in DNA cloning |

[6]T – 3' nontranslated transcriptional termination sequence and polyadenylation signal sequences.

```
 1          MVEGKVKWFN SEKGFGFIEV EGQDDVFVHF SAIQGEGFKT LEEGQAVSFE
51          IVEGNRGPQA ANVTKEA
```

**Figure IV-1. Deduced Amino Acid Sequence of the Full Length CSPB Protein Present in MON 87460**

The amino acid sequence of the CSPB was deduced from the full-length *cspB* coding sequence present in PV-ZMAP595.

```
  1   ATGATTGAAC AAGATGGATT GCACGCAGGT TCTCCGGCCG CTTGGGTGGA
 51   GAGGCTATTC GGCTATGACT GGGCACAACA GACAATCGGC TGCTCTGATG
101   CCGCCGTGTT CCGGCTGTCA GCGCAGGGGC GCCCGGTTCT TTTTGTCAAG
151   ACCGACCTGT CCGGTGCCCT GAATGAACTG CAGGACGAGG CAGCGCGGCT
201   ATCGTGGCTG GCCACGACGG GCGTTCCTTG CGCAGCTGTG CTCGACGTTG
251   TCACTGAAGC GGGAAGGGAC TGGCTGCTAT TGGGCGAAGT GCCGGGGCAG
301   GATCTCCTGT CATCTCACCT TGCTCCTGCC GAGAAAGTAT CCATCATGGC
351   TGATGCAATG CGGCGGCTGC ATACGCTTGA TCCGGCTACC TGCCCATTCG
401   ACCACCAAGC GAAACATCGC ATCGAGCGAG CACGTACTCG GATGGAAGCC
451   GGTCTTGTCG ATCAGGATGA TCTGGACGAA GAGCATCAGG GGCTCGCGCC
501   AGCCGAACTG TTCGCCAGGC TCAAGGCGCG CATGCCCGAC GGCGAGGATC
551   TCGTCGTGAC GCATGGCGAT GCCTGCTTGC CGAATATCAT GGTGGAAAAT
601   GGCCGCTTTT CTGGATTCAT CGACTGTGGC CGGCTGGGTG TGGCGGACCG
651   CTATCAGGAC ATAGCGTTGG CTACCCGTGA TATTGCTGAA GAGCTTGGCG
701   GCGAATGGGC TGACCGCTTC CTCGTGCTTT ACGGTATCGC CGCTCCCGAT
751   TCGCAGCGCA TCGCCTTCTA TCGCCTTCTT GACGAGTTCT TCTGA
```

**Figure IV-2. Nucleotide Sequence Encoding the NPTII Protein in PV-ZMAP595**

```
  1      MIEQDGLHAG SPAAWVERLF GYDWAQQTIG CSDAAVFRLS AQGRPVLFVK
 51     TDLSGALNEL QDEAARLSWL ATTGVPCAAV LDVVTEAGRD WLLLGEVPGQ
101     DLLSSHLAPA EKVSIMADAM RRLHTLDPAT CPFDHQAKHR IERARTRMEA
151     GLVDQDDLDE EHQGLAPAEL FARLKARMPD GEDLVVTHGD ACLPNIMVEN
201     GRFSGFIDCG RLGVADRYQD IALATRDIAE ELGGEWADRF LVLYGIAAPD
251     SQRIAFYRLL DEFF
```

**Figure IV-3. Deduced Amino Acid Sequence of the Full Length NPTII Protein Present in MON 87460**

The amino acid sequence of the NPTII was deduced from the full-length *nptII* coding sequence present in PV-ZMAP595.

### IV.B.2. The cspB Regulatory Sequences

Adjacent to the right border region of plasmid PV-ZMAP595 is the *cspB* expression cassette. The *cspB* coding sequence is under the regulatory control of the *Ract1* promoter and leader from the actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1990). Located between the *Ract1* promoter and the *cspB* coding sequence is the I-*Ract1* nontranslated intron from the actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1991). Following the *cspB* coding sequence is the 3' nontranslated sequence of *transcript* 7 gene from *Agrobacterium tumefaciens* (T-*tr7*) that directs polyadenylation (Dhaese et al., 1983).

### IV.B.3. The nptII-loxP Coding Sequence and NPTII Protein

The *nptII* cassette contains the *nptII* coding sequence flanked by *loxP* sites. The NPTII protein in MON 87460 confers resistance to kanamycin, which was used to facilitate the selection process. The *loxP* sites were inserted to facilitate the potential excision of the *nptII* cassette using CRE recombinase. The DNA sequence of the *nptII* coding region from the vector PV-ZMAP595 is shown in Figure IV-2. The deduced full-length amino acid sequence is shown in Figure IV-3.

### IV.B.4. The nptII Regulatory Sequences

Adjacent to the left border region of plasmid PV-ZMAP595 is the *nptII* expression cassette. The *nptII* coding sequence is under the regulatory control of the *35S* promoter from the Cauliflower Mosaic Virus (Odell et al., 1985). Following the *nptII* coding sequence is the 3' nontranslated sequence of the *nopaline synthase* (NOS) gene from *Agrobacterium tumefaciens* (T-*nos*) which terminates transcription and directs polyadenylation (Bevan et al., 1983).

### IV.B.5. T-DNA Borders

Plasmid PV-ZMAP595 contains right border and left border regions that delineate the T-DNA to be transferred into corn and are involved in the efficient transfer of the T-DNA into the corn genome. These border regions (Figure III-1 and Table IV-1) were derived from *Agrobacterium tumefaciens* plasmids (Depicker et al., 1982; Barker et al., 1983).

## IV.C. Genetic Elements Outside the T-DNA Borders

Four genetic elements exist outside of the T-DNA borders that are essential for the maintenance and selection of the vector PV-ZMAP595 in bacteria. They include: *OR-ori V,* origin of replication for the maintenance of the plasmid in *Agrobacterium* (Stalker et al., 1981); CS-*rop,* coding sequence of repressor of primer (ROP) protein for the maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989); *OR-ori-pBR322,* origin of replication from pBR322 for the maintenance of the plasmid in *E. coli* (Sutcliffe, 1979); and *aadA*, a bacterial promoter and coding sequence of an enzyme from transposon Tn7 that confers spectinomycin and streptomycin resistance for molecular cloning and selection purposes (Fling et al., 1985) prior to plant transformation. As these elements are outside of the border regions, they are not expected to be transferred into the corn genome. The absence of the backbone sequence in MON 87460 has been confirmed by Southern blot analyses (Sections V.B and V.D.2).

## IV.D. References


Barker, R.F., K.B. Idler, D.V. Thompson and J.D. Kemp. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTil5955. Plant Molecular Biololgy.2:335-350.

Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss and H. Schaller. 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon *Tn5*. Gene. 19:327-336.

Bevan, M., W.M. Barnes and M. Chilton. 1983. Structure and transcription of the nopaline synthase gene region of T-DNA. Nucleic Acids Research. 11:369-385.

Depicker, A., S. Stachel, P. Dhaese, P. Zambryski and H.M. Goodman. 1982. Nopaline synthase: transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics. 1:561-573.

Dhaese, P., H. d.Greve, J. Gielen, J. Seurinck, M. v.Montague and J. Schell. 1983. Identification of sequences involved in polyadenylation of higher plant nuclear transcripts using *Agrobacterium* T-DNA genes as models. EMBO Journal . 2:419-426.

Fling, M.E., J. Kopf and C. Richards. 1985. Nucleotide sequence of the transposon Tn*7* gene encoding an aminoglycoside-modifying enzyme, 3′(9)-*O*-nucleotidyltransferase. Nucleic Acids Research. 13:7095-7106.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L. Hoffmann and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proceedings of the National Academy of Sciences USA. 80:4803-4807.

Giza, P.E. and R.C.C. Huang. 1989. A self-inducing runaway-replication plasmid expression system utilizing the Rop protein. Gene. 78:73-84.

McElroy, D., W. Zhang, J. Cao and R. Wu. 1990. Isolation of an efficient actin promoter for use in rice transformation. Plant Cell. 2:163-171.

McElroy, D., A.D. Blowers, B. Jenes and R. Wu. 1991. Construction of expression vectors based on the rice actin 1(*Act1*) 5′ region for use in monocot transformation. Molecular and General Genetics. 231:150-160.

Odell, J.T., F. Nagy and N-H. Chua. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature. 313:810-812.

Phadtare, S., M. Inouye and K. Severinov. 2002. The nucleic acid melting activity of *Escherichia coli* CspE is critical for transcription antitermination and cold acclimation of cells. Journal of Biological Chemistry 277:7239-7245.

Russell, S.H., J.L. Hoopes and J.T. Odell. 1992. Directed excision of a transgene from the plant genome. Molecular and General Genetics. 234:49-59.

Stalker, D.M., C.M. Thomas and D.R. Helinski. 1981. Nucleotide sequence of the region of the origin of replication of the broad host range plasmid RK2. Molecular and General Genetics. 181:8-12.

Sutcliffe, J.G. 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symposium. 43:77-103.

Willimsky, G., H. Bang, G. Fischer and M.A. Marahiel. 1992. Characterization of *cspB*, a *Bacillus subtilis* inducible cold shock gene affecting cell viability at low temperatures. Journal of Bacteriology. 174:6326-6335.

## V. Genetic Analysis

Molecular analyses were performed to characterize the integrated DNA in MON 87460. Southern blot analyses were used to determine the number of integration sites within the corn genome (insert number), the number of copies within one insert (copy number), and the absence of plasmid backbone sequences in the plant. The Southern strategy was used to insure that all detectable fragments would have been detected in the analysis. The corn genome was assayed with probes that spanned the entire transformation plasmid and all probes were less than 2 kb in length in order to retain a high level of sensitivity. The high level of sensitivity was demonstrated for each blot by including and detecting a $1/10^{th}$ genome equivalent of the positive control. Two restriction enzyme sets were specifically chosen to independently confirm the presence of the insert and minimize the possibility that two DNA fragments could comigrate on the gel. In addition, at least one enzyme cut in each of the known regions of flanking DNA to insure that at least one of the 5′ border fragments and one of the 3′ border fragments were sequenced. The results of these analyses show that a single copy of the T-DNA inserted at a single locus of the genome.

The stability of the DNA insert across multiple generations was also demonstrated by Southern blot fingerprint analysis. Seven generations of MON 87460 were digested with one of the enzyme sets utilized for the copy number analysis and were hybridized with probes that would detect the entire insert (two hybridization bands). This fingerprint strategy consists of two border fragments that assay not only the stability of the insert, but also the stability of genomic DNA directly adjacent to the insert.

The DNA sequencing analyses compliment the Southern analyses. While Southern blot data demonstrated the presence of a single insert in MON 87460, the sequencing of the insert and the genomic DNA directly adjacent to the insert determined exactly what DNA was inserted during transformation. In addition, genomic rearrangements at the insertion site were assessed by comparing the insert and flanking sequence to the insertion site in conventional corn. Taken together, the data confirms that a single copy of the T-DNA inserted in a single locus in the corn genome and was associated with a 22 base pair deletion.

Genomic DNA from MON 87460 was digested with appropriate restriction enzymes and subjected to Southern blot analyses to characterize the T-DNA that was integrated into the corn genome. For each digest, there were duplicated samples which consisted of an equal amount of digested DNA with one set of samples run for a longer period of time (Long Run) than the second set (Short Run). The long run allows for greater resolution of high molecular weight DNA, whereas the short run allows the detection of low molecular weight DNA. Genomic DNA samples from conventional corn were used as the negative controls on the blots to determine potential non-specific hybridization signals. The positive controls for Southern blots were generated by digestion of plasmid DNA with enzyme combinations to produce the DNA banding patterns that were most relevant to the molecular assessment of MON 87460. Probe templates generated from the plasmid DNA were also used as positive controls. In addition, DNA markers were included to provide size estimation of the hybridized bands on Southern blots. Minor

differences in the sizes of the hybridization bands according to the DNA marker were observed compared to the expected sizes. The altered migrations may be due to the difference in salt concentrations between the DNA sample and the molecular weight marker (Sambrook and Russell, 2001).

The genetic elements present in MON 87460 are listed in Table V-1. The insert is identical to the T-DNA sequence of PV-ZMAP595, from the proximal end of the promoter to the Left Border. The information and results derived from the molecular analyses were used to construct a linear map of the insert in MON 87460. This linear map depicts restriction sites identified in the insert and the flanking corn genomic DNA flanking the insert, and provides information on the expected banding patterns and sizes of the DNA fragments after restriction enzyme digestion. The linear map is shown in Figure V-1. Based on the insert linear map and the plasmid map, a table summarizing the expected DNA fragments for Southern analyses is presented in Table V-2. The probes used in the Southern analyses and the map of PV-ZMAP595 are presented in Figure V-2 and Figure V-3. The materials and methods used in the analyses are presented in Appendix A.

**Table V-1. Summary of Genetic Elements in MON 87460**

| Genetic Element[1] | Location in Sequence[2] | Function (Reference) |
|---|---|---|
| **Sequence flanking 5' end of the insert** | 1-1121 | Corn genomic DNA |
| **P[3]-*Ract1*[87460]** | 1122-1312 | Truncated promoter and leader from the rice actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1990) |
| **I[4]-*Ract1*** | 1313-1789 | Intron from the rice actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1991) |
| Intervening Sequence | 1790-1791 | Sequence used in DNA cloning |
| **CS[5]-*cspB*** | 1792-1995 | Codon optimized coding sequence of the *cspB* gene from *Bacillus subtilis* encoding CSPB (Willimsky et al., 1992) |
| Intervening Sequence | 1996-2025 | Sequence used in DNA cloning |
| **T[6]-*tr7*** | 2026-2533 | 3' nontranslated sequence of *transcript* 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation (Dhaese et al., 1983) |
| Intervening Sequence | 2534-2607 | Sequence used in DNA cloning |
| ***loxP*** | 2608-2641 | Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al., 1992) |
| Intervening Sequence | 2642-2667 | Sequence used in DNA cloning |
| **P-*35S*** | 2668-2960 | Promoter for the 35S RNA of the Cauliflower mosaic virus (Odell et al., 1985) |
| Intervening Sequence | 2961-3024 | Sequence used in DNA cloning |
| **CS-*nptII*** | 3025-3819 | Coding sequence from *Tn5* (Beck et al., 1982) in *E. coli* encoding neomycin and kanamycin resistance (Fraley et al., 1983) |
| Intervening Sequence | 3820-3850 | Sequence used in DNA cloning |

[1] Flanking sequences and intervening sequences are not functional genetic elements
[2] Numbering includes the insert in MON 87460 and adjacent genomic DNA
[3] P – Promoter
[4] I – Intron
[5] CS – Coding Sequence
[6] T – 3' nontranslated transcriptional termination sequence and polyadenylation signal sequences

Table V-1 continues on next page.

**Table V-1 (cont.). Summary of Genetic Elements in MON 87460**

| Genetic Element | Location in Sequence | Function (Reference) |
|---|---|---|
| **T-*nos*** | 3851-4103 | 3' nontranslated sequence of the *nopaline synthase* (NOS) gene from *Agrobacterium tumefaciens* which terminates and directs polyadenylation (Bevan et al., 1983) |
| Intervening Sequence | 4104-4128 | Sequence used in DNA cloning |
| ***loxP*** | 4129-4162 | Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al., 1992) |
| Intervening Sequence | 4163-4182 | Sequence used in DNA cloning |
| **B[7]-Left Border[87460]** | 4183-4430 | DNA from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983) |
| **Sequence flanking 3' end of the insert** | 4431-5214 | Corn genomic DNA |

[7]B – Border

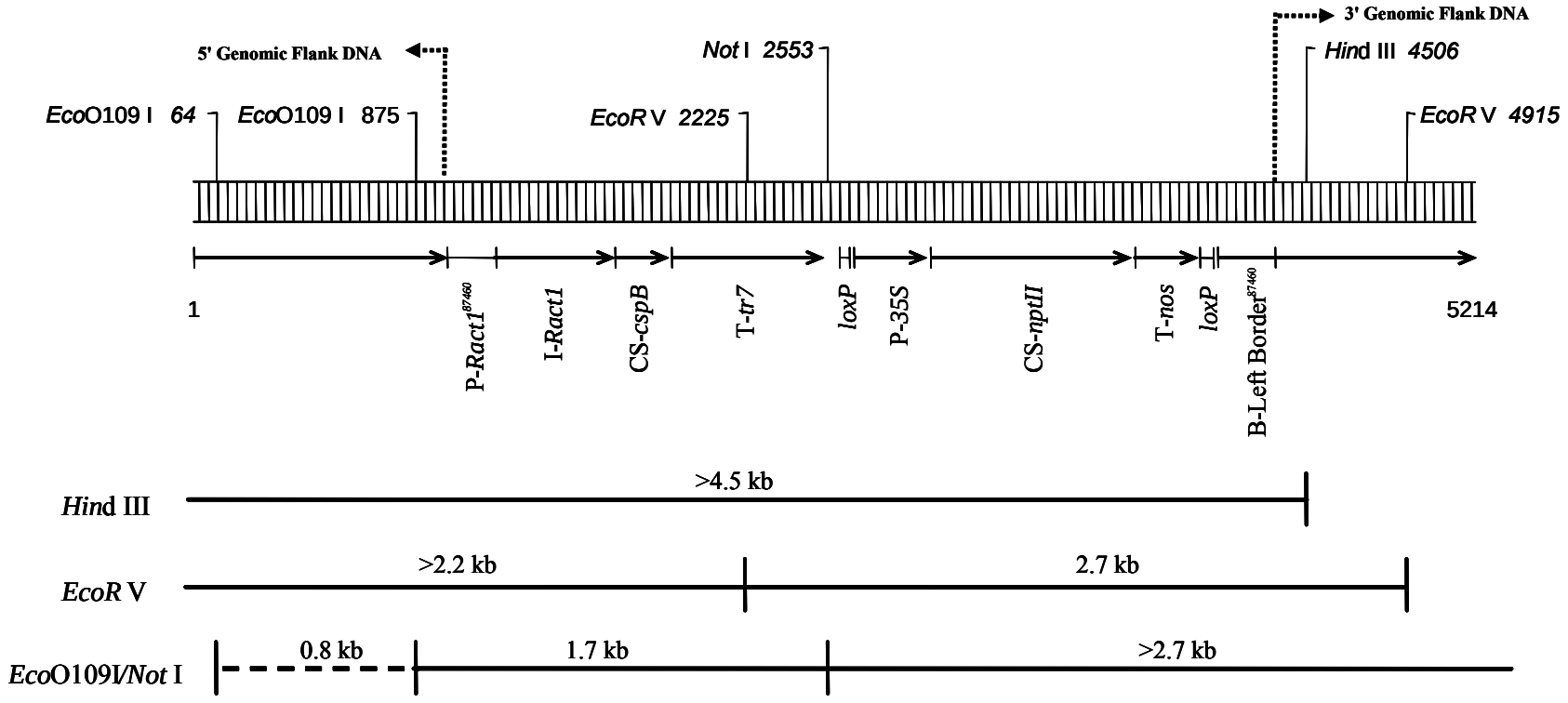
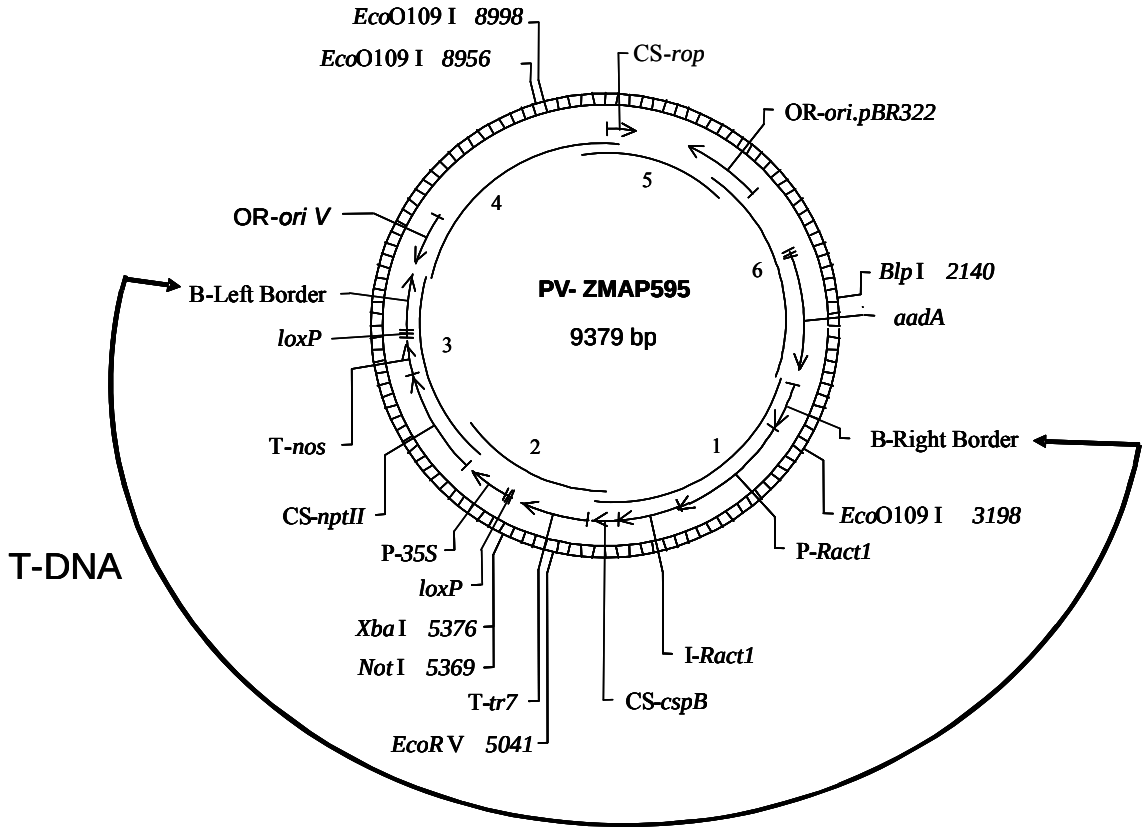



**Figure V-1. Schematic Representation of the Insert and Genomic Flanking Sequences in MON 87460**

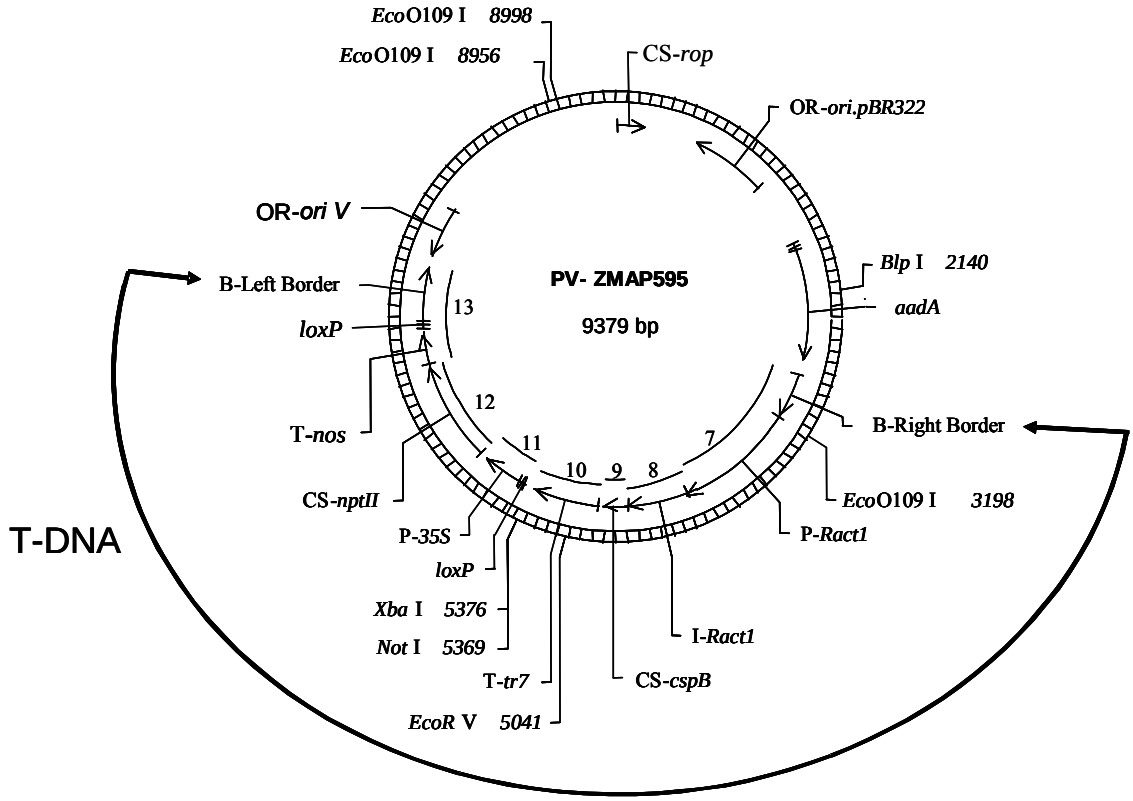
A linear map of the insert and known genomic DNA flanking the insert in MON 87460 is shown. Identified on the map are genetic elements within the insert, as well as restriction sites with positions relative to the size of the linear map for enzymes used in the Southern analyses. Shown on the lower portion of the map are the expected sizes of the DNA fragments after digestions with respective restriction enzyme or combination of enzymes. The dotted line indicates the additional DNA fragment that would be present if partial digestion of the internal *Eco*O109 I restriction site occurs. Arrows with dotted lines indicate the end of the insert and the beginning of corn genomic flanking sequence.




| Probe | DNA Probe | Start Position | Stop Position | Total Length (~kb) |
|---|---|---|---|---|
| 1 | T-DNA Probe 1 | 2816 | 4782 | 2.0 |
| 2 | T-DNA Probe 2 | 4670 | 6085 | 1.4 |
| 3 | T-DNA Probe 3 | 5839 | 7440 | 1.6 |
| 4 | Backbone Probe 1 | 7441 | 66 | 2.0 |
| 5 | Backbone Probe 2 | 9241 | 1245 | 1.4 |
| 6 | Backbone Probe 3 | 1094 | 2815 | 1.7 |

**Figure V-2. Circular Map of Plasmid PV-ZMAP595 Showing Probes 1-6**

Plasmid PV-ZMAP595 containing the T-DNA used in Agrobacterium-mediated transformation to produce MON 87460. Locations of the genetic elements are depicted by arrows on the interior of the map with their annotations shown on the exterior of the map. Restriction sites for enzymes used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The overlapping T-DNA and backbone probes used in the Southern analyses (labeled 1-6 within the interior of the map) are detailed in the accompanying table.




| Probe | DNA Probe | Start Position | Stop Position | Total Length (~kb) |
|---|---|---|---|---|
| 7 | P-*Ract1* Probe | 2816 | 4128 | 1.3 |
| 8 | I-*Ract1* Probe | 4129 | 4607 | 0.5 |
| 9 | CS-*cspB* Probe | 4608 | 4811 | 0.2 |
| 10 | T-*tr7* Probe | 4842 | 5354 | 0.5 |
| 11 | *loxP* + P-*35S* Probe | 5424 | 5785 | 0.36 |
| 12 | CS-*nptII* Probe | 5839 | 6635 | 0.8 |
| 13 | T-*nos* + *loxP* + Left Border Probe | 6667 | 7440 | 0.8 |

**Figure V-3. Circular Map of Plasmid PV-ZMAP595 Showing Probes 7-13**

Plasmid PV-ZMAP595 containing the T-DNA used in Agrobacterium-mediated transformation to produce MON 87460. Locations of the genetic elements are depicted by arrows on the interior of the map with their annotations shown on the exterior of the map. Restriction sites for enzymes used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The genetic element probes used in the Southern analyses (labeled 7-13 within the interior of the map) are detailed in the accompanying table.

**Table V-2. Summary Chart of the Expected DNA Fragments using Combinations of Restriction Enzymes and Probes**

| **Probes used** | **1, 2, 3** | **4, 5, 6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| **Southern blot in Figure** | V-4 | V-5 | V-6 | V-7 | V-8 | V-9 | V-10 | V-11 | V-12 |
| | | | | | | | | | |
| **Plasmid** | | | | | | | | | |
| *Blp*I + *Xba* I | 3.2 kb + 6.1 kb | 3.2 kb + 6.1 kb | 3.2 kb | 3.2 kb | 3.2 kb | 3.2 kb | 6.1 kb | 6.1 kb | 6.1 kb |
| | | | | | | | | | |
| **Probe templates** [1] | 1.4 kb + 1.6 kb + 2.0 kb | 1.4 kb + 1.7 kb + 2.0 kb | --[2] | -- | -- | -- | -- | -- | -- |
| | | | | | | | | | |
| **MON 87460** | | | | | | | | | |
| *Hind* III | > 4.5 kb | -- | -- | -- | -- | -- | -- | -- | -- |
| *Eco*R V | 2.7 kb + > 2.2 kb | no band | > 2.2 kb | > 2.2 kb | > 2.2 kb | 2.7 kb + > 2.2 kb | 2.7 kb | 2.7 kb | 2.7 kb |
| *Eco*O109 I and *Not* I | -- | no band | 1.7 kb | 1.7 kb | 1.7 kb | 1.7 kb | 1.7 kb | > 2.7 kb | >2.7 kb |

[1] Probe templates were spiked when multiple probes were used in Southern blot analysis.
[2] '--' Indicates that the particular restriction enzyme or the combination of the enzymes was not used in the analysis.

## V.A. Insert and Copy Number

The number of T-DNA inserts (insert number) in the MON 87460 genome was evaluated by digesting the test and control DNA with *Hind* III, a restriction enzyme that does not cleave within the T-DNA. Therefore, *Hind* III releases a restriction fragment containing the entire T-DNA and adjacent plant genomic DNA (Figure V-1). Therefore, the number of restriction fragments detected indicates the number of inserts present in MON 87460. The number of copies of the T-DNA (copy number) integrated at a single locus was determined by digesting test and control genomic DNA samples with the restriction enzyme *EcoR* V, which cleaves once within the insert (Figure V-1). If MON 87460 contains one copy of the T-DNA, probing with the entire T-DNA will result in two bands, each representing a portion of the T-DNA along with adjacent plant genomic DNA.

The Southern blot used to determine insert and copy number of the T-DNA (Figure V-4) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with the T-DNA, the blot contained conventional corn genomic DNA digested with *Hind* III (Figure V-4, lanes 1 and 8) or *EcoR* V (Figure V-4, lanes 3 and 10). The conventional control DNA digested with *Hin*d III or *Eco*R V produced several hybridization signals. These hybridization signals result from the

probes hybridizing to endogenous sequences residing in the corn genome and are not specific to the inserted DNA. These signals were produced in both test and control lanes, and therefore the bands are considered to be endogenous background.

To ensure that each of the T-DNA probes was able to hybridize to their respective targets, probe template spikes (Figure V-2, probes 1-3) that were generated from plasmid PV-ZMAP595 and mixed at different concentrations (1 copy: one genomic copy equivalence; 0.1 copy: one tenth genomic copy equivalence) with the control DNA pre-digested with *Eco*R V were included on the blot (Figure V-4, lanes 5-6). The expected hybridization bands at approximately 1.4, 1.6, and 2.0 kb were detected. The approximately 0.1 and 1 copies of the 1.4 kb band were faint in comparison to the 1.6 and 2.0 kb bands, but were clearly detectable. The detection of the probe template positive hybridization controls demonstrates that all three probes are hybridizing to the target DNA. To ensure that the T-DNA probes hybridize to the plasmid used for transformation, plasmid PV-ZMAP595 digested with a combination of *Blp* I and *Xba* I was spiked in the control DNA pre-digested with *Eco*R V. The expected hybridization bands of approximately 3.2 and 6.1 kb (Figure V-4, lane 7) were detected (refer to Figure V-2 and Figure V-3 plasmid map).

MON 87460 DNA digested with *Hin*d III (Figure V-4, lanes 2 and 9) and hybridized with the T-DNA probes produced a single unique band of approximately 6.8 kb. This is consistent with the expected band being greater than 4.5 kb (Figure V-1) and confirms that MON 87460 contains one insert located within a 6.8 kb *Hin*d III restriction fragment. MON 87460 DNA digested with *Eco*R V and hybridized with the T-DNA probes produced two bands (Figure V-4, lanes 4 and 11) of approximately 2.7 and 7.2 kb. The approximately 2.7 kb band is the expected size for the border fragment containing the 3' end of the inserted DNA (T-DNA) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure V-1). The approximately 7.2 kb band is consistent with the expected band being greater than 2.2 kb (Figure V-1). This band represents the 5' border fragment containing the 5' end of the inserted DNA along with the adjacent genomic DNA flanking the 5' end of the insert.

The results of MON 87460 presented in Figure V-4 show that MON 87460 contains a single copy of the T-DNA that resides at a single locus of integration on an approximately 6.8 kb *Hin*d III restriction fragment. Southern blot analyses described below using a combination of *Eco*O109 I and *Not* I which produced an expected 5′ border fragment further confirmed this conclusion.

**V.B. Presence or Absence of Plasmid PV-ZMAP595 Backbone**

Test and control DNAs were digested with either a combination of the restriction enzymes *Eco*O109 I and *Not* I or the restriction enzyme *Eco*R V to determine if PV-ZMAP595 sequences, other than the T-DNA region, were inserted in MON 87460. The samples were electrophoresed, blotted, and the blot was hybridized simultaneously with three overlapping probes (Figure V-2, probes 4-6) that spanned the backbone sequence of PV-ZMAP595. The results are shown in Figure V-5. Probe template spikes (Figure V-2, probes 4-6) generated from the plasmid PV-ZMAP595 were mixed with the

pre-digested control genomic DNA to serve as a positive hybridization control. Additionally, plasmid PV-ZMAP595 DNA previously digested with the combination of *Blp* I and *Xba* I was mixed with control genomic DNA digested with *Eco*R V and loaded on the gel to serve as a positive hybridization control.

The Southern blot used to determine the presence or absence of plasmid PV-ZMAP595 backbone sequences (Figure V-5) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with the backbone sequences, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-5, lanes 1 and 8) or *Eco*R V (Figure V-5, lanes 3 and 10). The conventional corn genomic DNA digested with *Eco*O109 I and *Not* I (Figure V-5, lanes 1 and 8) or *Eco*R V (Figure V-5, lanes 3 and 10) produced several hybridization signals. These hybridization signals result from the probes hybridizing to endogenous sequences residing in the corn genome and are not specific to the inserted DNA. These signals were produced in both test and control lanes, and therefore are considered to be endogenous background.

To ensure that each backbone probe was capable of hybridizing to its respective target, probe template spikes (Figure V-2, probes 4-6) that were generated from plasmid PV-ZMAP595 and mixed at different concentrations with the control DNA pre-digested with *Eco*R V were included on the blot (Figure V-5, lanes 5 and 6). The expected hybridization bands at 1.4, 1.7, and 2.0 kb were detected. The results show that the three probes hybridized, as expected, to the target DNA. To ensure that the backbone probes hybridize to the plasmid used for transformation, plasmid PV-ZMAP595 digested with *Blp* I/*Xba* I was mixed with conventional control DNA digested with *Eco*R V (Figure V-5, lane 7). The expected hybridization bands at 3.2 and 6.1 kb were detected, in addition to the endogenous background produced by the conventional control DNA (Figure V-5, lanes 3 and 10).

The results presented in Figure V-5 show that MON 87460 DNA digested with the combination of *Eco*O109 I and *Not* I (Figure V-5, lanes 2 and 9) or *Eco*R V (Figure V-5, lanes 4 and 11) showed no detectable hybridization signal besides the endogenous background, indicating that MON 87460 does not contain any detectable backbone sequence from the transformation vector PV-ZMAP595.

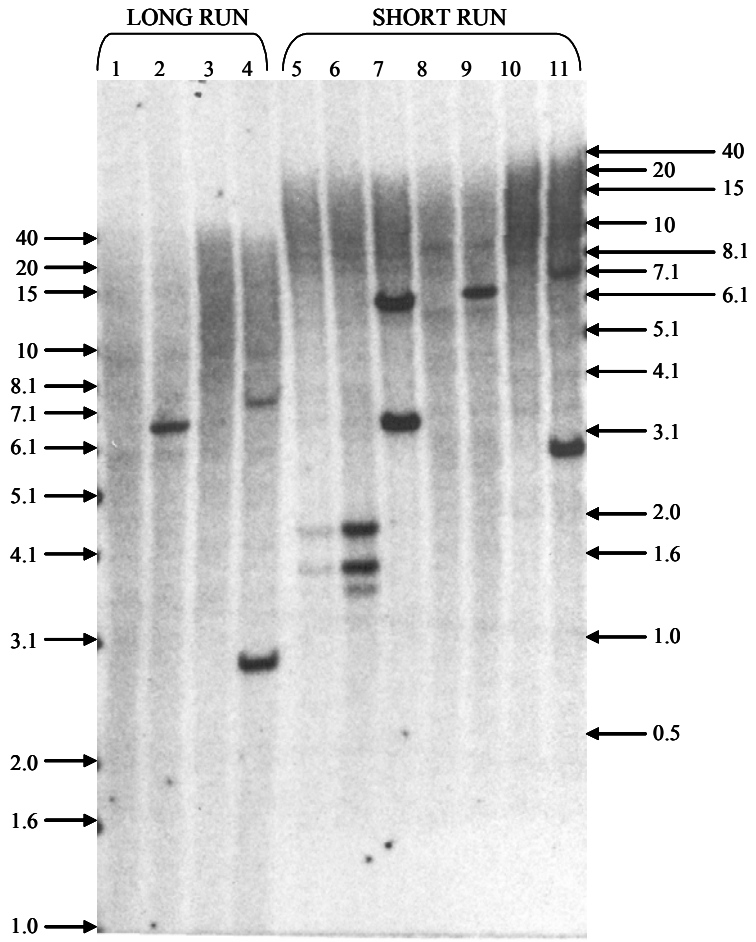



**Figure V-4. Southern Blot Analysis of MON 87460: Insert and Copy Number**

The blot was hybridized simultaneously with three overlapping $^{32}$P-labeled T-DNA probes that span the insert (Figure V-2, probes 1-3). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Hin*d III)
2: MON 87460 (*Hin*d III)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with probe templates [~0.1 copy]
6: Conventional (*Eco*R V) spiked with probe templates [~1 copy]
7: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
8: Conventional (*Hin*d III)
9: MON 87460 (*Hin*d III)
10: Conventional (*Eco*R V)
11: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

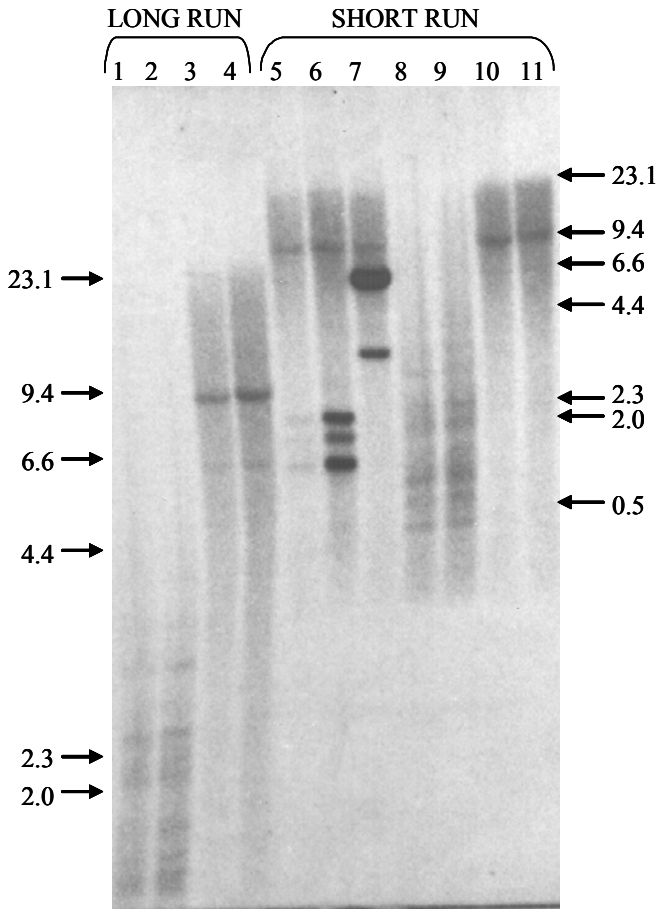



**Figure V-5. Southern Blot Analysis of MON 87460: PV-ZMAP595 Backbone**

The blot was hybridized simultaneously with three overlapping $^{32}$P-labeled probes that span the entire backbone sequence (Figure V-2, probes 4-6) of plasmid PV-ZMAP595. Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with probe templates [~0.1 copy]
6: Conventional (*Eco*R V) spiked with probe templates [~1 copy]
7: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
8: Conventional (*Eco*O109 I/*Not* I)
9: MON 87460 (*Eco*O109 I/*Not* I)
10: Conventional (*Eco*R V)
11: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

## V.C. Intactness of *cspB* and *nptII* Expression Cassettes

The copy number of the inserted *cspB* and *nptII* coding sequences and each of the associated genetic elements were assessed by digesting MON 87460 genomic DNA with restriction enzyme *Eco*R V or a combination of *Eco*O109 I and *Not* I and hybridizing Southern blots with probes covering the inserted *cspB* and *nptII* cassettes. The size of the genomic fragments and the T-DNA elements expected to be contained in each of those fragments is indicated below and summarized in Table V-2.

Digestion of MON 87460 genomic DNA with the combination of *Eco*O109 I and *Not* I was expected to generate two border fragments with expected sizes of 1.7 kb and greater than 2.7 kb (Figure V-1). The 1.7 kb restriction fragment contains genomic DNA flanking the 5' end of the insert, *Ract1*$^{87460}$ promoter and *Ract1* leader, *Ract1* intron, *cspB* coding sequence, and the *tr7* 3' nontranslated sequence. The restriction fragment greater than 2.7 kb contains the 5' *loxP* sequence, *35S* promoter, *nptII* coding sequence, *nos* 3' nontranslated sequence, 3' *loxP* sequence, left border and genomic DNA flanking the 3' end of the insert.

Digestion of MON 87460 genomic DNA with *Eco*R V was expected to release two border fragments with expected sizes of 2.7 kb and greater than 2.2 kb (Figure V-1). The restriction fragment greater than 2.2 kb contains genomic DNA flanking the 5' end of the insert, *Ract1*$^{87460}$ promoter and *Ract1* leader, *Ract1* intron, *cspB* coding sequence, and a portion of the *tr7* 3' nontranslated sequence. The approximately 2.7 kb restriction fragment contains the remaining portion of the *tr7* 3' nontranslated sequence, 5' *loxP* sequence, *35S* promoter, *nptII* coding sequence, *nos* 3' nontranslated sequence, 3' *loxP* sequence, left border, and genomic DNA flanking the 3' end of the insert.

Individual Southern blots were hybridized with the following probes: Right Border + *Ract1* promoter and leader probe, *Ract1* intron probe, *cspB* coding sequence probe, *tr7* 3' nontranslated sequence probe, *loxP* + *35S* promoter probe, *nptII* coding sequence probe, or *nos* 3′ nontranslated + *loxP* + Left Border sequence probe (Figure V-3, probes 7 – 13, respectively).

### V.C.1. Right Border + *Ract1* Promoter and Leader (Probe 7)

The Southern blot used to confirm the copy number of the Right Border + *Ract1* promoter and leader sequences (Figure V-6) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with probe 7, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-6, lanes 1 and 7) or *Eco*R V (Figure V-6, lanes 3 and 9). The conventional corn genomic DNA produced several hybridization signals resulting from the probes hybridizing to endogenous sequences residing in the corn genome and are not specific to the inserted DNA. These signals were produced in both test and control lanes, and therefore the bands are considered to be endogenous background.

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control

DNA. Results of this analysis show the expected hybridization band of approximately 3.2 kb (Figure V-6, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-6, lanes 2 and 8) produced the expected single unique band of approximately 1.7 kb (Figure V-1). The hybridization band in the long run (Figure V-6, lane 8) appears slightly larger than the corresponding band in the short run (Figure V-6, lane 2), most likely due to better resolution of the band in the longer run. MON 87460 DNA digested with *Eco*R V (Figure V-6, lanes 4 and 10) produced a single unique band of approximately 7.5 kb. This is consistent with the expected band being greater than 2.2 kb (Figure V-1). This band in the long run (Figure V-6, lane 10) appears slightly larger than the corresponding band in the short run (Figure V-6, lane 4), most likely due to better resolution of the band in the longer run. There were no additional bands detected using the Right Border, promoter and leader sequence probe. Based on the results presented in Figure V-6, MON 87460 contains no additional, detectable Right border, *Ract1* promoter and leader elements other than those associated with the *cspB* cassette.

### V.C.2. *Ract1* Intron (Probe 8)

The Southern blot used to confirm the copy number of the *Ract1* Intron sequence (Figure V-7) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with probe 8, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-7, lanes 1 and 7) or *Eco*R V (Figure V-7, lanes 3 and 9). The results of this analysis show no detectable hybridization bands.

As a positive control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with *Eco*R V pre-digested control DNA. Results of this experiment produced an expected band which migrated at approximately 3.1 kb (Figure V-7, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-7, lanes 2 and 8) that was electrophoresed, blotted, and hybridized with probe 8 produced the expected single unique band of approximately 1.7 kb (Figure V-1). This band in the long run appears slightly larger than the corresponding band in the short run, most likely due to better resolution of the band in the long run. MON 87460 DNA digested with *Eco*R V (Figure V-7, lanes 4 and 10) produced the single unique band of approximately 7.2 kb. This is consistent with the expected band being greater than 2.2 kb (Figure V-1). There were no additional hybridization bands detected using the *Ract1* intron probe. Based on the results presented in Figure V-7, MON 87460 contains no additional, detectable *Ract1* intron elements other than those associated with the *cspB* cassette.

### V.C.3 *cspB* Coding Sequence (Probe 9)

The Southern blot used to confirm the copy number of the *cspB* Coding Sequence (Figure V-8) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with probe 9, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I

(Figure V-8, lanes 1 and 7) or *Eco*R V (Figure V-8, lanes 3 and 9). The results of this analysis show no detectable hybridization bands, as expected for the negative control (Figure V-8, lanes 1, 3, 7, and 9).

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA. Results of this experiment produced an expected band which migrated at approximately 3.1 kb (Figure V-8, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-8, lanes 2 and 8) and hybridized with probe 9 produced the expected single unique band of approximately 1.7 kb (Figure V-1). This band in the long run appears slightly larger than the corresponding band in the short run, most likely due to better resolution of the band in the long run. MON 87460 DNA digested with *Eco*R V (Figure V-8, lanes 4 and 10) and hybridized with probe 9 produced the single unique band of approximately 7.2 kb. This is consistent with the expected band being greater than 2.2 kb (Figure V-1). There were no additional hybridization bands detected using the *cspB* coding sequence probe. Based on the results presented in Figure V-8, MON 87460 contains no additional, detectable *cspB* coding sequence elements other than those associated with the *cspB* cassette.

### V.C.4. *tr7* 3' Nontranslated Sequence (Probe 10)

The Southern blot used to confirm the copy number of the *tr7* 3' Nontranslated Sequence (Figure V-9) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with probe 10, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-9, lanes 1 and 7) or *Eco*R V (Figure V-9, lanes 3 and 9). The results of this analysis show no detectable hybridization bands, as expected for the negative control (Figure V-9, lanes 1, 3, 7, and 9).

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA. Results of this experiment produced an expected band which migrated at approximately 3.1 kb (Figure V-9, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-9, lanes 2 and 8) and hybridized with probe 10 produced the expected band of 1.7 kb (Figure V-1). Although difficult to observe in Figure V-9, overexposure of the Southern blots show a faint unexpected band of approximately 2.5 kb that is likely a result of partial digestion. The 2.5 kb band is consistent with partial digestion of genomic DNA because there is an *Eco*O109 I site at position 875 in the 5' flanking genomic DNA (Figure V-1). MON 87460 DNA digested with *Eco*R V (Figure V-9, lanes 4 and 10) and hybridized with probe 10 produced the expected bands of approximately 2.7 and 7.5 kb. The approximately 7.5 kb band is consistent with the expected band being greater than 2.2 kb and the approximately 2.7 kb band is the expected size for the 3′ border fragment (Figure V-1). The 2.7 kb band is less intense than the approximately 7.5 kb band probably due to a smaller portion of the *tr7* probe hybridizing to the 2.7 kb fragment.

There were no additional bands detected using the *tr7* 3' nontranslated probe. Based on the results presented in Figure V-9, MON 87460 contains no additional, detectable *tr7* 3' nontranslated sequence elements other than those associated with the intact *cspB* cassette.

### V.C.5. *loxP + 35S* Promoter (Probe 11)

The Southern blot used to confirm the copy number of the *loxP + 35S* Promoter sequences (Figure V-10) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with probe 11, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-10, lanes 1 and 7) or *Eco*R V (Figure V-10, lanes 3 and 9). The results of this analysis show no detectable hybridization bands, as expected for the negative control (Figure V-10, lanes 1, 3, 7, and 9).

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA. Results of this experiment produced the expected size band at 6.1 kb (Figure V-10, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-10, lanes 2 and 8) and hybridized with probe 11 produced the expected single unique band of approximately 3.2 kb. This is consistent with the expected band being greater than 2.7 kb (Figure V-1). MON 87460 DNA digested with *Eco*R V (Figure V-10, lanes 4 and 10) and hybridized with probe 11 produced the expected single unique band of 2.7 kb (Figure V-1). As there were no unexpected bands detected, the results presented in Figure V-10 show that MON 87460 contains no additional, detectable *loxP* sequence or *35S* promoter elements other than those associated with the intact *nptII* cassette.

### V.C.6. *nptII* Coding Sequence (Probe 12)

The Southern blot used to confirm the copy number of the *nptII* Coding Sequence (Figure V-11) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with probe 12, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-11, lanes 1 and 7) or *Eco*R V (Figure V-11, lanes 3 and 9). The results of this analysis show no detectable hybridization bands.

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA and hybridized with probe 12. Results of this experiment produced an expected band which migrated at approximately 5.5 kb (Figure V-11, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-11, lanes 2 and 8) and hybridized with probe 12 produced the expected single unique band of approximately 3.2 kb (Figure V-1). This band in the long run appears slightly larger than the corresponding band in the short run, most likely due to better resolution of the band in the long run. This band size is consistent with the expected band being greater than 2.7 kb. MON 87460 DNA digested with *Eco*R V (Figure V-11, lanes 4 and 10) and

hybridized with probe 12 produced the expected single unique band of 2.7 kb (Figure V-1). There were no additional bands detected using the *nptII* coding sequence probe. Based on the results presented in Figure V-11, MON 87460 contains no additional, detectable *nptII* coding sequence elements other than those associated with the intact *nptII* cassette.

### V.C.7. *nos* 3' Nontranslated sequence + *loxP* + Left Border Sequence (Probe 13)

The Southern blot used to confirm the copy number of the *nos* 3' Nontranslated sequence + *loxP* + Left Border Sequence (Figure V-12) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with probe 13, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-12, lanes 1 and 7) or *Eco*R V (Figure V-12, lanes 3 and 9). The results of this analysis show no detectable hybridization bands.

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA. Results of this experiment produced the expected size band at 6.1 kb (Figure V-12, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure V-12, lanes 2 and 8) and hybridized with probe 13 produced the expected single unique band of approximately 3.2 kb (Figure V-1). This is consistent with the expected band being greater than 2.7 kb (Figure V-1). MON 87460 DNA digested with *Eco*R V (Figure V-12, lanes 4 and 10) and hybridized with probe 13 produced the expected single unique band of 2.7 kb (Figure V-1). As there were no unexpected bands detected, the results presented in Figure V-12 show that MON 87460 contains no additional, detectable *nos* 3' nontranslated sequence, *loxP* sequence or left border sequence elements other than those associated with the intact *nptII* cassette.

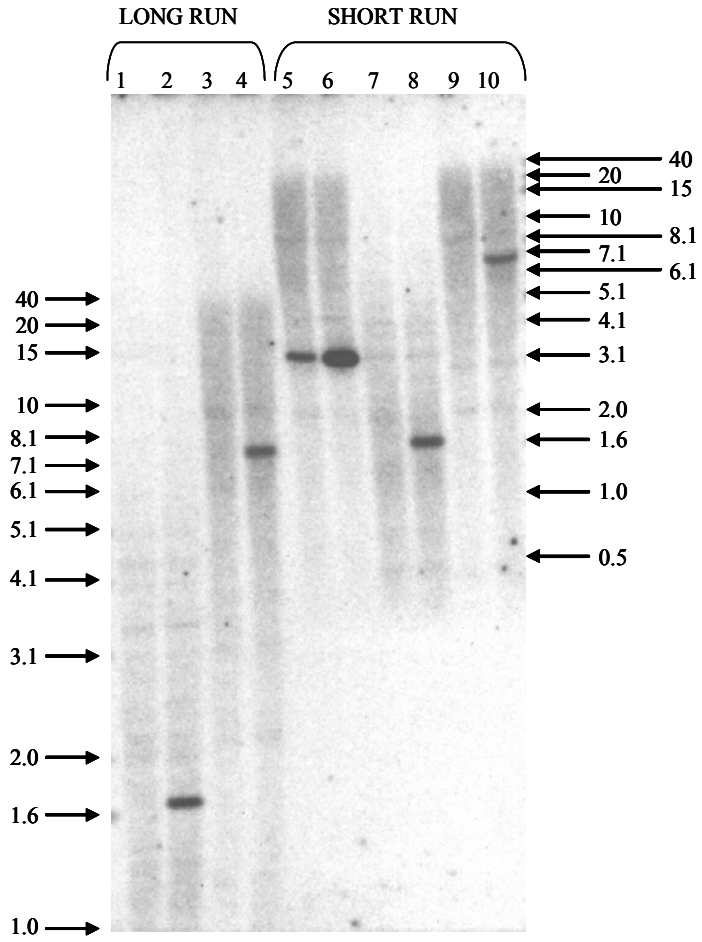



**Figure V-6. Southern Blot Analysis of MON 87460: P-*Ract1***

The blot was hybridized with a $^{32}$P-labeled probe that spanned the Right Border, *Ract1* promoter and leader (Figure V-3, probe 7). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

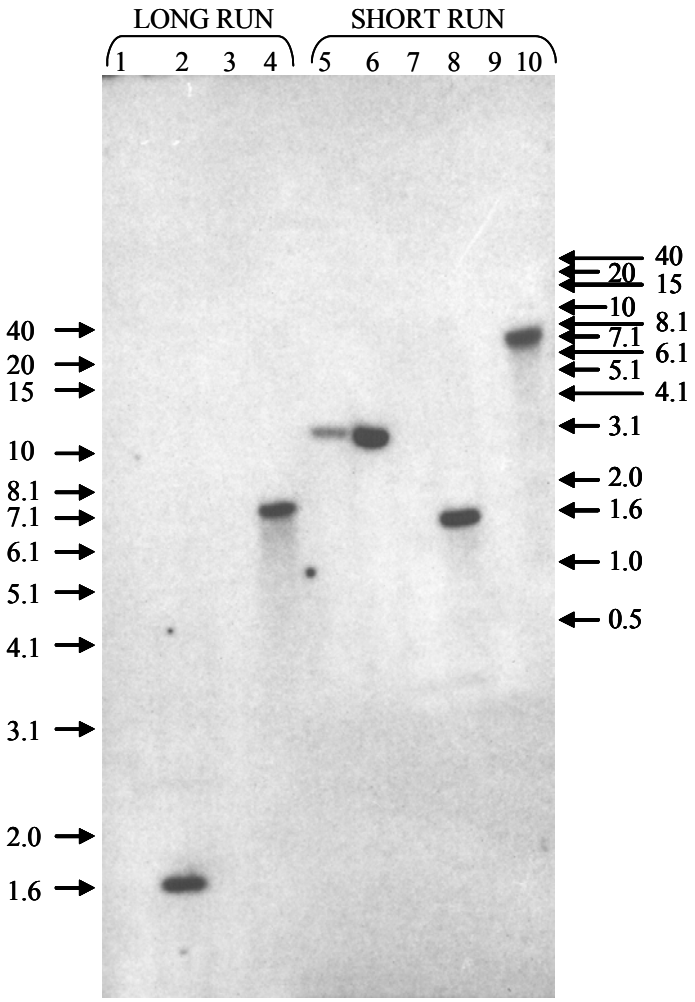



**Figure V-7. Southern Blot Analysis of MON 87460: I-*Ract1***

The blot was hybridized with a $^{32}$P-labeled probe that spanned the *Ract1* intron (Figure V-3, probe 8). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

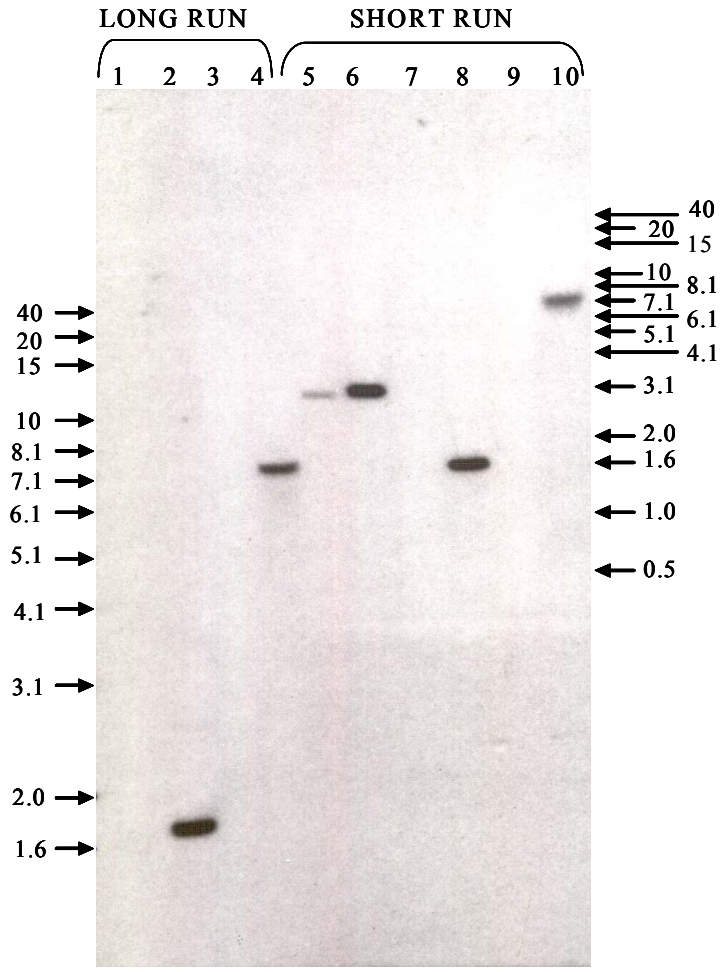



**Figure V-8. Southern Blot Analysis of MON 87460: CS-*cspB***

The blot was hybridized with a $^{32}$P-labeled probe that spanned the *cspB* coding sequence (Figure V-3, probe 9). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

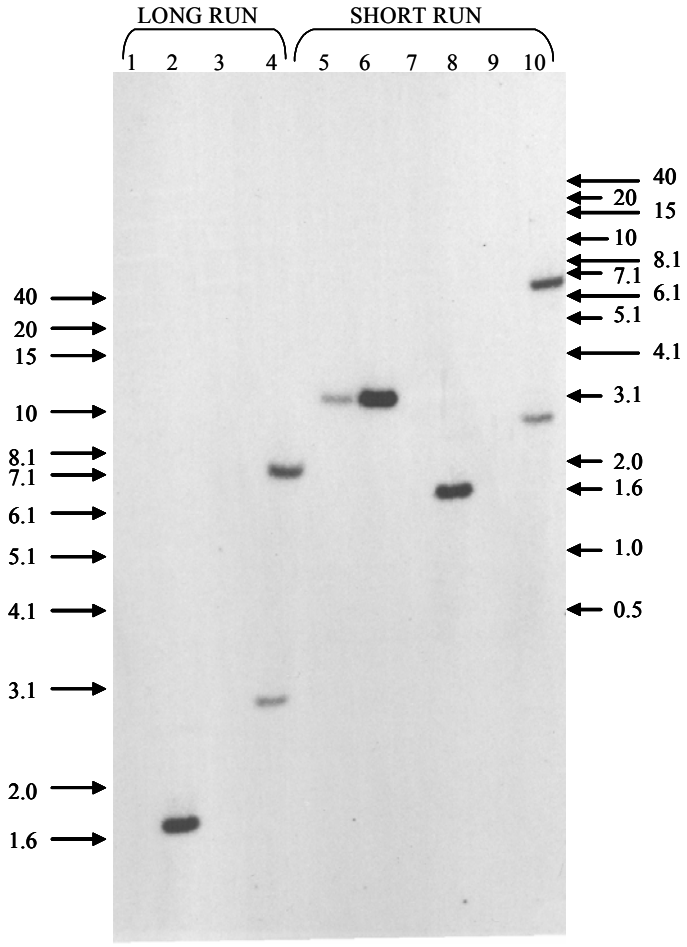



**Figure V-9. Southern Blot Analysis of MON 87460: T-*tr7***

The blot was hybridized with a $^{32}$P-labeled probe that spanned the *tr7* 3' nontranslated sequence (Figure V-3, probe 10). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

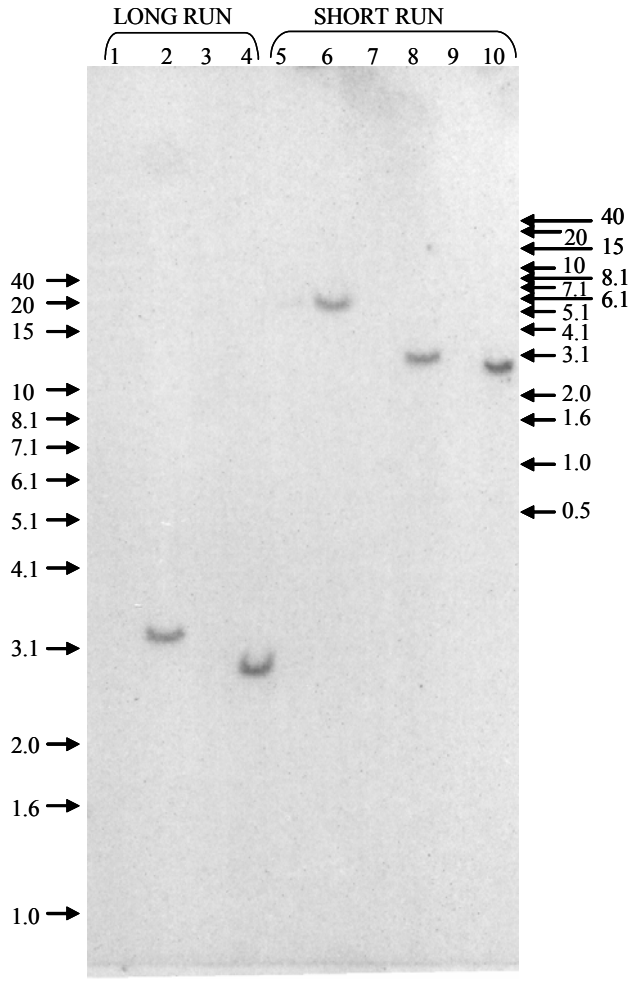



**Figure V-10. Southern Blot Analysis of MON 87460: *loxP* + P-*35S***

The blot was hybridized with a $^{32}$P-labeled probe that spanned the *loxP* sequence and *35S* promoter (Figure V-3, probe 11). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

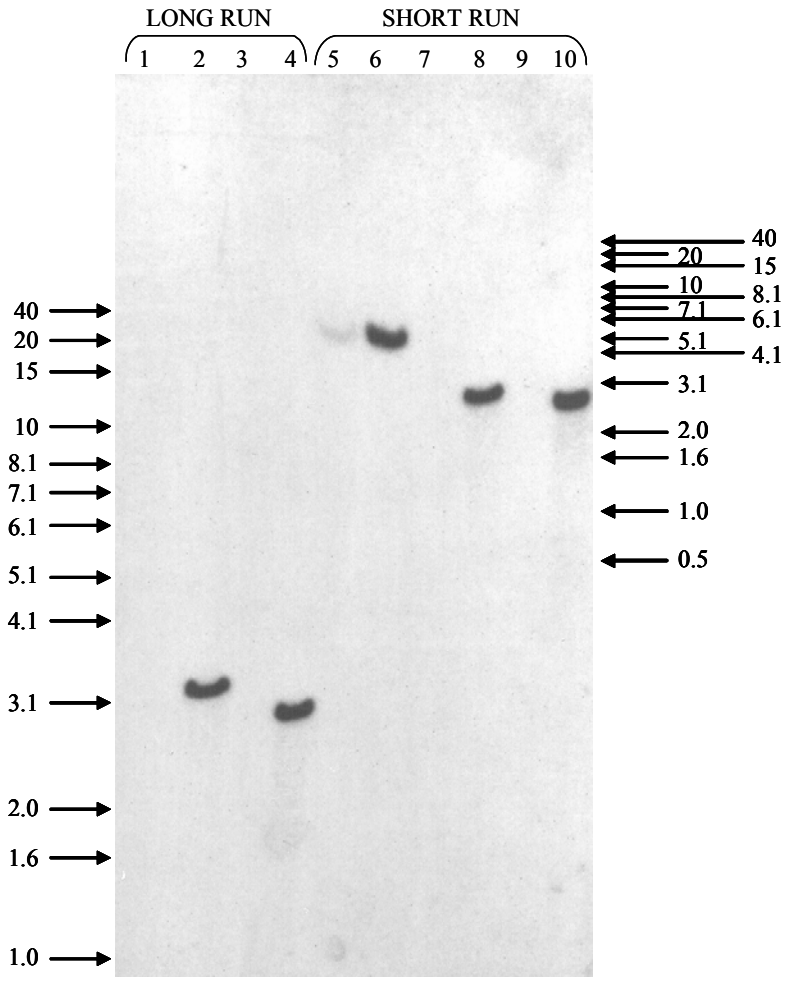



**Figure V-11. Southern Blot Analysis of MON 87460: CS-*nptII***

The blot was hybridized with a $^{32}$P-labeled probe that spanned the *nptII* coding sequence (Figure V-3, probe 12). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

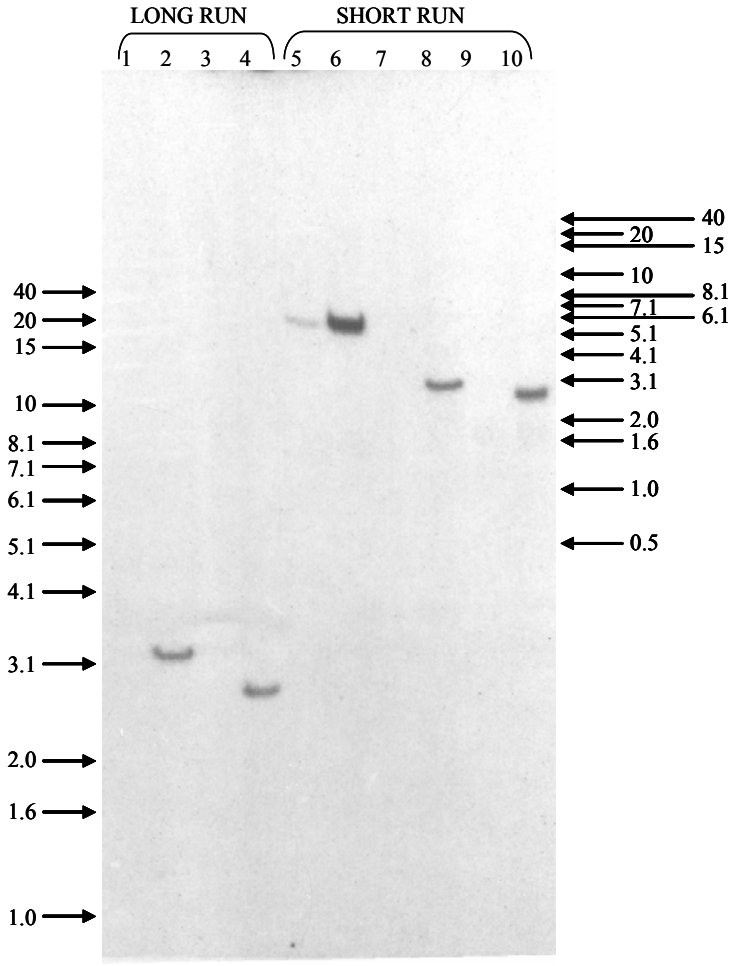



**Figure V-12. Southern Blot Analysis of MON 87460: T-*nos* + *loxP* + B-Left Border**

The blot was hybridized with a $^{32}$P-labeled probe that spanned the *nos* 3' nontranslated sequence, *loxP* sequence and Left Border sequence (Figure V-3, probe 13). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

## V.D. Southern Blot Analyses of MON 87460 across Multiple Generations

In order to assess the stability of the inserted DNA in MON 87460 across generations, Southern blot analyses were performed using DNA obtained from multiple generations of MON 87460. For reference, the breeding diagram of MON 87460 is presented in Figure V-13. The specific generations tested are indicated in the legends of Figures V-13, V-14 and V-15.

### V.D.1. Generational Stability of the Insert

DNA samples from seven generations of MON 87460 were isolated and subjected to digestion with *Eco*R V (refer to generations indicated in bold in Figure V-13). Digestion of the test materials with *Eco*R V was expected to release two border fragments with expected sizes of 2.7 kb and >2.2 kb (Figure V-1). The blot was hybridized simultaneously with three radiolabeled probes that span the entire T-DNA sequence of plasmid PV-ZMAP595 (Figure V-2, probes 1-3). The hybridization bands detected in each generation are compared to the fully characterized R3F1 [(LH59 R3×LH244)$F_1$] generation to determine insert stability.

The Southern blot used to confirm generational stability of the T-DNA (Figure V-14) contained several controls. To determine if any endogenous background hybridization bands were detected when hybridizing with the three radiolabeled probes that span the entire T-DNA, the blot contained conventional control DNA digested with *Eco*R V (Figure V-14, lane 1). The results of this analysis show several detectable hybridization bands. These hybridization signals result from the probes hybridizing to endogenous targets residing in the corn genome and are not specific to the inserted DNA.

To ensure that each probe was capable of hybridizing to its respective target, the blot contained probe template spikes (Figure V-2, probes 1-3) that were generated from plasmid PV-ZMAP595 and mixed at different concentrations with control DNA pre-digested with *Eco*R V (Figure V-14, lanes 2 and 3). When hybridized with three overlapping $^{32}$P-labeled probes that span the entire T-DNA (Figure V-2, probes 1-3), the expected hybridization bands at approximately 1.4, 1.6, and 2.0 kb were detected. The 0.1 and 1 copies of the 1.4 kb band are faint in comparison to the 1.6 and 2.0 kb bands, but were clearly detectable. The detection of the probe template positive hybridization controls demonstrates that all three probes are hybridizing to the target DNA. To ensure that the probes were capable of hybridizing to the plasmid used for transformation, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with control DNA pre-digested with *Eco*R V (Figure V-14, lane 4). Hybridization with probes 1-3 produced the expected size bands at approximately 3.2 and 6.1 kb in addition to the endogenous background.

MON 87460 DNA isolated from multiple generations of MON 87460 (Figure V-13), digested with restriction enzyme *Eco*R V, and hybridized with three overlapping $^{32}$P-labeled probes that span the entire T-DNA (Figure V-2, probes 1-3) produced two hybridization bands at 2.7 kb and approximately 7.2 kb (Figure V-14, lanes 5-11). The approximately 7.2 kb band is consistent with the 5' border fragment which was expected to be greater than 2.2 kb (Figure V-1). The 2.7 kb band is the expected size for the

border fragment containing the 3' end of the insert and adjacent flanking genomic DNA (Figure V-1). This is the same restriction pattern observed for the $F_1$ generation (LH59 R3 x LH244) shown in Figure V-4 (lanes 4 and 11). There were no additional unexpected bands detected, indicating that the single copy of T-DNA in MON 87460 is stable across the selected generations.

### V.D.2. Confirmation of the Absence of PV-ZMAP595 Backbone Sequence

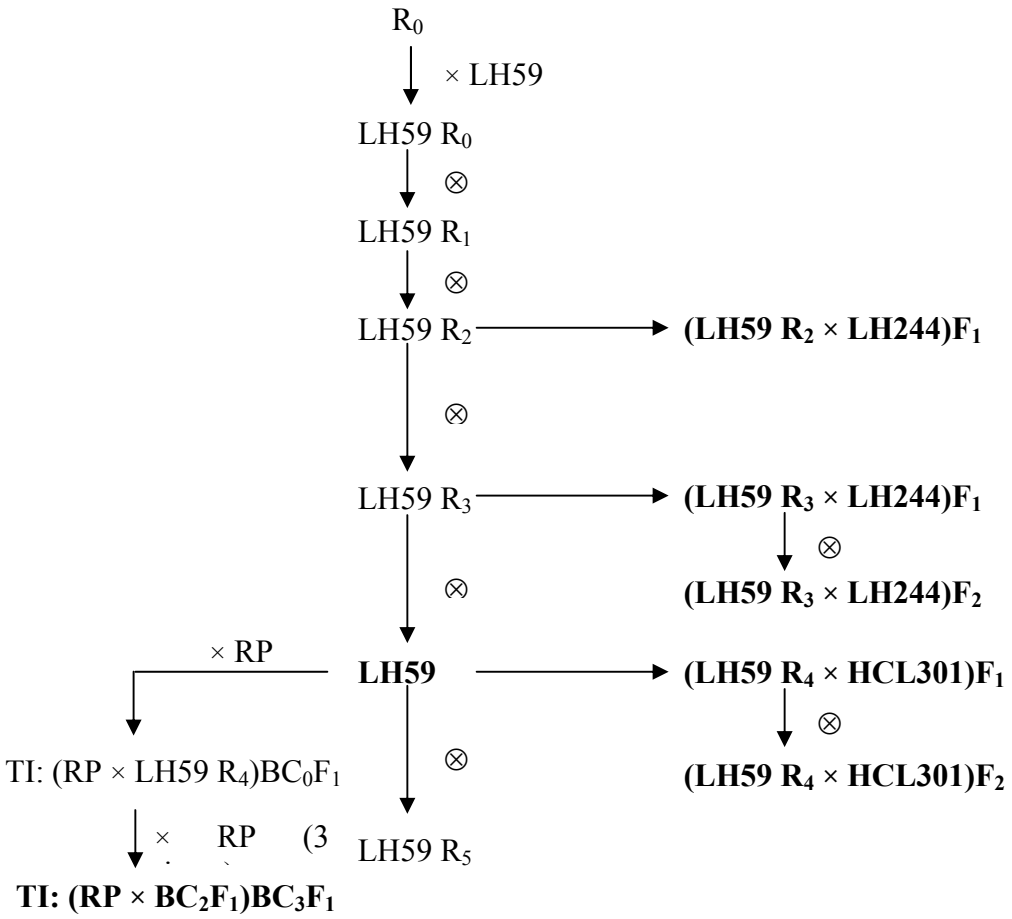
The generations of MON 87460 utilized to assess stability of the insert were also tested for the presence of backbone sequence by Southern blot analysis. Test and control DNA samples were digested with *Eco*R V and the blot was hybridized simultaneously with three radiolabeled probes that span the entire backbone sequence of plasmid PV-ZMAP595 (Figure V-2, probes 4-6).

The Southern blot used to confirm the absence of the PV-ZMAP595 backbone sequences (Figure V-15) contained several controls. To determine if any endogenous background hybridization bands were detected when hybridizing with the three radiolabeled backbone probes, the blot contained conventional control DNA digested with *Eco*R V (Figure V-15, lane 1). Several hybridization bands were detected. These signals were produced in all lanes, including those lanes containing the conventional control DNA material, and therefore they are considered endogenous background. These hybridization signals result from the probes hybridizing to endogenous targets residing in the corn genome and are not specific to the inserted DNA.

To ensure that each of the backbone probes was capable of hybridizing to its respective target, the blot contained probe template spikes (Figure V-2, probes 4-6) that were generated from plasmid PV-ZMAP595 and mixed with the control DNA pre-digested with *Eco*R V (Figure V-15, lanes 2 and 3). The expected sizes of the three bands are 1.4, 1.7, and 2.0 kb; however, the migrations of the approximately 1.7, 2.1 and 2.5 kb fragments as indicated by the molecular weight markers are slightly higher than expected. The altered migrations may be due to the difference in salt concentrations between the DNA sample and the molecular weight marker (Sambrook and Russell, 2001). The results show that the three probes hybridized to the target DNA. To ensure the probes were capable of hybridizing to the plasmid used for transformation, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with control DNA pre-digested with *Eco*R V (Figure V-15, lane 4). Hybridization with probes 4-6 produced the expected size bands at approximately 3.2 and 6.1 kb, in addition to the endogenous background produced by the conventional control DNA.

MON 87460 DNA isolated from multiple generations of MON 87460 (refer to Breeding History of MON 87460, Figure V-13), digested with restriction enzyme *Eco*R V, and hybridized with three overlapping $^{32}$P-labeled probes that span the backbone sequences of PV-ZMAP595 (Figure V-2, probes 4-6) show no detectable hybridization signals, besides the endogenous background bands (Figure V-15, lanes 5-11). Consistent with results depicted in Figure V-5 (lanes 4 and 11), these results indicate that the generations tested

do not contain any detectable backbone sequence from the transformation vector




R0 = transformed plant; F(#) = filial generation; ⊗ = self-pollination; BC(#) = backcross generation; RP = recurrent parent for commercial seed development; TI = trait integration for commercial seed development.

PV-ZMAP595.

**Figure V-13. MON 87460 Breeding Diagram**

The (LH59 $R_3$ × LH244)$F_1$ generation was used for the molecular characterization of MON 87460. The (LH59 $R_2$ × LH244) $F_1$, (LH59 $R_3$ × LH244) $F_1$, (LH59 $R_3$ × LH244) $F_2$, LH59 $R_4$, (LH59 $R_4$ × HCL301) $F_1$, (LH59 $R_4$ × HCL301) $F_2$, and (RP×$BC_2F_1$) $BC_3F_1$ generations were used for generational stability (indicated in bold). The (LH59 $R_3$ × LH244) $F_2$ and (LH59 $R_4$ × HCL301) $F_2$ generations were used for expression and composition analyses.

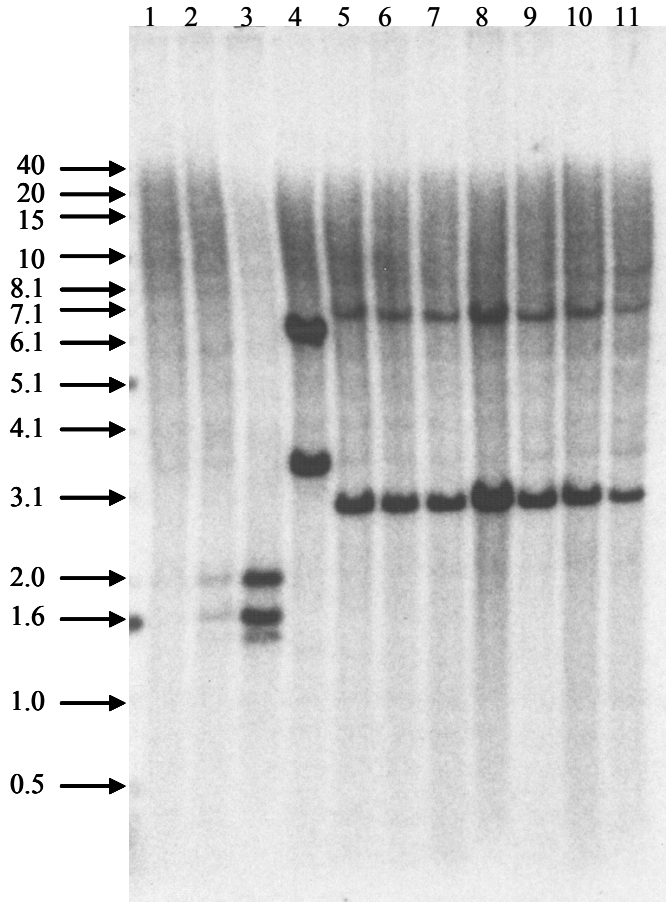



**Figure V-14. Generational Stability of MON 87460: Insert and Copy Number**

The blot was hybridized with three overlapping $^{32}$P-labeled probes that spanned the entire backbone sequence (Figure V-2, probes 1-3). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. The breeding history of MON 87460 is illustrated in Figure V-13.

Lane designations are as follows:

1: Conventional (*Eco*R V)
2: Conventional (*Eco*R V) spiked with probe templates [~0.1 copy]
3: Conventional (*Eco*R V) spiked with probe templates [~1 copy]
4: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
5: MON 87460 [(LH59 $R_2$ x LH244)$F_1$, *Eco*R V]
6: MON 87460 [(LH59 $R_3$ x LH244)$F_1$, *Eco*R V]
7: MON 87460 [(LH59 $R_3$ x LH244)$F_2$, *Eco*R V]
8: MON 87460 (LH59 $R_4$, *Eco*RV)
9: MON 87460 [(LH59 $R_4$ x HCL301)$F_1$, *Eco*R V]
10: MON 87460 [(LH59 $R_4$ x HCL301)$F_2$, *Eco*R V]
11: MON 87460 [(RP x $BC_2F_1$)$BC_3F_1$, *Eco*R V]

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

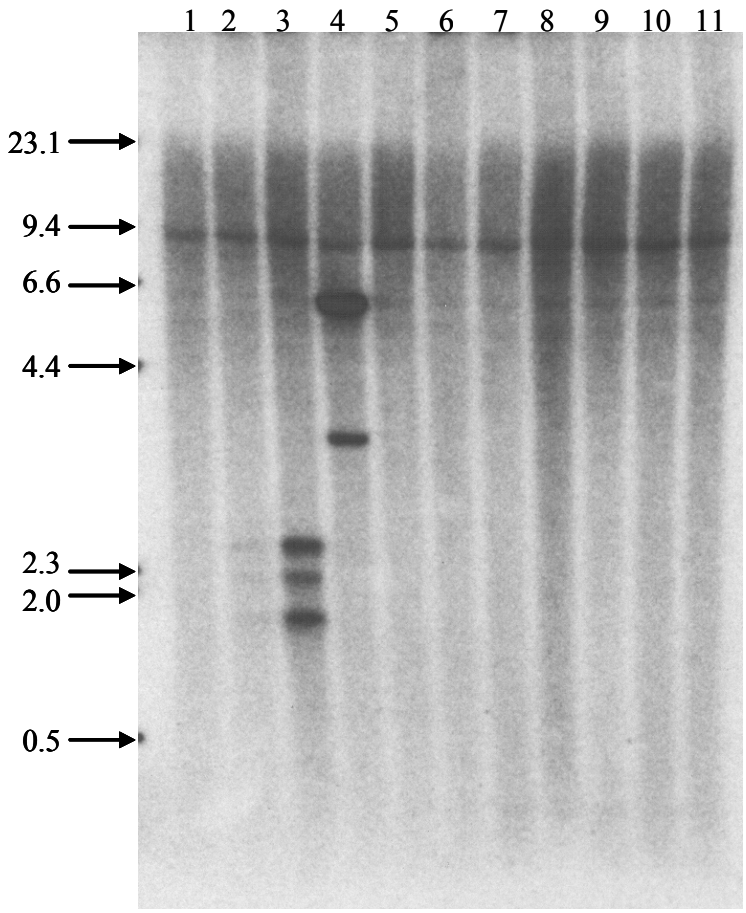



**Figure V-15. Generational Stability of MON 87460: PV-ZMAP595 Backbone**

The blot was hybridized with three overlapping $^{32}$P-labeled probes that spanned the T-DNA (Figure V-2, probes 4-6). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. The breeding history of MON 87460 is illustrated in Figure V-13. Lane designations are as follows:

1: Conventional (*Eco*R V)
2: Conventional (*Eco*R V) spiked with probe templates [~0.1 copy]
3: Conventional (*Eco*R V) spiked with probe templates [~1 copy]
4: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
5: MON 87460 [(LH59 $R_2$ x LH244)$F_1$, *Eco*R V]
6: MON 87460 [(LH59 $R_3$ x LH244)$F_1$, *Eco*R V]
7: MON 87460 [(LH59 $R_3$ x LH244)$F_2$, *Eco*R V]
8: MON 87460 (LH59 $R_4$, *Eco*RV)
9: MON 87460 [(LH59 $R_4$ x HCL301)$F_1$, *Eco*R V]
10: MON 87460 [(LH59 $R_4$ x HCL301)$F_2$, *Eco*R V]
11: MON 87460 [(RP x $BC_2F_1$)$BC_3F_1$, *Eco*R V]

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

## V.E. Organization of the Inserted Genetic Elements of MON 87460

The organization of the genetic elements within the insert of MON 87460 was confirmed by DNA sequence analyses. Several polymerase chain reaction (PCR) primers were designed to amplify overlapping DNA fragments spanning the entire length of the insert. The amplified DNA fragments were subjected to DNA sequence analyses. The DNA sequence of the insert contains 3309 base pairs beginning at base 3938 of PV-ZMAP595 located in the P-*Ract1* element region, and ending at base 7246 in the Left Border region of PV-ZMAP595. There are 733 base pairs of the P-*Ract1* element region of PV-ZMAP595 (base 3205-3937) absent in the MON 87460 insert and this molecular rearrangement presumably resulted from double-strand break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998). In addition to the insert DNA sequence, 1121 base pairs of corn genomic DNA flanking the 5' end of the insert and 784 base pairs of corn genomic DNA flanking the 3' end of the insert were also determined. Results confirm the presence and that the organization of the insert genetic elements is as depicted in Table V-1.

## V.F. Inheritance of the Genetic Insert in MON 87460

During the development of the MON 87460, trait segregation data were generated and analyzed. Chi-square analysis was performed over two generations to confirm the segregation and stability of the *cspB* gene in MON 87460. The Chi-square analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles. The $R_0$ plant was self-pollinated to produce $R_1$ seed, which is expected to segregate 1:2:1 (1 homozygote: 2 hemizygous: 1 null segregant) for the gene. A homozygous selection ($R_1$ plant) was identified from the segregating population by using an NPTII based Invader assay. The selected $R_1$ plant was self-pollinated again to produce $R_2$ seed, which was expected to be fixed for the trait, meaning, all seed are homozygous for the gene.

In additional tests, plants were backcrossed to produce $BC_3F_1$, $BC_3F_2$, $BC_3F_3$, $BC_4F_1$, and $BC_5F_1$ seed. These generations are derived from the R4 generation identified in Figure V-13. The $BC_3F_1$, $BC_4F_1$, and $BC_5F_1$ generations were expected to segregate 1:1 (1 positive:1 negative). The BC3F2 generation was expected to segregate 1:2:1 (1 homozygote:2 hemizygous:1 null segregant) for the gene. The $BC_3F_3$ generation was expected to be fixed for the trait.

The Chi-square test was computed as:

$$x^2 = \sum \frac{(o-e)^2}{e}$$

where o = observed frequency of the genotype and e = expected frequency of the genotype. The critical Chi-square value at $\alpha = 0.05$ and 1 degree of freedom is 3.841.

The segregation patterns reported in Table V-3 are based on PCR-based assays. The Chi-square values for the $R_1$, $BC_3F_1$, $BC_3F_2$, $BC_4F_1$, and $BC_5F_1$ generations indicated no significant differences between the observed and expected segregation ratios. The data for the $R_2$ and $BC_3F_3$ generations confirmed that the populations were fixed and that all plants tested positive for the *cspB* gene. These results are consistent with molecular characterization data indicating single insertion site of the gene and confirm that the *cspB/nptII* cassette within MON 87460 follows the expected Mendelian pattern of segregation.

**Table V-3. Segregation Patterns of *cspB* between Generations of MON 87460**

| Generation | Number of Plants | Observed Positives | Observed Negatives | Expected Positives | Expected Negatives | Chi-Square* | Probability ($\alpha = 0.05$) |
|---|---|---|---|---|---|---|---|
| $R_1$ | 36 | 26 | 10 | 27 | 9 | 0.1481 | NS |
| $R_2$ | 89 | 89 | 0 | 89 | 0 | Fixed | — |
| $BC_3F_1$ | 178 | 84 | 94 | 89 | 89 | 0.562 | NS |
| $BC_3F_2$ | 154 | 124 | 30 | 115.5 | 38.5 | 2.502 | NS |
| $BC_3F_3$ | 474 | 474 | 0 | 474 | 0 | Fixed | — |
| $BC_4F_1$ | 80 | 44 | 36 | 40 | 40 | 0.800 | NS |
| $BC_5F_1$ | 82 | 44 | 38 | 41 | 41 | 0.439 | NS |

* The critical Chi-square value at $\alpha = 0.05$ and 1 degree of freedom is 3.841.
*cspB* – Gene encoding cold shock protein B from *Bacillus subtilis*.
NS – not significant.

### V.G. Conclusions of Molecular Characterization

Molecular analyses show that one intact copy of the *cspB* and *nptII* expression cassette was integrated at a single chromosomal locus contained within a ~6.8 kb *Hind* III restriction fragment. No additional elements from the transformation vector PV-ZMAP595, linked or unlinked to the intact DNA insert, were detected in the genome of MON 87460. Additionally, backbone sequence from PV-ZMAP595 was not detected. Generational stability analysis demonstrate that the expected Southern blot fingerprint of MON 87460 has been maintained across seven generations of breeding, thereby confirming the stability of the DNA insert over multiple generations. These generations were also shown not to contain any detectable backbone sequence from plasmid PV-ZMAP595. In addition, DNA sequence analyses confirmed the sequence identity between the MON 87460 insert and the portion of the T-DNA from PV-ZMAP595 that was integrated into the corn genome. These results also confirmed the organization of the genetic elements within the *cspB* and *nptII* expression cassettes of MON 87460, which was identical to that in plasmid PV-ZMAP595. Analysis of the T-DNA insertion

site indicates that there is a 22-bp deletion of genomic DNA at the insert-to-plant DNA junction. Segregation analyses show heritability and stability of the *cspB* and *nptII* genes occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert at a single chromosomal locus.

### V.H. References


Barker, R.F., K.B. Idler, D.V. Thompson and J.D. Kemp. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTil5955. Plant Molecular Biology. 2:335-350.

Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss and H. Schaller. 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon *Tn5*. Gene. 19:327-336.

Bevan, M., W.M. Barnes and M. Chilton. 1983. Structure and transcription of the nopaline synthase gene region of T-DNA. Nucleic Acids Research. 11:369-385.

Dhaese, P., H. d.Greve, J. Gielen, J. Seurinck, M. v.Montague and J. Schell. 1983. Identification of sequences involved in polyadenylation of higher plant nuclear transcripts using *Agrobacterium* T-DNA genes as models. The EMBO Journal. 2:419-426.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L. Hoffmann and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proceedings of the National Academy of Sciences. USA. 80:4803-4807.

McElroy, D., W. Zhang, J. Cao and R. Wu. 1990. Isolation of an efficient actin promoter for use in rice transformation. Plant Cell. 2:163-171.

McElroy, D., A.D. Blowers, B. Jenes and R. Wu. 1991. Construction of expression vectors based on the rice actin 1(*Act1*) 5′ region for use in monocot transformation. Molecular and General Genetics. 231:150-160.

Odell, J.T., F. Nagy and N-H. Chua. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature. 313:810-812.

Russell, S.H., J.L. Hoopes and J.T. Odell. 1992. Directed excision of a transgene from the plant genome. Molecular and General Genetics. 234:49-59.

Salomon, S. and H. Puchta. 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO Journal. 17:6086-6095.

Sambrook, J. and D. Russell. 2001. Chapter 5 Protocol 1: Agarose gel electrophoresis. Pages 5.4 to 5.13 in Molecular cloning: a laboratory manual. 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Willimsky, G., H. Bang, G. Fischer and M.A. Marahiel. 1992. Characterization of *cspB*, a *Bacillus subtilis* inducible cold shock gene affecting cell viability at low temperatures. Journal of Bacteriology. 174:6326-6335.

## VI. Characterization of the Introduced CSPB and NPTII Proteins

Characterization of the introduced proteins is important to establishing the basic components of the food, feed and environmental safety assessments. This section summarizes the evaluation of the CSPB and NPTII proteins produced in MON 87460 including: (a) equivalence of the *in planta*-produced proteins to the recombinant *E. coli*-produced proteins used in protein safety studies, (b) the expression levels of the proteins determined in corn tissues, and (c) a summary of the food and feed safety assessment of the CSPB and NPTII proteins.

### VI.A. CSPB Protein

#### VI.A.1. Identity and Function of the CSPB Protein

The CSPB protein in MON 87460 belongs to the cold-shock protein (CSP) family and is identical in amino acid sequence to the native CSPB protein produced in *B. subtilis* with the exception of one amino acid change in the second position from leucine to valine that was necessary for cloning purposes. Bacterial CSPs are composed of approximately 67-73 amino acid residues (Graumann et al., 1997) and although typically acidic in nature, contain several positively charged amino acid residues that may facilitate binding to the negatively charged backbones of polynucleotides. Accumulation of the CSPB protein in *B. subtilis* cells occurs after transition from exponential growth to stationary phase (Graumann et al., 1997; Graumann and Marahiel, 1999), indicating that CSPB accumulation in cells can be triggered under several stress conditions that share a common signal such as inactivation of ribosomes (Schindler et al., 1999; Graumann et al., 1997). Stability of the protein both *in vivo* and *in vitro* depends on the protein's ability to form a complex with nucleic acids, most likely mRNAs (Schindler et al., 1999). In the absence of polynucleic acids, the CSPB protein has a very low thermodynamic stability and is susceptible to rapid proteolytic degradation (Schindler et al., 1999).

The structure of CSPB protein has been previously described (PDB accession number 1NMF) (Schindelin et al., 1993; Schindelin et al., 1994). The CSPB protein in MON 87460 consists of 66 amino acids and has an isoelectric point of 4.31. The protein is composed of five antiparallel β-strands forming a five-strand β-barrel similar to the structure of CSPA protein from *E. coli* (PDB accession number 1MJC) (Schindelin at al., 1993; Newkirk et al., 1994). All CSPs possess binding sites for single stranded nucleic acids called RNA-binding ribonucleoprotein (RNP) motifs (Newkirk et al., 1994; Schröder et al., 1995). CSPB protein, like other CSPs, contains two conserved RNP motifs: RNP1 and RNP2 (Figure VI-1, shown in boxes). Within the CSPB RNP domains four aromatic amino acids F15, F27, H29, and F30 (Figure VI-1, highlighted in bold and underlined) are required for the double-stranded polynucleotide unwinding or "melting" activity exhibited by CSPB. These amino acids are conserved in all CSPs and thought to be essential for protein function in bacteria (Phadtare et al., 2002). *In vitro* studies suggest that by binding to RNA secondary structures, CSPs reduce the free energy required for misfolded RNA to unfold and adopt the correct configuration (Herschlag, 1995). Experimental evidence suggests that CSPs bind at the single-stranded mRNA loop and then progressively cover this region, forcing the stem to open (Phadtare et al., 2002). It was suggested that CSPs bind to single stranded nucleic acids, RNA and

ssDNA, but do not appear to bind to dsDNA (Max et al., 2006). The stable association of CSPs with nucleic acids has been confirmed by co-crystallization of the *B. subtilis* CSPB protein in a complex with single stranded polynucleotides (Bienert et al., 2004; Max et al., 2007). The crystal structure data revealed the stoichiometry and sequence determinants of the binding of single-stranded nucleic acids to a preformed site on CSPB. These findings together with the described mechanism of RNA destabilization led to the classification of CSPs as RNA chaperones.

The nucleic acid unfolding ("melting") function of CSPB can be demonstrated in an *in vitro* assay using a molecular beacon system (Phadtare and Severinov, 2005). The hairpin-shaped molecule beacon is labeled with a fluorophore at the 5'- and quencher at the 3'-terminus. Due to the close proximity of the fluorescent tag and quencher in the hairpin conformation, the fluorescence is efficiently quenched. When a CSPB protein "melts" the hairpin conformation, the fluorescent tag and quencher are spatially separated which permits fluorescence. This assay has been broadly utilized to characterize the specificity of a variety of CSPs including cold-shock domain (CSD)-containing proteins identified in bacteria and plants (Karlson et al., 2002; Kim et al., 2007; Phadtare et al., 2002).

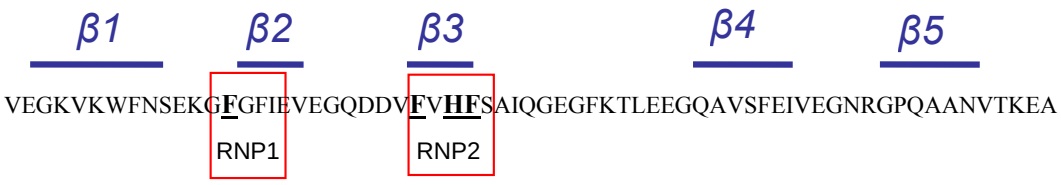



**Figure VI-1. Protein Sequence of *Bacillus subtilis* CSPB as Expressed in MON 87460**

The relative position of the β–sheets regions and the conserved RNA binding motifs RNP1 and RNP2 are indicated.

### VI.A.2. Characterization of CSPB Protein Produced in MON 87460

The expression levels of CSPB protein in different tissues of MON 87460 are relatively low. Therefore, it was necessary to produce the protein in a high-expressing, recombinant microorganism in order to obtain sufficient quantities of the protein for safety studies. A recombinant CSPB protein was produced in *E. coli*, the sequence of which was engineered to match that of CSPB protein produced in MON 87460. The equivalence of the physicochemical characteristics and functional activity between the MON 87460-produced and *E. coli*-produced CSPB protein was confirmed by a panel of analytical techniques, including sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis, matrix assisted laser

desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), glycosylation analysis, and assay of biological activity. The details of the materials, methods, and results are described in Appendix B**,** while the conclusions are summarized as follows.

A comparison of the MON 87460-produced CSPB to the *E. coli*-produced CSPB reference protein standard confirmed the identity of the MON 87460-produced CSPB protein and established the equivalence of the plant produced protein to the *E. coli*-produced CSPB reference protein standard. The molecular weight of the MON 87460- and *E. coli*-produced CSPB proteins was estimated by SDS-PAGE. The SDS-PAGE demonstrates that the proteins migrated at the same molecular weight, indicating that the CSPB proteins from both sources are equivalent in their molecular weight. The electrophoretic mobility and immunoreactive properties of the MON 87460-produced CSPB protein were shown to be equivalent to those of the *E. coli*-produced CSPB reference standard. The N-terminus of the MON 87460-produced CSPB was consistent with the predicted amino acid sequence translated from the *cspB* coding sequence, and the MALDI-TOF mass spectrometry analysis yielded peptide masses consistent with the expected peptide masses from the translated *cspB* coding sequence. The MON 87460- and the *E. coli*-produced CSPB reference standard were also found to be equivalent based on the functional activities and the lack of glycosylation. Taken together, these data provide a detailed characterization of the CSPB protein isolated from MON 87460 and established its equivalence to the *E.coli*-produced CSPB reference protein standard.

## VI.B. NPTII Protein

### VI.B.1. Identity and Function of the NPTII Protein

The NPTII protein functions as a selectable marker in the initial laboratory stages of plant cell selection following transformation (Horsch et al., 1984; DeBlock et al., 1984). The NPTII enzyme uses ATP to phosphorylate neomycin and related aminoglycoside antibiotics, thereby inactivating them. Cells that produce the NPTII enzyme selectively survive exposure to these aminoglycosides. The *nptII* coding sequence is derived from the prokaryotic *E. coli* transposon Tn5 (Beck et al., 1982). The purpose of inserting the gene encoding the NPTII protein into corn cells along with CSPB was to have an effective method for selecting cells after transformation. In general, the efficiency of plant cells transformation is often low, ranging from $1x10^{-5}$ to $lx10^{-4}$ of cells treated (Fraley et al., 1983). Therefore, the selectable marker, NPTII, was used to facilitate the screening process.

### VI.B.2. Characterization of the NPTII Protein Produced in MON 87460

The NPTII protein produced in MON 87460 was characterized, and its equivalence to a previously characterized *E. coli*-produced NPTII reference substance was demonstrated. Demonstration of the equivalence between *E. coli*- and MON 87460-produced NPTII proteins allows utilization of previous safety assessment data performed on *E. coli*-produced NPTII to confirm the safety of the NPTII protein in MON 87460. The analyses employed for the characterization of MON 87460-produced NPTII protein and establishment of the equivalence between MON 87460- and *E. coli*-produced proteins

included western blot and SDS-PAGE analyses. The details of the materials, methods, and results are described in Appendix C.

The NPTII protein from MON 87460 was characterized and compared to the *E. coli*-produced NPTII reference protein standard. The results of this analysis confirmed the identity of the MON 87460-produced NPTII protein and established the equivalence of the plant produced protein to the *E. coli*-produced NPTII reference protein standard. A western blot analysis was utilized to compare the immunoreactivity and apparent molecular weight of the MON 87460-produced NPTII protein to that of the previously characterized *E. coli*-produced NPTII reference protein standard. The MON 87460- and *E. coli*-produced NPTII proteins displayed similar immunoreactivity with NPTII-specific antibody and had identical electromobility on SDS-PAGE. Taken together, these data establish equivalence between the MON 87460-produced and *E.coli*-produced NPTII reference protein standard.

**VI.C. Expression Levels of CSPB and NPTII Proteins in MON 87460**

The levels of the CSPB and NPTII proteins in various tissues of MON 87460 were determined using enzyme-linked immunosorbent assays (ELISA). The materials and methods for the ELISA analysis, as well as a description of the tissue types, are provided in Appendix D. To produce the tissues for analysis, MON 87460 and conventional corn were each planted in field studies conducted during two different growing seasons. The first season was conducted at six sites in the U.S. during 2006 under typical agronomic practices and water conditions. The second season was conducted at four sites in Chile during 2006/2007 using a strip-plot design to establish two water treatment levels (well-watered and water-limited) to assess for any changes in CSPB and NPTII protein levels under different soil moisture conditions. The sites were located in the major corn-growing regions of the U.S. and Chile. Additional information on the water management for each study is provided in Section VIII.C. Forage, stover, silk, pollen, and grain samples were collected at appropriate times of plant development. Over-season leaf (OSL), over-season root (OSR), and over-season whole plant (OSWP) samples were collected four times (1-4) over the season corresponding to plant growth stages V2-V4, V6-V8, V10-V12, and pre-VT (pre-tasseling), respectively. The expression levels of CSPB and NPTII proteins in these tissues are shown in Tables VI-1 to VI-4.

CSPB expression in MON 87460 is driven by the rice actin constitutive promoter and thus is expected to occur in all plant tissues at various levels. The protein was detected in all tissue types with the highest level of expression in pollen, followed by leaf, root, silk, forage, grain, stover, senescent root, and forage root. In general, the levels of CSPB protein declined over the growing season in samples from both field studies. Consistent with the constitutive nature of the rice actin promoter, no obvious difference was observed in CSPB protein levels in tissues collected from plants grown under well-watered or water-limited conditions.

NPTII expression in MON 87460 is driven by the constitutive CaMV 35S promoter, which directs expression across all plant tissues at various levels. The NPTII protein was detected in three out of four analyzed tissue types, with the highest level determined in

leaves, followed by roots and forage. The level of NPTII protein in grain was below the Limit of Quantitation (LOQ) of the method. As was observed with the CSPB levels and consistent with the constitutive nature of the CaMV 35S promoter, no obvious difference was observed in NPTII protein levels in tissues collected from plants grown under well-watered or water-limited conditions.

### VI.C.1. CSPB Expression Levels in MON 87460

Results from the U.S. 2006 study show the mean CSPB protein levels across all six sites were highest in pollen (13 µg/g dwt), followed by young leaf (OSL-1, 3.1 µg/g dwt), young root (OSR-1, 1.4 µg/g dwt), silk (1.2 µg/g dwt), forage (0.10 µg/g dwt), grain (0.072 µg/g dwt), stover (0.042 µg/g dwt), senescent root (0.041 µg/g dwt), and forage root (0.029µg/g dwt) (Table VI-1). In tissues harvested throughout the growing season, mean CSPB protein levels in MON 87460 across all sites ranged from 0.47 – 3.1 µg/g dwt in leaf, 0.24 – 1.4 µg/g dwt in root, and 0.67 – 2.8 µg/g dwt in whole plant.

Results from the Chile 2006/2007 study represent combined-site data from three (CL, CT, LUM) of the four sites. For a site to be included in the combined-site analysis, commercial reference hybrids in the water-limited plots had to exhibit a minimum 15% reduction in yield compared to the same reference hybrids planted in the well-watered plots. A detailed description of the site inclusion criteria applied to these data is provided in Section VIII.B.2**.** As described in Section VIII.C and Table VIII-3, the QUI site in the Chile 2006/2007 study was not established with the appropriate water stress treatments; therefore, data and analysis for the QUI site are presented in Appendix D. Results show the mean CSPB protein levels across the three sites that were subjected to either well-watered or water-limited conditions were highest in pollen (25 µg/g dwt well-watered and 27 µg/g dwt water-limited conditions), followed by young leaf (OSL1, 2.8 µg/g dwt for both well-watered and water-limited conditions), young root (OSR1, 1.3 µg/g dwt well-watered and 1.5 µg/g dwt water-limited conditions), silk (0.82 µg/g dwt well-watered and 1.1 µg/g dwt µg/g dwt water-limited conditions), forage (0.11 µg/g dwt µg/g dwt well-watered and 0.15 µg/g dwt water-limited conditions), grain (0.048 µg/g dwt µg/g dwt well-watered and 0.038 µg/g dwt water-limited conditions), stover (0.033 µg/g dwt well-watered and 0.072 µg/g dwt water-limited conditions), senescent root (0.031 µg/g dwt well-watered and 0.052 µg/g dwt water-limited conditions), and forage root (0.039 µg/g dwt µg/g dwt well-watered and 0.076 µg/g dwt water-limited conditions) (Table VI-2). In tissues harvested throughout the growing season, mean CSPB protein levels in MON 87460 across all sites ranged from 0.39 – 2.8 µg/g dwt in leaves harvested from plants grown under well-watered and from 0.44 – 2.8 µg/g dwt in leaves harvested from plants grown under water-limited conditions. The mean CSPB protein levels in roots harvested from MON 87460 plants across all sites ranged from 0.031 – 1.3 µg/g dwt in well-watered and 0.052 – 1.5 µg/g dwt in water-limited conditions. In whole plants of MON 87460, the mean CSPB protein levels were 0.67 – 3.2 µg/g dwt in well-watered and 0.70 – 2.9 µg/g dwt in water-limited conditions.

### VI.C.2. NPTII Expression Levels in MON 87460

Results from the U.S. 2006 study show the mean NPTII protein levels across all sites under typical agronomic practices were highest in young leaf (OSL1, 2.6 µg/g dwt),

followed by roots (OSR1, 0.47 µg/g dwt), and forage (0.12µg/g dwt) (Table VI-3). The levels of NPTII protein in grain were below the NPTII assay Limit of Quantitation (LOQ), which was 0.0047 µg/g fwt for grain. The range in NPTII protein levels for MON 87460 in leaf, forage, and grain were 0.21 – 0.63, 0.017 – 0.053, and <LOQ µg/g fresh weight, respectively.

Results from the Chile 2006/2007 study show the mean NPTII protein levels across the three sites were highest in young leaf (OSL1, 2.4 µg/g dwt well-watered and 2.6 µg/g dwt water-limited), followed by root (OSR1, 0.51 µg/g dwt in well-watered and 0.48 µg/g dwt water-limited conditions), and forage (0.16 µg/g dwt in well-watered and 0.17 µg/g dwt water-limited conditions) (Table VI-4). The levels of NPTII protein in grain were below the NPTII assay LOQ (0.0047 µg/g fwt for grain) for tissue collected from plants under both well-watered and water-limited conditions. The NPTII protein levels in MON 87460 were lower than NPTII levels determined for the equivalent tissue types in MON 863 that also relied on NPTII as a selectable marker. The range of NPTII protein levels in MON 863 leaf, forage, and grain were 0.74 – 1.4, 0.17 – 0.23 and <LOQ µg/g fwt, respectively. The range in NPTII protein levels for MON 87460 in leaf, forage, and grain were 0.84-5.0 µg/g dwt well-watered and 0.98-4.0 µg/g dwt water-limited, 0.13-0.19 µg/g dwt well-watered and 0.14-0.22 µg/g dwt water-limited, and LOD/LOQ µg/g fresh weight for well-watered and water-limited, respectively.

**Table VI-1. CSPB Protein Levels in Tissues Collected from MON 87460 Produced in the U.S. during 2006 under Typical Agronomic Conditions**

| Tissue Type[1] | Mean (SD)[2] (µg/g fwt)[3] | Range[4] (µg/g fwt) | Mean (SD) (µg/g dwt)[5] | Range (µg/g dwt) | LOQ / LOD (µg/g fwt) |
|---|---|---|---|---|---|
| **OSL-1** | 0.45 (0.14) | 0.24 – 0.77 | 3.1 (0.93) | 2.0 – 5.1 | 0.015 / 0.0069 |
| **OSL-2** | 0.40 (0.21) | 0.18 – 0.80 | 2.2 (1.2) | 1.0 – 4.4 | 0.015/ 0.0069 |
| **OSL-3** | 0.21 (0.067) | 0.10 – 0.29 | 1.0 (0.30) | 0.54 – 1.5 | 0.015 / 0.0069 |
| **OSL-4** | 0.10 (0.047) | 0.034 – 0.19 | 0.47 (0.25) | 0.16 – 0.96 | 0.015 / 0.0069 |
| **OSR-1** | 0.13 (0.052) | 0.060 – 0.24 | 1.4 (0.55) | 0.55 – 2.4 | 0.0020 / 0.0018 |
| **OSR-2** | 0.11 (0.052) | 0.030 – 0.21 | 1.0 (0.43) | 0.25 – 1.6 | 0.0020 / 0.0018 |
| **OSR-3** | 0.059 (0.026) | 0.015 – 0.11 | 0.43 (0.20) | 0.077 – 0.84 | 0.0020 / 0.0018 |
| **OSR-4** | 0.035 (0.015) | 0.012 – 0.063 | 0.24 (0.10) | 0.098 – 0.42 | 0.0020 / 0.0018 |
| **OSWP-1** | 0.30 (0.13) | 0.11 – 0.46 | 2.8 (1.4) | 1.1 – 5.1 | 0.0045 / 0.0043 |
| **OSWP-2** | 0.18 (0.075) | 0.096 – 0.31 | 1.9 (0.87) | 0.86 – 3.4 | 0.0045 / 0.0043 |
| **OSWP-3** | 0.11 (0.041) | 0.067 – 0.22 | 0.88 (0.29) | 0.45 – 1.4 | 0.0045 / 0.0043 |
| **OSWP-4** | 0.091 (0.049) | 0.015 – 0.18 | 0.67 (0.36) | 0.11 – 1.3 | 0.0045 / 0.0043 |
| **Forage Root** | 0.0055 (0.0034) | 0.0020 – 0.015 | 0.029 (0.013) | 0.014 – 0.055 | 0.0020 / 0.0018 |
| **Senescent Root** | 0.0040 (0.0013) | 0.0022 – 0.0068 | 0.041 (0.084) | 0.014 – 0.36 | 0.0020 / 0.0018 |
| **Forage** | 0.027 (0.0095) | 0.011 – 0.046 | 0.10 (0.032) | 0.041– 0.17 | 0.0045 / 0.0043 |
| **Stover** | 0.018 (0.013) | 0.0056 – 0.047 | 0.042 (0.024) | 0.013 – 0.090 | 0.0045 / 0.0043 |
| **Silk** | 0.099 (0.024) | 0.031 – 0.13 | 1.2 (0.32) | 0.31 – 1.8 | 0.0075 / 0.0047 |
| **Pollen** | 7.3 (1.6) | 5.3 – 10 | 13 (2.3) | 10 – 17 | 0.050 / 0.045 |
| **Grain** | 0.063 (0.014) | 0.040 – 0.089 | 0.072 (0.015) | 0.045 – 0.10 | 0.0038 / 0.0017 |

[1]Over-season leaf (OSL), over-season root (OSR), and over-season whole plant (OSWP) samples were collected four times (1-4) over the season corresponding to plant growth stages V2-V4, V6-V8, V10-V12, and pre-VT (pre-tasseling), respectively.

[2]The arithmetic mean and standard deviation (SD) were calculated for each tissue type across all sites (n=18 for all tissues except OSL-2, OSWP-2 and forage where n=17, and senescent root where n=16).

[3]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.

[4]Minimum and maximum values were determined for each tissue type across all sites.

[5]Protein levels are expressed as "µg/g" of tissue on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.

**Table VI-2. CSPB Protein Levels in Tissues Collected from MON 87460 Produced in Chile during 2006/2007 under Well-Watered and Water-Limited Conditions**

| Tissue Type[1] | Well-Watered | | Water-Limited | | LOQ / LOD (µg/g fwt) |
|---|---|---|---|---|---|
| | Mean (SD)[2] Range[3] (µg/g fwt)[4] | Mean (SD) Range (µg/g dwt)[5] | Mean (SD) Range (µg/g fwt) | Mean (SD) Range (µg/g dwt) | |
| **OSL-1** | 0.50 (0.19)<br>0.28 - 0.80 | 2.8 (1.0)<br>1.7 - 4.5 | 0.50 (0.20)<br>0.26 - 0.80 | 2.8 (0.95)<br>1.7 - 4.2 | 0.015/0.0069 |
| **OSL-2** | 0.48 (0.18)<br>0.21 - 0.69 | 2.6 (1.2)<br>0.96 - 3.8 | 0.47 (0.15)<br>0.23 - 0.62 | 2.6 (1.0)<br>1.1 - 3.6 | 0.015/0.0069 |
| **OSL-3** | 0.13 (0.10)<br>0.023 - 0.33 | 0.56 (0.48)<br>0.10 - 1.5 | 0.11 (0.073)<br>0.023 - 0.25 | 0.45 (0.32)<br>0.086 - 1.1 | 0.015/0.0069 |
| **OSL-4** | 0.10 (0.041)<br>0.040 - 0.14 | 0.39 (0.13)<br>0.18 - 0.58 | 0.11 (0.054)<br>0.050 - 0.20 | 0.44 (0.17)<br>0.22 - 0.69 | 0.015/0.0069 |
| **OSR-1** | 0.13 (0.029)<br>0.079 - 0.18 | 1.3 (0.29)<br>0.79 - 1.8 | 0.14 (0.034)<br>0.10 - 0.20 | 1.5 (0.43)<br>0.95 - 2.2 | 0.0020/0.0018 |
| **OSR-2** | 0.086 (0.025)<br>0.070 - 0.13 | 0.86 (0.25)<br>0.70 - 1.4 | 0.10 (0.015)<br>0.082 - 0.12 | 0.82 (0.092)<br>0.74 - 0.95 | 0.0020/0.0018 |
| **OSR-3** | 0.061 (0.012)<br>0.035 - 0.075 | 0.49 (0.12)<br>0.27 - 0.62 | 0.054 (0.012)<br>0.036 - 0.076 | 0.41 (0.13)<br>0.24 - 0.63 | 0.0020/0.0018 |
| **OSR-4** | 0.045 (0.012)<br>0.032 - 0.067 | 0.31 (0.076)<br>0.22 - 0.45 | 0.058 (0.016)<br>0.036 - 0.084 | 0.40 (0.087)<br>0.28 - 0.52 | 0.0020/0.0018 |
| **OSWP-1** | 0.32 (0.11)<br>0.18 - 0.52 | 3.2 (0.98)<br>1.8 - 4.8 | 0.30 (0.092)<br>0.20 - 0.42 | 2.9 (0.84)<br>1.8 - 3.8 | 0.0045/0.0043 |
| **OSWP-2** | 0.19 (0.036)<br>0.12 - 0.24 | 2.3 (0.54)<br>1.4 - 3.0 | 0.18 (0.046)<br>0.12 - 0.25 | 2.2 (0.61)<br>1.4 - 3.1 | 0.0045/0.0043 |
| **OSWP-3** | 0.10 (0.042)<br>0.065 - 0.17 | 0.89 (0.34)<br>0.59 - 1.4 | 0.091 (0.032)<br>0.067 - 0.15 | 0.71 (0.25)<br>0.44 - 1.1 | 0.0045/0.0043 |
| **OSWP-4** | 0.11 (0.026)<br>0.076 - 0.17 | 0.67 (0.16)<br>0.48 - 0.98 | 0.13 (0.037)<br>0.10 - 0.20 | 0.70 (0.16)<br>0.55 - 1.0 | 0.0045/0.0043 |
| **Forage Root** | 0.0052 (0.0018)<br>0.0026 - 0.0088 | 0.039 (0.015)<br>0.017 - 0.068 | 0.011 (0.0039)<br>0.0056 - 0.016 | 0.076 (0.029)<br>0.035 - 0.12 | 0.0020/0.0018 |
| **Senescent Root** | 0.0040 (0.0017)<br>0.0026 - 0.0073 | 0.031 (0.015)<br>0.020 - 0.061 | 0.0067 (0.0051)<br>0.0026 - 0.017 | 0.052 (0.040)<br>0.019 - 0.14 | 0.0020/0.0018 |
| **Forage** | 0.026 (0.0041)<br>0.018 - 0.034 | 0.11 (0.018)<br>0.077 - 0.14 | 0.035 (0.0078)<br>0.022 - 0.047 | 0.15 (0.040)<br>0.087 - 0.22 | 0.0045/0.0043 |
| **Stover** | 0.011 (0.0023)<br>0.0071 - 0.014 | 0.033 (0.0070)<br>0.018 - 0.040 | 0.021 (0.010)<br>0.011 - 0.036 | 0.072 (0.033)<br>0.035 - 0.12 | 0.0045/0.0043 |
| **Silk** | 0.073 (0.019)<br>0.050 - 0.12 | 0.82 (0.28)<br>0.50 - 1.5 | 0.13 (0.048)<br>0.054 - 0.22 | 1.1 (0.38)<br>0.49 - 1.8 | 0.0075/0.0047 |
| **Pollen** | 18 (5.6)<br>7.0 - 24 | 25 (7.4)<br>8.9 - 33 | 18 (6.5)<br>12 - 31 | 27 (10)<br>18 - 48 | 0.050/0.045 |
| **Grain** | 0.041 (0.012)<br>0.028 - 0.065 | 0.048 (0.014)<br>0.033 - 0.075 | 0.033 (0.0067)<br>0.021 - 0.045 | 0.038 (0.0079)<br>0.024 - 0.053 | 0.0038/0.0017 |

[1]Over-season leaf (OSL), root (OSR), and whole plant (OSWP) samples were collected four times (1-4) corresponding to plant growth stages V2-V4, V6-V8, V10-V12, and pre-VT (pre-tasseling), respectively.

[2]The arithmetic mean and standard deviation (SD) were calculated for each tissue type across all sites (n=9 for well-watered and n=9 for water-limited, except OSR-2 where n=6 for under both well-watered and water-limited conditions and senescent root where n=6 for well-watered).

[3]Minimum and maximum values were determined for each tissue type across all sites.

[4]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.

[5]Protein levels are expressed as "µg/g" of tissue on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.

**Table VI-3. NPTII Protein Levels in Tissues Collected from MON 87460 Produced in the U.S. during 2006 under Typical Agronomic Conditions**

| Tissue Type[1] | NPTII (µg/g fwt) Mean (SD)[2] | Range (µg/g fwt)[3] | NPTII (µg/g dwt) Mean (SD)[4] | Range (µg/g dwt) | LOQ / LOD (µg/g fwt) |
|---|---|---|---|---|---|
| **OSL-1** | 0.37 (0.12) | 0.21–0.63 | 2.6 (0.92) | 1.3–4.2 | 0.0047 / 0.0090 |
| **OSR-1** | 0.041 (0.011) | 0.024–0.068 | 0.47 (0.12) | 0.30–0.85 | 0.0075 / 0.0043 |
| **Forage** | 0.034 (0.011) | 0.017–0.053 | 0.12 (0.049) | 0.053–0.20 | 0.0056/ 0.0024 |
| **Grain** | <LOQ | | N/A[5] | | 0.0047 / 0.0024 |

[1]Over-season leaf (OSL-1) and over-season root (OSR-1) samples were collected at the V2-V4 plant growth stage.
[2]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis. The arithmetic mean and standard deviation (SD) were calculated for each tissue type across all sites (n=18).
[3]Minimum and maximum values were determined for each tissue type across sites.
[4]Protein levels are expressed as µg/g of tissue on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.
[5]N/A – Not applicable; dry weight values were not calculated if fresh weight values were less than the LOQ.

**Table VI-4. NPTII Protein Levels in Tissues Collected from MON 87460 Produced in Chile during 2006/2007 under Well-Watered and Water-Limited Conditions**

| | Well-Watered | | Water-Limited | | |
|---|---|---|---|---|---|
| Tissue Type[1] | Mean (SD)[2] Range[3] (µg/g fwt)[4] | Mean (SD) Range (µg/g dwt)[5] | Mean (SD) Range (µg/g fwt) | Mean (SD) Range (µg/g dwt) | LOQ / LOD (µg/g fwt) |
| **OSL-1** | 0.42 (0.23)<br>0.15 - 0.85 | 2.4 (1.3)<br>0.84 - 5.0 | 0.46 (0.18)<br>0.16 - 0.68 | 2.6 (0.98)<br>0.98 - 4.0 | 0.047/0.0090 |
| **OSR-1** | 0.051 (0.0083)<br>0.041 - 0.064 | 0.51 (0.083)<br>0.41 - 0.64 | 0.046 (0.0075)<br>0.035 - 0.057 | 0.48 (0.097)<br>0.39 - 0.64 | 0.0075/0.0043 |
| **Forage** | 0.037 (0.0041)<br>0.031 - 0.044 | 0.16 (0.020)<br>0.13 - 0.19 | 0.039 (0.0048)<br>0.034 - 0.048 | 0.17 (0.028)<br>0.14 - 0.22 | 0.0056/0.0024 |
| **Grain** | <LOQ(N/A[6])<br><LOD-0.0057 | N/A (N/A)<br>N/A | <LOQ (N/A)<br><LOD-0.0051 | N/A (N/A)<br>N/A | 0.0047/0.0024 |

[1]Over-season leaf (OSL-1) and root (OSR-1) samples were collected at the V2-V4 plant growth stage.
[2]The mean and standard deviation (SD) were calculated across sites (n=9 for well-watered and n=9 for water-limited, except OSL-1 where n=8 for water-limited).
[3]Minimum and maximum values were determined for each tissue type across sites.
[4]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
[5]Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt by the dry weight conversion factors obtained from moisture analysis data.
[6]N/A – Not applicable; dry weight values were not calculated if fresh weight values were less than the LOQ or LOD.

**VI.D. Food and Feed Safety Assessment Summary of the CSPB and NPTII Proteins**

Numerous factors have been considered in the safety assessment of the CSPB and NPTII proteins that are expressed in MON 87460. A comprehensive assessment of safety of these proteins was submitted to the FDA. The assessment leads to the following conclusions.

a) The donor organism of the CSPB protein, *B. subtilis*, is not pathogenic, is often used as a food additive, is present in many fermented foods, and has a history of safe consumption. In 1999, FDA designated enzyme preparations from this organism as GRAS (generally recognized as safe, FDA, 1999). Proteins containing cold shock domains are ubiquitous in nature, being present in many plants and common bacteria including species that are normally present in gastrointestinal flora. Cold shock proteins have no known toxicity and are not associated with pathogenicity.

b) The *B. subtilis* CSPB shares a high percent of identity with CSPs present in other bacterial species broadly used by the food industry and with CSD-containing proteins in plant species used as a food. Many foods prepared with the help of *B. subtilis* have been consumed for a long time with no documented history of any adverse effects to human health. CSPB protein present in MON 87460 shares amino acid identity to other naturally occurring CSD-containing proteins found in food and food products. The amino acid identity ranges from 35% to 98.5% across different plant and bacterial species. The CSPB protein is homologous to the CSP proteins found in the genera *Lactobacillus*, *Lactococcus* and *Bifidobacterium*, and *E. coli*, which are normally present in gastrointestinal flora and, therefore, considered to be safe. The strains of lactic acid bacteria, *Bifidobacterium* and *Lactobacillus*, are the most common type of bacteria used in the dairy industry for preparation of probiotic products containing live bacterial cultures. These bacteria resist gastric acid, bile salts and pancreatic enzymes, and, thus, readily colonize the intestinal tract (Rolfe, 2000). In addition, *Bacillus*, *Lactobacillus, and Lactococcus* species containing CSPs are involved in many fermentation processes of milk, meats, cereals and vegetables.

c) A dietary safety assessment was conducted to evaluate the risks to humans and animals from the CSPB and NPTII proteins present in the foods and feeds derived from MON 87460. Risks are quantified as a margin of exposure (MOE), which is defined as the ratio of the No Observable Effect Level (NOEL) from an acute mouse gavage study to estimates of the dietary intake of the respective proteins. Acute oral toxicity studies with mice demonstrated that the two proteins are not acutely toxic and do not cause any observed adverse effects even at the highest tested dose levels, which are 4.7 and 5,000 mg/kg body weight for CSPB and NPTII proteins, respectively. The dietary safety assessment showed that the MOEs for the overall U.S. population were greater than or equal to 26,700 and 454,000,000 for the CSPB and NPTII proteins, respectively. For children aged 1-6 years old, an age group with the highest corn consumption on a body weight basis, the MOEs were greater than or equal to 11,400 and 208,000,000 for the CSPB and NPTII proteins, respectively. Dietary exposures in animals will also be

low, with chickens, swine, and dairy cows consuming only nanogram quantities of each protein per kilogram of body weight.

d) Digestive fate experiments conducted with the CSPB protein demonstrated that the full-length protein is rapidly digested in SGF, a characteristic shared among many proteins with a history of safe consumption. A small transiently stable CSPB protein fragment was very quickly degraded during short exposure to SIF. Rapid digestion of the full-length CSPB protein in SGF and SIF, together with rapid degradation of the small transiently stable fragment in SIF, indicates that it is highly unlikely that the CSPB protein and its fragment will reach absorptive cells of the intestinal mucosa. Proteins that are rapidly digestible in mammalian gastrointestinal systems are unlikely to be allergens when consumed. Finally, the CSPB protein represents no more than 0.00007% of the total protein in the grain of MON 87460.

e) CSPB and NPTII proteins do not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which have adverse effects to mammals. This has been shown by extensive assessments with bioinformatic tools, such as FASTA sequence alignment tool and an eight-amino acid sliding window search.

f) The safety of the NPTII protein and its donor organism, *E. coli*, have been recognized by regulatory agencies and well documented. All existing data suggest that the NPTII protein represents a negligible hazard to human health and is safe for consumption.

Using the guidance provided by the FDA, a conclusion of "no concern" is reached for the donor organisms and the CSPB and NPTII proteins. The food and feed products containing MON 87460 or made of MON 87460 are safe for human and animal consumption.

## VI.E. References

Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss and H. Schaller. 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon *Tn5*. Gene. 19:327-336.

Bienert, R., M. Zeeb, L. Dostál, A. Feske, C. Magg, K. Max, H. Welfle, J. Balbach and U. Heinemann. 2004. Single-stranded DNA bound to bacterial cold-shock proteins: preliminary crystallographic and Raman analysis. Acta Crystallographica Section D. 60:755-757.

DeBlock, M., L. Herrera-Estrella, M.V. Montagu, J. Schell and P. Zambryski. 1984. Expression of foreign genes in regenerated plants and in their progeny. EMBO Journal. 3:1681-1689.

FDA. 1999. Carbohydrase and protease enzyme preparations derived from *Bacillus subtilis* or *Bacillus amyloliquefaciens*; affirmation of GRAS status as direct food ingredients, Final Rule. Federal Register 64:19887-19895, U.S. Food and Drug Administration, Washington, D.C.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L. Hoffmann and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proceedings of the National Academy of Sciences USA. 80:4803-4807.

Graumann, P.L., T.M. Wendrich, M.H.W. Weber, K. Schroder and M.A. Marahiel. 1997. A family of cold shock proteins in *Bacillus subtilis* is essential for cellular growth and for efficient protein synthesis at optimal and low temperatures. Molecular Microbiology. 25:741-756.

Graumann, P.L. and M.A. Marahiel. 1999. Cold shock proteins CspB and CspC are major stationary-phase-induced proteins in *Bacillus subtilis*. Archives of Microbiology. 171:135-138.

Herschlag, D. 1995. RNA chaperones and the RNA folding problem. Journal of Biological Chemistry 270(36): 20871-20874.

Horsch, R.B., R.T. Fraley, S.G. Rogers, P.R. Sanders, A. Lloyd and N. Hoffmann. 1984. Inheritance of functional foreign genes in plants. Science. 223:496-498.

Karlson, D., K. Nakaminami, T. Toyomasu and R. Imai. 2002. A cold-regulated nucleic acid-binding protein of winter wheat shares a domain with bacterial cold shock proteins. Journal of Biological Chemistry. 277:35248-35256.

Kim, J.S., S.J. Park, K.J. Kwak, Y.O. Kim, J.Y. Kim, J. Song, B. Jang, C.-H. Jung and H. Kang. 2007. Cold shock domain proteins and glycine-rich RNA-binding proteins from *Arabidopsis thaliana* can promote the cold adaptation process in *Escherichia coli*. Nucleic Acids Research. 35:506-516.

Max, K.E.A., M. Zeeb, R. Bienert, J. Balbach and U. Heinemann. 2006. T-rich DNA single strands bind to a preformed site on the bacterial cold shock protein *Bs*-CspB. Journal of Molecular Biology. 360:702-714.

Max, K.E.A., M. Zeeb, R. Bienert, J. Balbach and U. Heinemann. 2007. Common mode of DNA binding to cold shock domains. Crystal structure of hexathymidine bound to the domain-swapped form of a major cold shock protein from *Bacillus aldolyticus*. FEBS Journal 274:1265-1279.

Newkirk, K., W. Feng, W. Jiang, R. Tejero, S.D. Emerson, M. Inouye and G.T. Montelione. 1994. Solution NMR structure of the major cold shock protein (CspA) from *Escherichia coli*: identification of a binding epitope for DNA. Proceedings of the National Academy of Sciences USA. 91:5114-5118.

Phadtare, S., and K. Severinov. 2005. Nucleic acid melting by *Escherichia coli* CspE. Nucleic Acids Research. 33:5583-5590.

Phadtare, S., S. Tyagi, M. Inouye and K. Severinov. 2002. Three amino acids in *Escherichia coli* CspE surface-exposed aromatic patch are critical for nucleic acid melting activity leading to transcription antitermination and cold acclimation of cells. Journal of Biological Chemistry. 277:46706-46711.

Rolfe, R.D. 2000. The role of probiotic cultures in the control of gastrointestinal health. Journal of Nutrition. 130:396S-402S.

Schindelin, H., M.A. Marahiel and U. Heinemann. 1993. Universal nucleic acid-binding domain revealed by crystal structure of the *B. subtilis* major cold-shock protein. Nature. 364:164-168.

Schindelin, H., W. Jiang, M. Inouye and U. Heinemann. 1994. Crystal structure of CspA, the major cold shock protein of *Escherichia coli*. Proceedings of the National Academy of Sciences USA. 91:5119-5123.

Schindler, T., P.L. Graumann, D. Perl, S.F. Ma, F.X. Schmid and M.A. Marahiel. 1999. The family of cold shock proteins of *Bacillus subtilis*: stability and dynamics *in vitro* and *in vivo*. Journal of Biological Chemistry. 274:3407-3413.

Schröder, K., P.L. Graumann, A. Schnuchel, T.A. Hlak and M.A. Marahiel. 1995. Mutational analysis of the putative nucleic acid-binding surface of the cold-shock domain, CspB, revealed an essential role of aromatic and basic residues in binding of single-stranded DNA containing the Y-box motif. Molecular Microbiology. 16:699-708.

## VII. Compositional Assessment

Compositional analyses were conducted to assess the nutrient, anti-nutrient and key secondary metabolite levels in the forage and grain tissues derived from MON 87460. Because MON 87460 reduces yield loss under water-limited conditions, field studies were designed to evaluate the composition of MON 87460 across a broad range of soil moisture and environmental conditions relevant to where commercial production would be expected. In each assessment, MON 87460 was compared to an appropriate conventional control, which had a genetic background similar to MON 87460 but did not possess the drought tolerance trait. In addition, multiple conventional corn hybrids (references) were included in the analysis to establish a range of natural variability for each analyte, where the range of variability is defined by a 99% tolerance interval for that particular analyte. Results of the comparisons indicate that MON 87460 is compositionally and nutritionally equivalent to conventional corn hybrids that have a history of safe consumption that are currently in commerce.

The compositional assessment was conducted on forage and grain samples harvested from two different growing seasons using two different water management regimes. The first season was conducted in the U.S. during 2006 under typical agronomic practices and water conditions (Section VII.A). The second season was conducted in Chile during 2006/2007 using a strip-plot design to establish two water treatment levels (well-watered and water-limited) to assess for any changes in compositional equivalence under different soil moisture conditions (Section VII.B). Results from these assessments were published by Harrigan et al. (2009). Samples from the Chile 2006/2007 study were also analyzed for 11 additional secondary metabolites that are potentially associated with drought stress (Section VII.C). Square brackets in the tables presented in this section denote ranges for the test and control or 99% tolerance intervals for the reference materials. Materials and methods for the compositional analyses are provided in Appendix E.

The commercial reference hybrids selected for each study were adapted to the geographic region in which they were grown with selections based on agronomic characteristics such as relative maturity and drought tolerance ratings. Relative maturity was an important consideration in order to ensure that the test, control and reference materials would be at comparable stages of development at each data or sample collection time point. Drought tolerance ratings typically spanned a range of ratings exhibited by conventional hybrids.

### VII.A. U.S. 2006 Composition Study under Typical Agronomic Conditions

Forage and grain tissues of MON 87460 and control corn were harvested from plants grown under typical agronomic practices with three replicates at each of six field sites in the U.S. within corn production regions. Two sites were in Iowa (IAE, IAW), one each was in Illinois (IL), Indiana (IN), Kansas (KS) and Nebraska (NE). Each site received water as is typical of the growing area. Four sites were rainfed (IAE, IAW, IL, IN) and two (KS, NE) received supplemental irrigation. These conditions provide a comparison of MON 87460 and the control under conditions common to corn production. Table VII-1 presents temperature data and applied water from the production period. Three different conventional commercial corn hybrids were also grown at each of the six sites.

This allowed harvest of forage and grain from a total of 18 commercial references to provide information on natural variation in the levels of analyzed nutrients and anti-nutrients. Compositional analysis included the significant nutrients, anti-nutrients, and key secondary metabolites, consistent with OECD guidelines (OECD, 2002).

The experiment was arranged in a randomized complete block design with three replicates per block. Tissue was collected from MON 87460 and the control from all three blocks; tissue from the three different commercial references grown at each site was collected from a single block. Forage was collected at the early dent (R5) plant growth stage, and grain was collected at physiological maturity.

The compositional data set was examined for evidence of statistically significant differences between MON 87460 and the control. Seven sets of statistical analyses were made, six based on the data from each of the replicated field sites and the seventh based on data from a combination of all six field sites. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods.

Commercial references were included to provide data for the development of a 99% tolerance interval for each component analyzed. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial corn. The tolerance interval illustrates the compositional variability that currently occurs in corn grown commercially. It allows statistically significant differences between MON 87460 and the control to be placed in biological perspective. This comparative evaluation also considers natural ranges in corn component levels published in the literature or in the International Life Sciences Institute (ILSI) Crop Composition Database (http://www.cropcomposition.org).

### VII.A.1. Assessment of Nutrients, Anti-Nutrients, and Key Secondary Metabolites

Forage and grain samples were harvested from all plots and analyzed for nutritional and anti-nutrient components. Compositional analyses of the forage samples included measurement of proximates (moisture, fat, protein, ash), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium, and phosphorus. Compositional analyses of the grain samples included measurement of proximates (moisture, fat, protein, ash), carbohydrates by calculation, ADF, NDF, total dietary fiber (TDF), total amino acid composition, fatty acid composition (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc), vitamins (vitamin B1 [thiamine], vitamin B2 [riboflavin], vitamin B6 [pyridoxine], vitamin E, niacin, folic acid), anti-nutrients (phytic acid, raffinose), and secondary metabolites (ferulic acid, furfural, *p*-coumaric acid). Methods for analysis were based on internationally-recognized procedures and literature publications (Appendix E).

In total, 77 different analytical components were measured (9 in forage, 68 in grain). Of these evaluated components, 15 had more than 50% of the observations below the assay limit of quantitation (LOQ). Components with more than 50% of observations below the assay LOQ were excluded from statistical analysis. These included 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0

pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, sodium, and furfural. These components are known to be present at low levels in corn grain (OECD, 2002). Therefore, 62 components (9 in forage and 53 in grain) were statistically assessed using a mixed model analysis of variance method.

Statistical evaluation of the composition data involved a comparison of the forage and grain from MON 87460 to those of the control. There were a total of 434 comparisons made (seven sets of comparisons × 53 components from grain and seven sets of comparisons × nine components from forage).

A summary of significant differences ($p<0.05$) between test and control in both the combined-site and individual site (total of 6 sites) analyses is presented in Table VII-2. Mean values, ranges, standard error values and statistical analyses for the combined-site data are presented in Appendix E (Tables E-5 through E-11). The standard error values presented in Appendix E are calculated from the ANOVA. Thus, each test and control mean for a given analyte mean has the same standard error. Literature and ILSI Crop Composition Database ranges for corn components are presented in Table E-30.

The combined-site analysis show that there were no statistically significant differences ($p>0.05$) for 59 (95.2%) of the 62 comparisons between the mean component values of MON 87460 and the control. Of the three significant differences, mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. The individual site analysis show that there were no statistically significant differences ($p>0.05$) for 348 (93.5%) of the 372 comparisons. Individual site differences were not reproducible across multiple sites and, where differences were observed, mean component values of the test and control substances were within the 99% tolerance interval (Table VII-2). Therefore, these differences were within the natural variability of corn for these components.

### VII.A.2. Levels of Nutrients

Corn forage and grain contain a variety of key nutrients that provide much of this crop's value as a food and feed. The OECD consensus document on compositional considerations for corn describes the nutrients present in corn grain or processed corn products and includes proximates, fiber, minerals, total amino acids, fatty acids (FA) and vitamins (OECD, 2002). A comprehensive comparison of MON 87460 and the control confirms that the two materials are compositionally equivalent with respect to nutrients.

In the combined-site analysis of forage, no significant differences were found between MON 87460 and the control. In the combined-site analysis of grain, 50 of the 53 comparisons were not significantly different ($p>0.05$). The three differences were detected in the values for ash, 18:0 stearic acid and 20:1 eicosenoic acid (Table VII-2). However, values for 18:0 stearic acid were not significantly different ($p>0.05$) at any of the individual sites. The magnitude of the combined-site difference in the 18:0 stearic acid value was small (0.069% of total FA) and the mean component values for the test

and control substances were within the 99% tolerance interval established from the commercial reference hybrids grown at the same sites. Only one out of the six individual site comparisons for ash and 20:1 eicosenoic acid values showed a significant difference ($p<0.05$). The magnitude of the differences in ash (0.082 % DW) and 20:1 eicosenoic acid (0.0078% of total FA) was extremely small, and the mean values for these two components were within the 99% tolerance interval established from the commercial references grown at the same sites. Furthermore, this lack of reproducibility across multiple sites established that the differences observed in the combined-site analysis in values for these two components were not biologically meaningful. These findings confirmed that these minor differences reflected the natural variability of conventional corn.

In the individual-site analysis of forage, 51 of the 54 comparisons were not significantly different ($p>0.05$). The three differences were in the values for carbohydrates by calculation, moisture, and protein, with each component difference being observed at only a single site. This lack of reproducibility across multiple sites indicated that there were no meaningful trends in differences in the values for these three components and that this limited number of differences constituted no biological significance. For grain nutrients, individual site differences in components not recorded in the combined-site analysis included values for moisture (two sites), and cystine, histidine, lysine, methionine, valine, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 22:0 behenic acid, ADF, TDF, thiamine, folic acid, and riboflavin (each at a single site only). The lack of reproducibility in differences across multiple sites and the fact that the mean values for these components at these sites were within the 99% tolerance interval established from the commercial references confirmed that the limited number of site differences in values for these components were not biologically meaningful.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of nutrient components from MON 87460 and the control. The limited number of differences observed in this study reflected the natural variation of conventional corn and supported the compositional equivalence of MON 87460 to conventional corn.

### VII.A.3. Levels of Key Anti-Nutrients and Secondary Metabolites

The OECD consensus document on compositional considerations for corn describes the anti-nutrients and secondary metabolites present in corn grain or processed corn products (OECD, 2002). The anti-nutrients assessed included phytic acid and raffinose. Phytic acid is widely distributed in plants and can limit the uptake of minerals such as calcium in higher animals (Lott et al., 2000; Novak and Haslberger, 2000). Raffinose is a nondigestible oligosaccharide that is considered to be an anti-nutrient due to gas production and the resulting flatulence caused by its consumption (Voragen, 1998). The secondary metabolites included ferulic acid, *p*-coumaric acid, and furfural. Ferulic acid and *p*-coumaric acid are derived from the aromatic amino acids, phenylalanine and tyrosine (Douglas, 1999), and serve as precursors for a large group of phenylpropanoid compounds. The non-starch polysaccharide pentosans are a major source of furfural (Adams et al., 1997).

No combined-site differences ($p>0.05$) between values for grain anti-nutrient components and secondary metabolites in MON 87460 and the control were recorded. Individual site differences ($p<0.05$) were observed in values for raffinose, phytic acid, and ferulic acid. For each component, these differences were observed at a single site only. As only one out of six individual site comparisons recorded a significant difference for each of these components, these differences represented no meaningful trend and were considered to be not biologically meaningful. The limited number of differences recorded in this study reflected the natural variation of conventional corn.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of anti-nutrient components and secondary metabolites in MON 87460 and the control. Thus, a comprehensive evaluation of anti-nutrient components and key secondary metabolites supported the compositional equivalence of MON 87460 to conventional corn.

### VII.A.4. Conclusions for U.S. 2006 under Typical Agronomic Practices

The overall dataset was evaluated for evidence of biologically relevant changes. The combined-site analysis show that there were no statistically significant differences ($p>0.05$) for 59 (95.2%) of the 62 comparisons between the mean component values of MON 87460 and the control. Of the three significant differences observed, mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. The individual site analysis show that there were no statistically significant differences ($p>0.05$) for 348 (93.5%) of the 372 comparisons. Individual site differences were not reproducible across multiple sites and, where differences were observed, mean component values test and control substances were within the 99% tolerance interval. Furthermore, the limited number of component differences was characterized by small differences in magnitude.

These factors established that the limited number of differences observed in this study were within the natural variability of corn for these components, reflected no meaningful trends, and were of no biological significance.

Therefore, the corn grain and forage derived from MON 87460, and consequently the foods and feeds derived from MON 87460, can be considered compositionally equivalent to those derived from conventional corn with a history of safe consumption.

**Table VII-1. Monthly Temperature and Accumulated Water Data for the U.S. 2006 Study**

| Site[1] | Measurement | May | June | July | August | September | October |
|---|---|---|---|---|---|---|---|
| **IAE** | Accumulated water (in.) | 2.5 | 2.3 | 2.3 | 6.2 | 2.4 | 2.1 |
| | Avg Max temp (°F) | 72 | 81 | 86 | 86 | 76 | 59 |
| | Avg Min temp (°F) | 51 | 60 | 65 | 62 | 49 | 37 |
| | Range[2] (°F) | 30 - 98 | 45 - 97 | 54 - 95 | 55 - 96 | 32 - 94 | 19 - 94 |
| **IAW** | Accumulated water (in.) | 1.8 | 0.4 | 2.8 | 4.9 | 8.7 | 1.5 |
| | Avg Max temp (°F) | 72 | 84 | 88 | 83 | 71 | 60 |
| | Avg Min temp (°F) | 50 | 59 | 63 | 66 | 48 | 38 |
| | Range[2] (°F) | 34 - 91 | 49 - 93 | 52 - 99 | 58 - 93 | 34 - 86 | 23 - 90 |
| **IL** | Accumulated water (in.) | 2.1 | 1.5 | 3.7 | 3.1 | 2.8 | 2.7 |
| | Avg Max temp (°F) | 72 | 81 | 88 | 82 | 75 | 60 |
| | Avg Min temp (°F) | 51 | 57 | 64 | 62 | 48 | 36 |
| | Range[2] (°F) | 36 - 94 | 46 - 92 | 46 - 97 | 51 - 96 | 34 - 88 | 19 - 92 |
| **IN** | Accumulated water (in.) | 4.8 | 5.5 | 3.4 | 5.6 | 2.4 | 5.9 |
| | Avg Max temp (°F) | 71 | 80 | 86 | 83 | 75 | 63 |
| | Avg Min temp (°F) | 51 | 58 | 65 | 63 | 53 | 41 |
| | Range[2] (°F) | 40 - 92 | 52 - 89 | 50 - 96 | 55 - 93 | 37 - 87 | 25 - 90 |
| **KS** | Accumulated water (in.) | 5.3 | 8.7 | 8 | 10.9 | 2 | 1.9 |
| | Avg Max temp (°F) | 81 | 92 | 96 | 92 | 80 | 68 |
| | Avg Min temp (°F) | 53 | 62 | 66 | 67 | 51 | 42 |
| | Range[2] (°F) | 37 - 100 | 52 - 102 | 54 - 109 | 52 - 107 | 36 - 92 | 23 - 96 |
| **NE** | Accumulated water (in.) | 1.9 | 3.8 | 5.6 | 9.5 | 4.8 | 0.8 |
| | Avg Max temp (°F) | 79 | 88 | 91 | 86 | 76 | 63 |
| | Avg Min temp (°F) | 51 | 61 | 65 | 63 | 50 | 37 |
| | Range[2] (°F) | 37 - 96 | 52 - 101 | 55 - 103 | 52 - 100 | 36 - 93 | 18 - 96 |

[1] Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; KS = Pawnee County, KS; NE = York County, NE.

[2] The range is the absolute maximum and minimum temperature in each month.

**Table VII-2. Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control in the U.S. during 2006 under Typical Agronomic Conditions**

| Tissue/Site/ Components (Units)[1] | Mean MON 87460 | Mean Control | Mean Diff (% of Control) | Signif. (p-value) | MON 87460 (Range) | 99% Tolerance Interval[2] |
|---|---|---|---|---|---|---|
| **Forage** | | | | | | |
| **IL** | | | | | | |
| Carbohydrates (% DW) | 87.31 | 85.75 | 1.81 | 0.007 | (86.23 - 88.01) | [82.09, 90.80] |
| **IN** | | | | | | |
| Moisture (% FW) | 66.23 | 67.80 | -2.31 | 0.008 | (64.70 - 67.40) | [59.32, 81.14] |
| Protein (% DW) | 6.75 | 7.23 | -6.60 | 0.031 | (6.68 - 6.90) | [4.92, 10.30] |
| **Grain** | | | | | | |
| **Combination of all sites** | | | | | | |
| Ash (% DW) | 1.54 | 1.46 | 5.60 | 0.041 | (1.33 - 1.83) | [0.55, 2.30] |
| 18:0 Stearic (% Total FA) | 2.05 | 1.98 | 3.50 | 0.024 | (1.88 - 2.34) | [1.00, 2.51] |
| 20:1 Eicosenoic (% Total FA) | 0.18 | 0.19 | -4.05 | 0.007 | (0.17 - 0.19) | [0.15, 0.33] |
| **IAE** | | | | | | |
| Moisture (% FW) | 9.78 | 9.22 | 6.08 | 0.049 | (9.57 - 10.00) | [7.58, 12.13] |
| Histidine (% DW) | 0.31 | 0.32 | -2.68 | 0.032 | (0.30 - 0.32) | [0.20, 0.36] |
| Methionine (% DW) | 0.21 | 0.20 | 2.45 | 0.035 | (0.20 - 0.21) | [0.14, 0.28] |
| Valine (% DW) | 0.51 | 0.53 | -3.71 | 0.028 | (0.48 - 0.52) | [0.33, 0.62] |
| 18:3 Linolenic (% Total FA) | 1.22 | 1.25 | -2.45 | 0.040 | (1.17 - 1.25) | [0.39, 1.85] |
| Total Dietary Fiber (% DW) | 12.23 | 11.60 | 5.40 | 0.029 | (11.94 - 12.52) | [8.11, 17.95] |
| Raffinose (% DW) | 0.20 | 0.15 | 27.28 | 0.009 | (0.19 - 0.20) | [0.039, 0.26] |
| **IAW** | | | | | | |
| 22:0 Behenic (% Total FA) | 0.24 | 0.21 | 12.99 | 0.015 | (0.20 - 0.25) | [0, 0.37] |
| Thiamine HCl (mg/kg DW) | 2.85 | 2.48 | 14.81 | 0.011 | (2.77 – 2.9) | [1.84, 4.94] |
| Ferulic Acid (µg/g DW) | 1753.10 | 1847.90 | -5.13 | 0.003 | (1661.13 - 1914.78) | [395.96, 3485.38] |

Table VII-2 continues on the next page.

**Table VII-2 (cont.). Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control in the U.S. during 2006 under Typical Agronomic Conditions**

| Tissue/Site/ Components (Units)[1] | Mean MON 87460 | Mean Control | Mean Diff (% of Control) | Signif. (p-value) | MON 87460 (Range) | 99% Tolerance Interval[2] |
|---|---|---|---|---|---|---|
| **IL** | | | | | | |
| Moisture (% FW) | 9.94 | 10.43 | -4.73 | 0.013 | (9.71 - 10.30) | [7.58, 12.13] |
| Folic Acid (mg/kg DW) | 0.28 | 0.27 | 4.19 | 0.033 | (0.27 - 0.29) | [0.13, 0.38] |
| **IN** | | | | | | |
| Cystine (% DW) | 0.19 | 0.20 | -5.55 | 0.039 | (0.19 - 0.20) | [0.15, 0.27] |
| 18:1 Oleic (% Total FA) | 20.05 | 20.63 | -2.83 | 0.025 | (19.96 - 20.13) | [11.92, 39.78] |
| 18:2 Linoleic (% Total FA) | 63.81 | 63.03 | 1.24 | 0.026 | (63.67 - 63.95) | [45.91, 72.47] |
| Acid Detergent Fiber (% DW) | 2.55 | 3.63 | -29.68 | 0.036 | (2.52 - 2.60) | [0.62, 5.72] |
| Riboflavin/Vitamin B2 (mg/kg DW) | 1.52 | 1.09 | 39.64 | 0.039 | (1.45 - 1.57) | [0.047, 2.91] |
| Phytic Acid (% DW) | 0.63 | 0.77 | -17.45 | 0.007 | (0.60 - 0.66) | [0.50, 1.11] |
| **KS** | | | | | | |
| Lysine (% DW) | 0.33 | 0.31 | 4.51 | 0.045 | (0.31 - 0.34) | [0.22, 0.36] |
| 20:1 Eicosenoic (% Total FA) | 0.18 | 0.19 | -7.81 | 0.043 | (0.17 - 0.18) | [0.15, 0.33] |
| **NE** | | | | | | |
| Ash (% DW) | 1.57 | 1.38 | 14.01 | 0.007 | (1.49 - 1.62) | [0.55, 2.30] |

[1]DW= dry weight; FW=fresh weight, FA = fatty acid.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.
Tables E-5 through E-11 in Appendix E present the full results of the combined site analysis for the compositional analyses from the U.S. 2006 production including means, standard error values and p-values.

## VII.B. Chile 2006/2007 Composition Study under Well-Watered and Water-Limited Conditions

Forage and grain samples of MON 87460 and its conventional control were harvested from plants grown in three replicates at each of four field sites in Chile in 2006 - 2007. These field sites were located in commercial corn production regions of Chile. The sites were Calera de Tango (CT), Colina (CL), Lumbreras (LUM) and Quillota (QUI). These sites are well-suited for corn production, but as they typically do not receive any rainfall during the growing season, all water received at each site occurred through controlled irrigation. At each site, a strip-plot design was used allowing comparisons of the test and control substances under two separate irrigation regimens, well-watered and water-limited. The well-watered treatment was managed to provide optimal grain yield. The water-limited treatment was managed to impose a drought stress by withholding irrigation during approximately the V10 – R2 growth stages, which represents the growth stages when corn grain yield potential is most susceptible to drought stress (Campos et al., 2006). Table VII-3 presents temperature data and applied water from the production period. In addition to MON 87460 and its conventional control, four different conventional commercial corn hybrids were also grown at each site. This allowed harvest of forage and grain from a total of 16 commercial references from each water treatment to provide information on natural variation in the levels of analyzed nutrient and anti-nutrients under well-watered and water-limited conditions. Compositional analysis included components consistent with OECD guidelines.

The experiment was arranged in a strip-plot design with three replicates per site, with irrigation treatment (well-watered or water-limited) as the whole plot and substance type as the sub-plot. The whole plot factor was arranged as a randomized complete block design. The strip-plot factor consisted of the test, control, and reference substances.

Tissue was collected from MON 87460 and the control from all three blocks for each treatment; tissue from the four different commercial references grown at each site was collected from a single block for each treatment. Forage was collected at the early dent (R5) plant growth stage; grain was collected at physiological maturity.

Within each treatment, the composition of forage and grain of MON 87460 was compared to that of the conventional control across sites (combined-site analysis) and within site (individual site analysis). Results from the Chile 2006/2007 study represent combined-site data from three (CL, CT, LUM) of the four sites. For a site to be included in the combined-site analysis, commercial reference hybrids in the water-limited plots had to exhibit a minimum 15% reduction in yield compared to the same reference hybrids planted in the well-watered plots. A detailed description of the site inclusion criteria applied to these data is provided in Section VIII.B.2. As described in Section VIII.C and Table VIII-3, the QUI site in the Chile 2006/2007 study was not established with the appropriate water stress treatments; therefore, data for the QUI site are presented in Appendix E, Section E-8.

Statistical comparisons between MON 87460 and the control were performed within each irrigation treatment. A range of component values and a statistical population were determined for the commercial references within each irrigation treatment. Thus, four sets of statistical analyses were made for each treatment, three based on the data from each of the

replicated field sites and the fourth based on data from the combined sites. Statistically significant differences were determined at the 5% level of significance ($\alpha = 0.05$) using established statistical methods.

Commercial references were included to provide data for the development of a 99% tolerance interval for each component analyzed. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial corn. The tolerance interval illustrates the compositional variability that occurs in corn currently grown commercially. It allows statistically significant differences between MON 87460 and the control to be placed in biological perspective. This comparative evaluation can also consider natural ranges in corn component levels published in the literature or in the International Life Sciences Institute (ILSI) Crop Composition Database (http://www.cropcomposition.org).

### VII.B.1. Assessment of Nutrients, Anti-Nutrients, and Key Secondary Metabolites under Well-Watered Conditions

The well-watered plots provide a compositional comparison between MON 87460 and a conventional control grown under conditions that are optimal for corn growth and development. Results confirm that MON 87460 and the control are compositionally equivalent when produced under well-watered conditions.

Forage and grain samples were harvested from all well-watered plots and analyzed for nutritional and anti-nutrient components as described in Section VII.A.1. In total, 77 different analytical components were measured (nine in forage, 68 in grain). Of these evaluated components, 16 had more than 50% of the observations below the assay limit of quantitation (LOQ). Components with more than 50% of observations below the assay LOQ were excluded from statistical analysis. These included 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, sodium, and furfural. These components are known to be present at low levels in corn grain (OECD, 2002). Therefore, 61 components (9 in forage, 52 in grain) were statistically assessed using a mixed model analysis of variance method.

Statistical evaluation of the composition data involved a comparison of the forage and grain from MON 87460 to those of the control. There were a total of 244 comparisons made (four sets of comparisons × 52 components from grain and four sets of comparisons × nine components from forage).

A summary of significant differences ($p<0.05$) between test and control in both the combined-site and individual site (total of 3 sites) analyses for the well-watered treatment is presented in Table VII-4. Mean values, ranges, standard error values and statistical analyses for the combined-site data are presented in Appendix E (Tables E-12 through E-18). The standard error values presented in Appendix E are calculated from the ANOVA. Thus, each test and control mean for a given analyte mean has the same standard error. Literature and ILSI Crop Composition Database ranges for corn components are presented in Table E-30.

The combined-site analysis show that there were no statistically significant differences ($p>0.05$) for 59 (96.7%) of the 61 comparisons between the mean component values of MON 87460 and the control. Of the two significant differences observed, mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. The individual site analysis show that there were no statistically significant differences ($p>0.05$) for 171 (93.4%) of the 183 comparisons. Individual site differences were not reproducible across multiple sites and, where differences were observed, mean component values of the test and control substances were within the 99% tolerance interval (Table VII-4). Therefore, these differences were within the natural variability of corn for these components.

### VII.B.2. Levels of Nutrients under Well-Watered Conditions

A description of nutrients present in corn grain is provided in the OECD consensus document on compositional considerations for corn (OECD, 2002). A comparative assessment of levels of proximates, fiber, minerals, total amino acids, fatty acids, and vitamins follows.

In the combined-site analysis of forage, no significant differences were found between MON 87460 and the control. In the combined-site analysis of grain, 50 of the 52 comparisons were not significantly different ($p>0.05$). Differences included values for total fat and magnesium. Individual site comparisons between values for total fat and magnesium in MON 87460 and the control grain show a significant difference ($p<0.05$) only at a single site. This lack of reproducibility across multiple sites established that the differences observed in the combined-site analysis in values for these two components were of no biological significance. The magnitude of the differences in total fat (0.17% DW) and magnesium (0.01% of DW) were extremely small, and the mean values for these two components were within the 99% tolerance interval established from the commercial references grown at the same sites. These findings confirmed that these minor differences reflected the natural variability of conventional corn.

In the individual-site analysis of forage, 23 of the 27 comparisons were not significantly different ($p>0.05$). Differences included values for carbohydrates by calculation, moisture, ADF, and calcium, with each component difference being observed at only a single site. This lack of reproducibility across all sites established that there were no meaningful trends in values for these components and that this limited number of differences constituted no biological significance. For grain nutrients, individual site differences in components not recorded in the combined-site analysis included values for serine, threonine, 18:0 stearic acid, 18:2 linoleic acid, 18:3 linolenic acid, and vitamin E. For each component, these differences were observed at a single site only. This lack of reproducibility in observing these differences across multiple sites, and the fact that the mean values for these components at these sites were within the 99% tolerance interval established from the commercial references, support a conclusion that the limited number of site differences in values for these components were of no biological significance.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of nutrient components from MON 87460 and the control. The limited number of

differences recorded in this study reflected the natural variation of corn and supported the compositional equivalence of MON 87460 and conventional corn.

### VII.B.3. Levels of Anti-Nutrients and Key Secondary Metabolites under Well-Watered Conditions

A description of the anti-nutrients and secondary metabolites present in corn grain is provided in the OECD consensus document on compositional considerations for corn (OECD, 2002). The anti-nutrients and key secondary metabolites analyzed in this study are the same as those listed in Section VII.A.3**.**

The statistical analysis highlighted no differences within or across sites in the levels of anti-nutrient components and secondary metabolites in MON 87460 and the control. Thus, a comprehensive evaluation of anti-nutrient components and key secondary metabolites supported the compositional equivalence of MON 87460 and conventional corn.

### VII.B.4. Conclusions for Chile 2006/2007 Well-Watered Conditions

The overall dataset was evaluated for evidence of biologically relevant changes. The combined-site analysis show that there were no statistically significant differences ($p>0.05$) for 59 (96.7%) of the 61 comparisons between the mean component values of MON 87460 and the control. Of the two significant differences observed, mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. The individual site analysis show that there were no statistically significant differences ($p>0.05$) for 171 (93.4%) of the 183 comparisons. Individual site differences were not reproducible across multiple sites and, where differences were observed, mean component values test and control substances were within the 99% tolerance interval. Furthermore, the limited number of component differences was characterized by small differences in magnitude

These factors established that the limited number of differences observed in this study were within the natural variability of corn for these components, reflected no meaningful trends, and were of no biological significance.

Therefore, the corn grain and forage derived from MON 87460 grown under well-watered conditions, and consequently the foods and feeds derived from MON 87460, can be considered compositionally equivalent to those derived from conventional corn grown under the same conditions. MON 87460 grown under well-watered conditions is as safe as conventional corn with a history of safe consumption.

**Table VII-3. Monthly Temperature and Accumulated Water Data for the Chile 2006/2007 Study**

| Site[1] | Measurement | December | January | February | March | April | May |
|---|---|---|---|---|---|---|---|
| **CL** | Accumulated water (in.), well-watered | 0.9 | 10.3 | 8.5 | 9.4 | 2.8 | 0.0 |
| | Accumulated water (in.), water-limited[2, 3] | 0.9 | 10.3 | 4.7 | 5.6 | 2.8 | 0.0 |
| | Avg Max temp (°F) | NA[5] | 88 | 85 | 82 | 74 | 67 |
| | Avg Min temp (°F) | NA[5] | 53 | 49 | 47 | 41 | 33 |
| | Range[4] (°F) | NA[5] | 46 - 97 | 42 - 94 | 41 - 94 | 33 - 89 | 26 - 78 |
| **CT** | Accumulated water (in.), well-watered | 2.8 | 9.4 | 8.5 | 10.3 | 2.8 | 0.0 |
| | Accumulated water (in.), water-limited[2, 3] | 3.8 | 9.4 | 2.8 | 6.6 | 2.8 | 0.0 |
| | Avg Max temp (°F) | NA[5] | 84 | 79 | 79 | 71 | 66 |
| | Avg Min temp (°F) | NA[5] | 52 | 50 | 50 | 41 | 37 |
| | Range[4] (°F) | NA[5] | 46 - 91 | 42 - 90 | 42 - 88 | 31 - 87 | 29 – 77 |
| **LUM** | Accumulated water (in.), well-watered | 2.8 | 9.4 | 8.5 | 10.3 | 1.9 | 0.0 |
| | Accumulated water (in.), water-limited[2, 3] | 2.8 | 9.4 | 2.8 | 6.6 | 2.8 | 0.0 |
| | Avg Max temp (°F) | NA[5] | 81 | 78 | 79 | 73 | 67 |
| | Avg Min temp (°F) | NA[5] | 52 | 50 | 49 | 42 | 37 |
| | Range[4] (°F) | NA[5] | 47 - 89 | 42 - 89 | 42 - 94 | 32 - 87 | 28 - 77 |

[1] Site codes are as follows: CL = Colina; CT = Calera de Tango; LUM = Lumbreras.
[2]Water limitation began at the V10 growth stage which occurred at approximately February 7.
[3]Water limitation ended at the R2 growth stage which occurred at approximately March 13.
[4]The range is the absolute maximum and minimum temperature in each month.
[5]Temperature data are available from January 6 through May 25; planting occurred in late December and early January. Rainfall did not occur during the production period.

**Table VII-4. Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control in Chile during 2006/2007 under Well-Watered Conditions**

| Tissue/Site/ Components (Units)[1] | Mean MON 87460 | Mean Control | Mean Diff (% of Control) | Signif. (p-value) | MON 87460 (Range) | 99% Tolerance Interval[2] |
|---|---|---|---|---|---|---|
| **Forage** | | | | | | |
| **CT** | | | | | | |
| Moisture (% FW) | 72.53 | 76.10 | -4.69 | <0.001 | 70.90 - 75.00 | [69.22, 81.25] |
| Carbohydrates (% DW) | 87.22 | 86.14 | 1.26 | 0.048 | 86.98 - 87.46 | [82.51, 92.09] |
| Acid Detergent Fiber (% DW) | 22.84 | 29.47 | -22.51 | 0.002 | 17.95 - 31.28 | [16.01, 45.98] |
| Calcium (% DW) | 0.26 | 0.32 | -18.99 | 0.047 | 0.25 - 0.28 | [0.043, 0.46] |
| **Grain** | | | | | | |
| **Combination of all sites** | | | | | | |
| Total Fat (% DW) | 3.89 | 3.72 | 4.52 | 0.029 | 3.45 - 4.23 | [2.47, 4.68] |
| Magnesium (% DW) | 0.12 | 0.11 | 8.64 | 0.012 | 0.10 - 0.14 | [0.064, 0.16] |
| **CL** | | | | | | |
| Vitamin E (mg/kg DW) | 12.46 | 10.50 | 18.66 | 0.002 | 12.37 - 12.51 | [0, 19.32] |
| **CT** | | | | | | |
| 18:2 Linoleic (% Total FA) | 64.64 | 63.82 | 1.28 | 0.048 | 64.11 - 65.10 | [49.61, 73.18] |
| **LUM** | | | | | | |
| Total Fat (% DW) | 3.96 | 3.61 | 9.54 | 0.010 | 3.80 - 4.23 | [2.47, 4.68] |
| Magnesium (% DW) | 0.13 | 0.11 | 15.54 | 0.022 | 0.11 - 0.14 | [0.064, 0.16] |
| Serine (% DW) | 0.50 | 0.44 | 13.33 | 0.024 | 0.47 - 0.54 | [0.32, 0.65] |
| Threonine (% DW) | 0.35 | 0.32 | 10.08 | 0.047 | 0.33 - 0.37 | [0.23, 0.42] |
| 18:0 Stearic (% Total FA) | 1.83 | 1.71 | 6.83 | 0.024 | 1.74 - 1.97 | [0.60, 2.58] |
| 18:3 Linolenic (% Total FA) | 1.20 | 1.23 | -2.41 | 0.042 | 1.17 - 1.23 | [0.72, 1.66] |

[1]DW = dry weight; FW = fresh weight; FA = fatty acid.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.
Tables E-12 through E-18 in Appendix E present the full results of the combined site analysis for the compositional analyses from the Chile 2006/2007 production including means, standard error values and p-values.

### VII.B.5. Assessment of Nutrients, Anti-Nutrients, and Key Secondary Metabolites under Water-Limited Conditions

The water-limited treatment allowed a compositional comparison between MON 87460 and the conventional control with similar background genetics but lacking the introduced trait. Both were grown under conditions intended to impose drought stress by withholding irrigation during approximately the V10 – R2 growth stages, which represents the growth stages when corn grain yield potential is most susceptible to drought stress. Irrigation management in this treatment was intended to provide well-watered conditions before and after the V10 - R2 growth stages.

Forage and grain samples were harvested from all water-limited plots and analyzed for the same nutritional and anti-nutrient components assessed in the evaluation of samples from the well-watered plots. A summary of significant differences ($p<0.05$) between test and control in both the combined-site and individual site (total of 3 sites) analyses for the water-limited treatment is presented in Table VII-5. Mean values, ranges, standard error values, and statistical analyses for the combined-site data are presented in Appendix E (Tables E-19 through E-25). Literature and ILSI Crop Composition Database ranges for corn components are presented in Table E-30.

The combined-site analysis show that there were no statistically significant differences ($p>0.05$) for 59 (96.7%) of the 61 comparisons between the mean component values of MON 87460 and the control. Of the two significant differences observed, mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. The individual site analysis show that there were no statistically significant differences ($p>0.05$) for 174 (95.1%) of the 183 comparisons. Individual site differences were not reproducible across multiple sites and, where differences were observed, mean component values of the test and control substances were within the 99% tolerance interval (Table VII-5). Therefore, these differences were within the natural variability of corn for these components.

### VII.B.6. Levels of Nutrients under Water-Limited Conditions

In the combined-site analysis of forage, eight of the nine comparisons between MON 87460 and the control were not significantly different ($p>0.05$). The single difference was in total fat values. However, the mean values for total fat in the test and control substances were within the 99% tolerance interval established from the commercial reference hybrids grown at the same sites, indicating that the difference was within the natural variability of corn for this component. Values for total fat were not observed to be significantly different ($p>0.05$) at any of the individual sites. In the combined-site analysis of grain, 51 of the 52 comparisons were not significantly different ($p>0.05$). The single difference was in 20:1 eicosenoic acid values. However, the mean values for 20:1 eicosenoic acid in the test and control substances were within the 99% tolerance interval established from the commercial references grown at the same sites, indicating that the difference was within the natural variability of corn for this component. Values for 20:1 eicosenoic acid were observed to be significantly different ($p<0.05$) at only one of the individual sites.

For forage, 26 of the 27 individual site comparisons were not significantly different ($p>0.05$). Individual site differences included only a single value for moisture. This lack of reproducibility across multiple sites established that there are no meaningful trends in differences in values for this component and that the limited number of differences constituted no biological significance. For grain nutrients, individual site differences in components not recorded in the combined-site analysis included values for iron, phosphorus, 18:1 oleic acid, 22:0 behenic acid, folic acid, vitamin E, and phytic acid. For each component, these differences were observed at a single site only. The fact that the mean values for these components at multiple sites were within the 99% tolerance interval established from the commercial references and the lack of reproducibility in differences across these sites confirmed that the limited number of site differences in values for these components were within the natural variability of corn for these components.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of nutrient components from MON 87460 and the control. Those limited number of differences observed in this study reflected the natural variation of conventional corn and supported the compositional equivalence of MON 87460 and conventional corn.

### VII.B.7. Levels of Anti-Nutrients and Key Secondary Metabolites under Water-Limited Conditions

No combined-site differences between values for grain anti-nutrient components (phytic acid and raffinose) and secondary metabolites (*p*-coumaric acid and ferulic acid) in MON 87460 and the control were recorded. Individual site differences were observed for a single value for phytic acid. As only one out of six individual site comparisons was recorded for this component, this difference represented no meaningful trend and reflected the natural variation observed with conventional corn.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of anti-nutrient components and secondary metabolites in MON 87460 and the conventional control. Thus, a comprehensive evaluation of anti-nutrient components and key secondary metabolites supported the compositional equivalence of MON 87460 and conventional corn.

### VII.B.8. Conclusions for Chile 2006/2007 Water-Limited Conditions

The overall dataset was evaluated for evidence of biologically relevant changes. The combined-site analysis show that there were no statistically significant differences ($p>0.05$) for 59 (96.7%) of the 61 comparisons between the mean component values of MON 87460 and the control. Of the two significant differences observed, mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. The individual site analysis show that there were no statistically significant differences ($p>0.05$) for 174 (95.1%) of the 183 comparisons. Individual site differences were not reproducible across multiple sites and, where differences were observed, mean component values of the test and control substances were within the 99% tolerance interval. Furthermore, the limited number of component differences was characterized by small differences in magnitude.

These factors established that the limited number of differences observed in this study were within the natural variability of corn for these components, reflected no meaningful trends, and were of no biological significance.

Therefore, the corn grain and forage derived from MON 87460 grown under water-limited conditions, and the intended foods and feeds derived from MON 87460, can be considered compositionally equivalent to those derived from conventional corn grown under the same conditions. MON 87460 grown under limited water availability is as safe as conventional corn with a history of safe consumption.

**Table VII-5. Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control in Chile during 2006/2007 under Water-Limited Conditions**

| Tissue/Site/ Components (Units)[a] | Mean MON 87460 | Mean Control | Mean Diff (% of Control) | Signif. (p-value) | MON 87460 (Range) | 99% Tolerance Interval[b] |
|---|---|---|---|---|---|---|
| **Forage** | | | | | | |
| **Combination of all sites** | | | | | | |
| Total Fat (% DW) | 1.32 | 0.84 | 56.03 | 0.045 | 0.50 - 1.92 | [0, 3.25] |
| **CT** | | | | | | |
| Moisture (% FW) | 72.13 | 74.23 | -2.83 | 0.005 | 72.00 - 72.30 | [70.85, 80.94] |
| **Grain** | | | | | | |
| **Combination of all sites** | | | | | | |
| 20:1 Eicosenoic (% Total FA) | 0.1763 | 0.1845 | -4.43 | 0.042 | 0.1617 - 0.1916 | [0.11, 0.34] |
| **CL** | | | | | | |
| 22:0 Behenic (% Total FA) | 0.17 | 0.11 | 57.68 | 0.029 | 0.13 - 0.20 | [0, 0.32] |
| Vitamin E (mg/kg DW) | 13.34 | 11.16 | 19.54 | 0.001 | 12.57 - 14.24 | [0, 22.61] |
| Phytic Acid (% DW) | 0.87 | 0.69 | 25.47 | 0.012 | 0.84 - 0.89 | [0.40, 1.12] |
| **CT** | | | | | | |
| Iron (mg/kg DW) | 17.61 | 18.81 | -6.34 | 0.046 | 17.06 -18.24 | [7.05, 30.38] |
| Phosphorus (% DW) | 0.32 | 0.35 | -8.35 | 0.027 | 0.32 - 0.32 | [0.25, 0.42] |
| 20:1 Eicosenoic (% Total FA) | 0.17 | 0.18 | -9.16 | 0.016 | 0.16 - 0.17 | [0.11, 0.34] |
| Folic Acid (mg/kg DW) | 0.30 | 0.25 | 19.36 | 0.046 | 0.25 - 0.37 | [0.098, 0.58] |
| **LUM** | | | | | | |
| 18:1 Oleic (% Total FA) | 20.38 | 20.89 | -2.46 | 0.030 | 20.20 - 20.48 | [12.15, 35.55] |

[a]DW= dry weight; FA=fatty acid.
[c]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.
Tables E-19 through E-25 in Appendix E present the full results of the combined site analysis for the compositional analyses from the Chile 2006/2007 production including means, standard error values and p-values.

## VII.C. Additional Secondary Metabolites from the Chile 2006/2007 Composition Study under Well-Watered and Water-Limited Conditions

As part of the comparative assessment approach described previously, the OECD consensus documents suggest that additional components relevant to the trait can be considered for characterization of food and feed derived from new products (OECD, 2002). Because MON 87460 is expected to be grown in regions subjected to frequent drought stress, additional secondary metabolites selected for further comparative evaluation included those known to be generally associated with stress responses in a range of plants and tissues, and thus possibly relevant in corn. There is no evidence in the literature for such components that are unique to corn.

Eleven additional secondary metabolites were selected for comparison between MON 87460 and the control. These selected metabolites included: osmoprotectants, such as sugars and polyols (sucrose, glucose, fructose, sorbitol, mannitol, and glycerol), free proline, glycine betaine and choline (Yancey, 2004 and 2005), as well as metabolites that are generally associated with stress responses such as salicylic acid (Yuan and Lin, 2008), and abscisic acid (Wasilewska, et al., 2008). These 11 metabolites were measured in forage and grain of MON 87460 and the control. No safety issues are evident for these metabolites and most represent an extremely minor fraction of corn biomass.

Field design and sampling are described in Section VII.B. Samples from test, control, and reference substances from all four sites in Chile were subjected to additional compositional analysis. This section describes results of combined-site data from three (CL, CT, LUM) of the four sites established. For a site to be included in the combined-site analysis, commercial reference hybrids in the water-limited plots had to exhibit a minimum 15% reduction in yield compared to the same reference hybrids planted in the well-watered plots. A detailed description of the site inclusion criteria applied to these data is provided in Section VIII.B.2. As described in Section VIII.C and Table VIII-3, the QUI site in the Chile 2006/2007 study was not established with the appropriate water stress treatments; therefore, data for the QUI site are presented in Appendix E.

Statistical comparisons between the test and control substances were performed within each irrigation treatment. A range of component values and a statistical population were determined for the reference substances within each irrigation treatment. Thus, four sets of statistical analyses were made for each treatment, three based on the data from each of the replicated field sites and the fourth based on data from a combination of all three field sites. Statistically significant differences were determined at the 5% level of significance ($\alpha = 0.05$) using established statistical methods.

### VII.C.1. Levels of Additional Secondary Metabolites under Well-Watered Conditions

Two of the 11 metabolites (sorbitol, mannitol) had more than half of the observations below the assay limit of quantitation (LOQ). Metabolites with more than half of the observations below the assay LOQ were excluded from statistical analysis. Therefore, nine metabolites in both forage and grain were statistically assessed using a mixed model analysis of variance method.

There were a total of 72 comparisons made (four sets of comparisons × nine components from grain and four sets of comparisons × nine components from forage). A summary of significant differences ($p<0.05$) between test and control in both the combined-site and individual site (total of 3 sites) analyses for the well-watered treatment is presented in Table VII-6. Mean values, ranges, standard error values and statistical analyses for the combined-site data are presented in Appendix E (Tables E-26 and E-27). The standard error values presented in Appendix E are calculated from the ANOVA. Thus, each test and control mean for a given analyte mean has the same standard error. Literature and ILSI Crop Composition Database ranges for corn components are presented in Table E-30.

The combined-site analysis show that there were no statistically significant differences ($p>0.05$) for 17 (94.4%) of the 18 comparisons between the mean component values of MON 87460 and the control. The single difference was for abscisic acid. However, the magnitude of the combined-site difference in the abscisic acid values was exceedingly small (21.37 ppb FW) and therefore considered to be within the natural variability of corn for this component. The individual site analysis show that there were no statistically significant differences ($p>0.05$) for 51 (94.4%) of 54 comparisons. Individual site differences were not reproducible across multiple sites and, where differences were observed, with the exception of the highly variable forage abscisic acid, mean component values test and control substances were within the 99% tolerance interval (Table VII-6). Therefore, these differences were within the natural variability of corn for these components.

### VII.C.2. Conclusions for Chile 2006/2007 Additional Secondary Metabolites Well-Watered Conditions

In summary, statistical analysis highlighted no consistent differences across sites in the levels of the additional secondary metabolites from MON 87460 and the control. The limited number of differences observed in this study reflected the natural variation of corn and support the conclusion that levels of key osmoprotectants and metabolites generally associated with stress do not differ between MON 87460 and conventional corn. This supports the assessment of MON 87460 as compositionally equivalent to conventional corn.

**Table VII-6. Summary of Significant Differences in Additional Secondary Metabolite Composition (p<0.05) Comparing MON 87460 to the Conventional Control in Chile during 2006/2007 under Well-Watered Conditions**

| Tissue/Site/ Components (Units)[1] | Mean MON 87460 | Mean Control | Mean Diff (% of Control) | Signif. (p-value) | MON 87460 (Range) | 99% Tolerance Interval[2] |
|---|---|---|---|---|---|---|
| **Forage** | | | | | | |
| **Combination of all sites** | | | | | | |
| Abscisic Acid (ppb FW) | 37.03 | 15.66 | 136.54 | 0.040 | (11.90 - 122.00) | [1.22, 33.02] |
| **CL** | | | | | | |
| Abscisic Acid (ppb FW) | 75.23 | 15.63 | 381.24 | 0.003 | (18.50 - 122.00) | [1.22, 33.02] |
| **CT** | | | | | | |
| Free Proline (% DW) | 0.027 | 0.019 | 40.22 | 0.025 | (0.023 - 0.031) | [0, 0.042] |
| Choline (ppm FW) | 135.67 | 108.97 | 24.50 | <0.001 | [114.00 - 148.00] | [76.96, 179.64] |

[1]DW= dry weight; FW=fresh weight.

[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Tables E-26 and E-27 in Appendix E present the full results of the combined site analysis for the compositional analyses from the Chile 2006/2007 production including means, standard error values and p-values.

### VII.C.3. Levels of Additional Secondary Metabolites under Water-Limited Conditions

A summary of significant differences ($p<0.05$) between test and control in both the combined-site and individual site (total of 3 sites) analyses for the water-limited treatment is presented in Table VII-7. Mean values, ranges, standard error values and statistical analyses for the combined-site data are presented in Appendix E (Tables E-28 and E-29). The standard error values presented in Appendix E are calculated from the ANOVA. Thus, each test and control mean for a given analyte mean has the same standard error. Literature and ILSI Crop Composition Database ranges for corn components are presented in Table E-30.

The combined-site analysis show that there were no statistically significant differences ($p>0.05$) for 17 (94.4%) of the 18 comparisons between the mean component values of MON 87460 and the control. For the single significant difference observed, mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. The individual site analysis show that there were no statistically significant differences ($p>0.05$) for 44 (81.5%) of the 54 comparisons. Individual site differences were not reproducible across multiple sites and, where differences were observed, mean component values test and control substances were within the 99% tolerance interval (Table VII-7). Therefore, these differences were within the natural variability of corn for these components.

### VII.C.4. Conclusions for Chile 2006/2007 Additional Secondary Metabolites Water-Limited Conditions

In summary, statistical analysis highlighted no consistent differences across sites in the levels of metabolite components from MON 87460 and the control. The limited number of differences observed in this study reflects the natural variation of corn and supports the conclusion that levels of key osmoprotectants and metabolites potentially associated with stress do not differ between MON 87460 and conventional corn. This supports the conclusion that MON 87460 is compositionally equivalent to conventional corn.

**Table VII-7. Summary of Significant Differences in Additional Secondary Metabolite Composition (p<0.05) Comparing MON 87460 to the Conventional Control in Chile during 2006/2007 under Water-Limited Conditions**

| Tissue/Site/ Components (Units)[1] | Mean MON 87460 | Mean Control | Mean Diff (% of Control) | Signif. (p-value) | MON 87460 (Range) | 99% Tolerance Interval[2] |
|---|---|---|---|---|---|---|
| **Forage** | | | | | | |
| **CL** | | | | | | |
| Glycine Betaine ppm FW) | 102.40 | 147.00 | -30.34 | 0.016 | [73.20 - 122.00] | [0, 357.15] |
| Salicylic Acid (ppm FW) | 0.11 | 0.33 | -68.43 | 0.002 | [0.10 - 0.11] | [0, 0.82] |
| **CT** | | | | | | |
| Abscisic Acid (ppb FW) | 42.27 | 23.57 | 79.35 | 0.043 | [32.90 - 54.30] | [0, 94.59] |
| Choline (ppm FW) | 157.00 | 144.00 | 9.03 | 0.024 | [153.00 - 160.00] | [66.54, 217.46] |
| **LUM** | | | | | | |
| Choline (ppm FW) | 167.67 | 151.67 | 10.55 | 0.035 | [153.00 - 181.00] | [66.54, 217.46] |
| Salicylic Acid (ppm FW) | 0.41 | 0.26 | 57.57 | 0.016 | [0.27 - 0.58] | [0, 0.82] |
| **Grain** | | | | | | |
| **Combination of all sites** | | | | | | |
| Sucrose (% DW) | 1.63 | 1.86 | -12.40 | 0.008 | [1.33 - 1.86] | [0.61, 2.84] |
| **CL** | | | | | | |
| Abscisic Acid (ppb FW) | 10.03 | 16.59 | -39.52 | 0.041 | [8.78 - 11.90] | [0, 176.41] |
| Glycerol (% DW) | 0.030 | 0.023 | 31.03 | 0.045 | [0.025 - 0.034] | [0, 0.060] |
| **CT** | | | | | | |
| Sucrose (% DW) | 1.71 | 2.03 | -15.85 | 0.010 | [1.44 - 1.86] | [0.61, 2.84] |
| **LUM** | | | | | | |
| Sucrose (% DW) | 1.41 | 1.58 | -10.55 | 0.040 | [1.33 - 1.54] | [0.61, 2.84] |

[1]DW= dry weight; FW=fresh weight.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.
Tables E-28 and E-29 in Appendix E present the full results of the combined site analysis for the compositional analyses from the Chile 2006/2007 production including means, standard error values and p-values.

## VII.D. Overall Conclusions from Compositional Analysis of MON 87460 from the U.S. 2006 and Chile 2006/2007 Studies

The compositional analyses of MON 87460 were based on forage and grain harvested from two different growing seasons, the first during 2006 in the U.S. conducted under typical agronomic practices and water conditions, and the second during 2006/2007 in Chile under well-watered and water-limited conditions. Thus, the multi-year study allowed a determination of whether food and feed derived from MON 87460 exhibits compositional equivalence to conventional corn under a broad range of environmental conditions.

Components evaluated in samples harvested from both studies included (1) moisture, protein, carbohydrates by calculation fat, fiber, and ash in a proximate analysis, (2) essential macro- and micro-nutrients in a nutritional analysis, and (3) known endogenous toxicants and anti-nutrients.

Overall, a comprehensive evaluation of key nutrient, anti-nutrients and secondary metabolites from MON 87460 and the control showed no biologically meaningful differences. The statistical differences were small in magnitude and not reproducible across multiple sites. All mean component values of the test and control substances were within the 99% tolerance interval established from commercial references. Therefore, the forage and grain from MON 87460 and the foods and feeds derived from such, can be considered compositionally equivalent to those derived from conventional corn.

A supplementary analysis of secondary metabolites associated with stress tolerance was conducted for samples from the Chile 2006/2007 study. Statistical comparisons between the test and control substances were performed within each water treatment and showed very similar results. The few detected differences were either exceedingly small in magnitude or the mean component values of MON 87460 and the control were within the 99% tolerance interval. Therefore, these differences were within the natural variability of corn for these components. The evaluation of these additional metabolites further supports the compositional equivalence of MON 87460 to conventional corn, which has a history of safe consumption.

## VII.E. References


Adams, T. B., J. Doull, J.I. Goodman, I.C. Munro, P. Newberne, P.S. Portoghese, R.L. Smith, B.M. Wagner, C.S. Weil, L.A. Woods and R.A. Ford. 1997. The FEMA GRAS assessment of furfural used as a flavour ingredient. Food and Chemical Toxicology. 35: 739-751.

Campos, H., M. Cooper, G.O. Edmeades, C. Loffler, J.R. Schussler, and M. Ibanez. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. Maydica. 51:369-381.

Douglas, C.J. 1999. Phenylpropanoid metabolism and lignin biosynthesis: from weeds to trees. Trends in Plant Science. 1: 171-178.

Harrigan, G.G., W.P. Ridley, K.D. Miller, R. Sorbet, S.G. Riordan, M.A. Nemeth, W. Reeves, T.A. Pester. 2009. The forage and grain of MON 87460, a drought-tolerant corn hybrid, are compositionally equivalent to that of conventional corn. J. Agric. Food Chem. ***57***: 9754–9763.

Lott, J.N.A., I. Ockenden, V. Raboy and G.D. Batten. 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Science Research. 10: 11-33.

Novak, W.K. and A.G. Haslberger. 2000. Substantial equivalence of antinutrients and inherent plant toxins in genetically modified novel foods. Food Chemical. Toxicology. 38: 473-483.

OECD. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. ENV/JM/MONO(2002)25. Organization for Economic Co-operation and Development, Series on the Safety of Novel Foods and Feeds, No. 6, Paris, France.

Voragen, A.G.J. 1998. Technological aspects of functional food-related carbohydrates. Trends in Food Science and Technology. 9: 328-335.

Wasilewska, A., F. Vlad, C. Sirichandra, Y. Redko, F. Jammes, C. Valon, N.F. dit Frey, and J. Leung. 2008. An update on abscisic acid signaling in plants and more… Molecular Plant. 1:198-217.

Yancey, P.H. 2004. Compatible and counteracting solutes: protecting cells from the Dead Sea to the deep sea. Science Progress. 87:1-24.

Yancey, P.H. 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. Journal of Experimental Biology. 208:2819-2830.

Yuan, S. and Lin, H.H. 2008. Role of salicylic acid in plant abiotic stress. Zeitschrift Fur Naturforschung Section C-a Journal of Bioscience. 63:313-320.

## VIII. Phenotypic, Agronomic, and Environmental Interactions Assessment

This section provides an assessment of the phenotypic and agronomic characteristics, and the environmental interactions of MON 87460. As an introduction to Monsanto's approach to the plant pest risk assessment of MON 87460, the first two sections provide a list of the characteristics measured among the assessment studies conducted (Section VIII.A) and the criteria used to interpret the data generated (Section VIII.B). The remaining Sections (VIII.C through VIII.F) present the results of several field, greenhouse, and laboratory studies that comprise the comprehensive assessment of MON 87460.

Phenotypic and agronomic characteristics of MON 87460 were evaluated in a comparative manner to assess plant pest potential (OECD, 1993). These assessments included 14 plant growth and development characteristics, five seed germination parameters, two pollen characteristics, and observations for plant-insect and plant-disease interactions and plant responses to abiotic stressors. Results from the phenotypic and agronomic assessments indicate that MON 87460 does not demonstrate increased plant pest characteristics compared to conventional corn. Data on environmental interactions indicate that MON 87460 does not confer any increased susceptibility or tolerance to specific diseases, insects, or abiotic stressors, with the exception of the drought tolerance trait.

### VIII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 87460, data were collected to evaluate specific aspects of altered plant pest potential based on requirements of USDA-APHIS set forth at 7 CFR § 340.6. The assessment encompasses five general data categories: (1) phenotypic growth and development, including vegetative and reproductive growth; (2) germination and dormancy; (3) pollen viability and morphology; (4) plant interactions with insects, diseases, and abiotic stressors; and (5) persistence in cultivated fields or areas outside of cultivation. An overview of the characteristics assessed is presented in Table VIII-1.

The phenotypic, agronomic, and environmental interactions data were evaluated on the basis of familiarity (OECD, 1993) and were comprised of a combination of field, greenhouse, and laboratory studies conducted by scientists who are familiar with the production and evaluation of corn. In each of these assessments, MON 87460 was compared to an appropriate conventional control, which had a genetic background similar to MON 87460 but did not possess the drought tolerance trait. In addition, multiple commercial corn hybrids (references) were included to provide a range of baseline values that are common to the existing commercial corn hybrids for each measured phenotypic, agronomic, and environmental interaction characteristic. The commercial reference hybrids selected for each study were adapted to the geographic region in which they were grown with selections based on agronomic characteristics such as relative maturity and drought tolerance ratings. Relative maturity was an important consideration in order to ensure that the test, control and reference materials would be at comparable stages of development at each data or sample collection time point. Data collected from the

commercial references reflect a range of selection and breeding for desirable characteristics including drought tolerance and can therefore provide context for interpreting experimental results.

The commercial reference hybrids selected for each study were adapted to the geographic region relevant to each study site. It is known that corn hybrids are variable in their response to drought stress (Campos et al., 2006). Breeders have been selecting for improved drought tolerance in conventional corn through traditional breeding and selection methods for decades. Monsanto and other seed companies commonly evaluate their commercial hybrids under a range of environmental conditions each year and assign drought tolerance ratings as a performance characteristic for each hybrid (http://www.asgrowanddekalb.com/seedresourceguide/search/seeds). Approximately 75% of the conventionally bred hybrids offered for planting in the dryer western areas of the Great Plains are rated as having very good to excellent drought tolerance and have been recommended for commercial production fields that regularly experience drought stress. The variation in yield response observed among MON 87460, the control, and commercial reference hybrids in the field studies discussed below is within normal levels expected for conventional corn grown under sub-optimal soil moisture environments.
The characteristics measured among the field, greenhouse, and laboratory studies are listed in Table VIII-1. The standard phenotypic, agronomic, and environmental interactions characteristics are well known to experts familiar with corn breeding and agronomic performance. MON 87460 is expected to provide reduced yield loss under water-limited conditions compared to conventional corn. Reduced yield loss is a desirable agronomic characteristic and is not *per se* considered to be associated with plant pest potential. Additional plant characteristics assessed included germination and dormancy, pollen characteristics collected under well-watered and water-limited conditions, abiotic stress tolerance to multiple levels of drought, cold, heat, and salt conditions, volunteer potential in a subsequent season under cultivated soil conditions, and survival ability under environments not cultivated for agricultural production. These characteristics are useful to assess plant pest potential of MON 87460. Specifically, certain characteristics can be used to assess weediness potential, including seed germination and dormancy (hard seed), pre-harvest seed loss characteristics (lodging and ear drop), and the potential to volunteer in cultivated areas or survive outside cultivation.

Because MON 87460 reduces yield loss under water-limited conditions, field studies were designed to evaluate the relevant characteristics of MON 87460 across a broad range of soil moisture and environmental conditions relevant to where commercial production would be expected. Agronomic and phenotypic characteristics data were collected from plants grown under a combination of three different water management regimes: (1) well-watered, (2) well-watered and water-limited treatments in the same field arranged in either a strip- or split-plot design, or (3) water managed according to typical local agronomic practices and water conditions. A detailed description of the three water management regimes is provided in Section VIII.C. In total, these data included more than 400 phenotypic and agronomic evaluations and more than 800 observations for plant-arthropod, plant-disease, and plant responses to abiotic stressor interactions.

**Table VIII-1. Characteristics Measured for Phenotypic, Agronomic and Environmental Interactions Assessment of MON 87460**

| Characteristic | Characteristics measured | Evaluation timing | Evaluation description (measurement endpoints) |
|---|---|---|---|
| Plant phenotypic and agronomic characteristics | Dormancy, Germination | After 4, 7, and 12 days | Percent normally germinated, abnormally germinated, viable hard (dormant), dead, and viable firm swollen seed |
| | Seedling vigor* | Stage V2 – V4 | Rated as: 1-3 = above average vigor, 7-9 = below average vigor (2007 scale) or, where 0 = dead, and 9 = above average vigor (2006 scale) |
| | Early stand count | Stage V2 – V4 | Number of emerged plants per plot |
| | Final stand count | Pre-harvest | Number of plants per plot |
| | Stay green* | Maturity | Rated as: 1 = 90-100% green tissue, 5 = 50-59% green tissue, 9 = 0-19% green tissue (2007 rating scale) or, 0 = entire plant dried, and 9 = entire plant green (2006 rating scale) |
| | Ear height | Maturity | Distance from the soil surface at the base of the plant to the ear attachment node |
| | Plant height | Maturity | Distance from the soil surface at the base of the plant to the flag leaf collar |
| | Stalk lodged plants | Pre-harvest | Number of plants per plot broken below the ear |
| | Root lodged plants | Pre-harvest | Number of plants per plot leaning at the soil surface at >30° from the vertical |
| | Days to 50% pollen shed | Pollen shed | Days from planting until 50% of the plants have begun to shed pollen |
| | Days to 50% silking | Silking | Days from planting until 50% of the plants have silks exposed |
| | Pollen viability | Tasseling | Viable and nonviable pollen based on pollen grain staining characteristics |
| | Pollen morphology | Tasseling | Diameter of viable pollen grains |
| | Grain moisture | Harvest | Moisture percentage of harvested shelled grain |
| | Test weight (lb/bu) | Harvest | Test weight of harvested shelled grain |
| | Yield (bu/ac) | Harvest | Harvested weight of shelled grain, adjusted to 15.5% moisture |
| | Dropped ears | Pre-harvest | Number of mature ears dropped from plants |
| Plant environmental interactions | Insect, disease and abiotic stressors | Variable, from planting to harvest | Qualitative assessment of each plot, with rating on a 0-9 scale for plant-insect, plant-disease, and plant response to abiotic stressor interactions |
| | Abiotic stress tolerance to drought, cold, heat, and salt | Stage V2 – V6 | Conducted in greenhouse and growth chamber experiments. Measurements included plant height, growth stage, vigor, chlorophyll content, and biomass. |
| | Volunteer potential | After fall planting and following spring | Number of plants present as volunteer corn in plots |
| | Survival outside of cultivation | Variable, from planting to harvest | Variable, phenotypic assessments from planting to harvest that includes early and final stand counts, vigor ratings, plant height, and number of ears and seed per plot |

*Rating scale changed in 2007 to be consistent with assessments used by corn breeders

## VIII.B. Interpretation of Assessment Data

Plant pest risk assessments for biotechnology-derived crops are, by standard, comparative assessments. The concept of familiarity is useful when designing a risk assessment and evaluating the plant pest potential of a biotechnology-derived plant compared to the conventional crop. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant variety whose biological properties and plant pest potential are known to experts. Familiarity considers the biology of the crop, the introduced trait, the receiving environment, and the interaction among these factors, and provides a basis for comparative risk assessment between a biotechnology-derived plant and its conventional counterpart.

Expert knowledge and experience with conventionally bred corn was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for corn. Thus, assessment of phenotypic and agronomic characteristics and environmental interactions was essential to compare the biotechnology-derived plant to the conventional counterpart. An overview of the characteristics assessed is presented in Table VIII-1. A subset of the data relating to well-understood weediness criteria (e.g., dormancy, lodging or pre-harvest seed loss characteristics, volunteer potential and survival outside cultivation) was used to assess whether there is an increased weediness potential, an element of APHIS's plant pest determination. Data on abiotic stress tolerance from the greenhouse and growth chamber assays were used to characterize the extent of stress tolerance imparted by the insertion of the *cspB* gene and determine whether any potential changes in tolerance required additional evaluation as a component of the plant pest risk assessment. Based on all of the data collected, an assessment was made whether the biotechnology-derived plant is likely to pose an increased plant pest risk compared to the conventional counterpart.

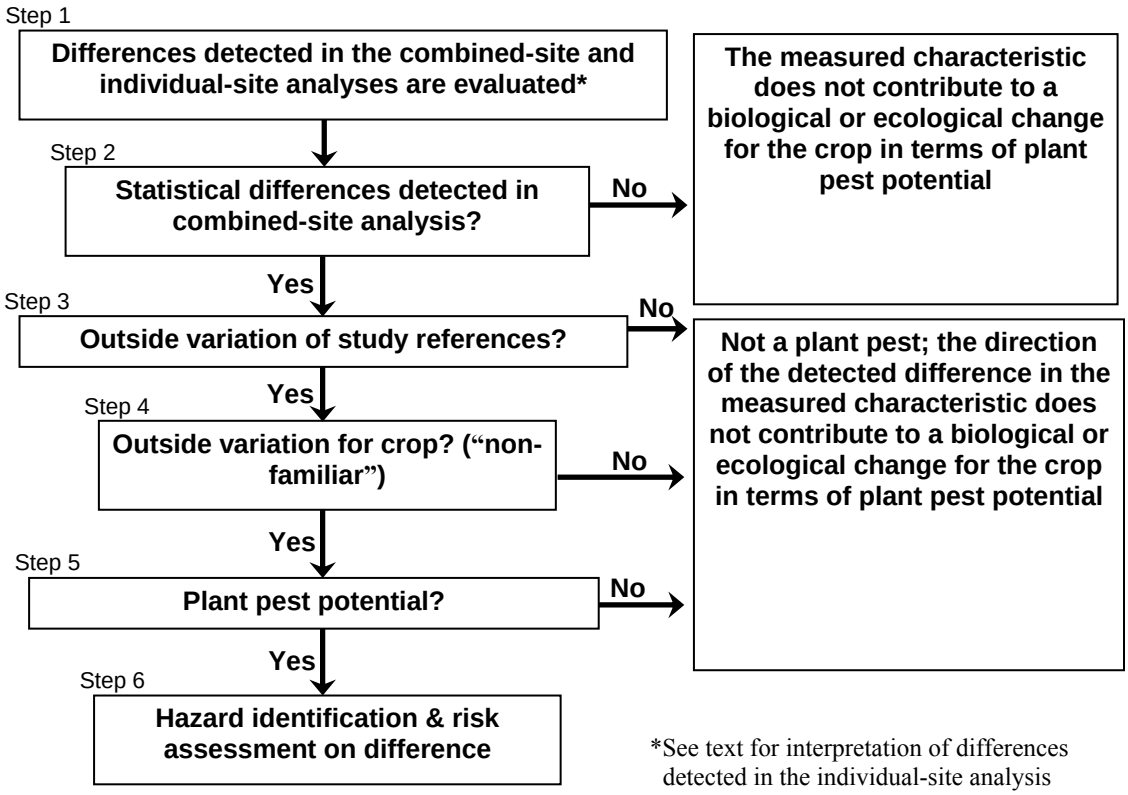
Agronomists familiar with the experimental design, evaluation criteria, and expected outcomes were involved in all steps of data collection, summarization, and analyses. This supervision ensured that measurements were taken appropriately, data were consistent with expectations based on experience with the crop, and that the experimental and field sites were carefully monitored. These scientists were expected to report any unexpected observations or issues during the course of the studies. The overall dataset was evaluated for evidence of biologically relevant changes and for possible evidence of an unexpected plant response and the data were subsequently submitted for statistical analysis.

### VIII.B.1. Interpretation of Detected Differences Criteria

Comparative plant characterization data between a biotechnology-derived crop and the control are interpreted in the context of contributions to increased plant pest potential as assessed by APHIS. Under the framework of familiarity, characteristics for which no differences are detected support a conclusion of no increased plant pest potential of the biotechnology-derived crop compared to the conventional crop. Characteristics for which differences are detected are considered in the step-wise method (Figure VIII-1). All detected differences for a characteristic are considered in the context of whether or not the difference would increase the plant pest potential of the biotechnology-derived

crop. Ultimately, a weight of evidence approach considering all characteristics and studies was used for the overall risk assessment of differences and their significance. In detail, Figure VIII-1 illustrates the stepwise assessment process employed:

- **Steps 1 & 2. Evaluate Detected Statistical Differences**. Combined-site and individual-site statistical analyses are conducted and evaluated on each measured characteristic. Differences detected in the individual-site analysis must be observed in the combined-site analysis to be considered further for plant pest potential. A difference in the combined-site analysis is further assessed regardless of whether or not the difference is detected in the individual-site analysis.
- **Step 3. Evaluate Differences Relative to Reference Range.** If a difference is detected in the combined-site analysis across multiple environments, then the test substance mean value is assessed relative to the range of values of the reference substances.
- **Step 4. Evaluate Difference in the Context of the Crop.** If the test substance mean is outside the variation of the reference substances (e.g., reference range or tolerance interval), the test substance mean is considered in the context of known values common for the crop.
- **Step 5. Evaluate Difference in the Context of Plant Pest Potential**. If the test substance mean is outside the range of values common for the crop, the detected difference is then assessed for plant pest potential.
- **Step 6. Conduct Risk Assessment on Identified Hazard.** If a hazard is identified, risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced plant pest potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for, and effects of trait transfer to feral populations of the crop or a sexually compatible species.




Note: A "no" answer at any step indicates that the characteristic does not contribute to a biological or ecological change for the crop in terms of plant pest potential and subsequent steps are not considered. If the answer is "yes" or uncertain the subsequent step is considered.

**Figure VIII-1. Decision Diagram for Interpretation of Detected Differences**

## VIII.B.2. Interpretation of Site Inclusion Criteria

Water management was a critical aspect in the field-based plant characterization evaluation of MON 87460. Because MON 87460 reduces yield loss under water-limited conditions, field studies were designed to evaluate the environmental consequences of MON 87460 across a broad range of soil moisture and environmental conditions relevant to where commercial production would be expected. Two distinct hypotheses were tested in this risk assessment: (1) no phenotypic differences between MON 87460 and the control under well-watered conditions, and (2) no phenotypic differences between MON 87460 and the control under water-limited conditions except for grain yield. As such, field studies were designed to enable assessment of the effects of defined water treatments on the phenotypic and agronomic characteristics of MON 87460 compared to the conventional control, and sites established with a defined water treatment had to meet set criteria to qualify as either well-watered (non-stressed) or water-limited (stressed) to be included in the assessment.

The well-watered treatment allowed a comparison of MON 87460 and the control in the absence of trait bias, where no statistical differences were expected between MON 87460 and the conventional control. The water-limited treatment imposed during susceptible growth stages (i.e., approximately V10 through R3) allowed a comparison of MON 87460 and the control under conditions where MON 87460 was expected to provide a yield benefit. Water treatment levels were managed by using a combination of either a water budgeting program (KanSched software, supported by Kansas State University Research and Extension), physical assessments for soil moisture (Appearance and Feel Test; Black and Rogers, 1989), or by applying water on a calendar basis following local agronomic practices.

Criteria were established to identify sites that were managed appropriately to impose the defined well-watered and water-limited treatment levels. Specifically, commercial reference hybrids in the water-limited plots had to exhibit a minimum 15% reduction in yield compared to the same reference hybrids planted in the well-watered plots. The 15% yield loss criteria represents a meaningful stress level, as moderate water deficits result in approximately a 15% yield loss annually for corn grown in both temperate and tropical regions (Barker et al., 2005). Reductions in plant height, ear height, and days to 50% silking are also phenotypic indicators of moisture deficit in corn (Campos et al., 2006); therefore, these characteristics were assessed to confirm the inclusion of sites that met the 15% yield reduction requirement.

Field sites designed to assess the effects of water limitation were established with defined well-watered and water-limited treatments. Each site was evaluated in a step-wise method (Figure VIII-2) using set criteria to determine if a differential water treatment was achieved and if a water treatment effect was observed in the plant responses of the reference substances. Only sites that met the inclusion criteria were included in the assessment. Data from sites that did not meet the inclusion criteria are presented in the appendices. In detail, Figure VIII-2 illustrates the stepwise assessment process employed:

- **Step 1. Confirm Differential Water Treatments Imposed**. A review of the water management practices and rainfall data is conducted for each site. All rainfall events and irrigation amounts during the water stress period are evaluated for compliance with soil moisture requirements. Precipitation and irrigation records should support the expectation that differential water treatments were imposed at the field site.

- **Step 2. Confirm Target Soil Moisture Levels Imposed.**

  *Well-watered treatment*: available moisture should be $\geq$50% of field capacity for the duration of the study to avoid drought stress conditions and provide optimal grain yield.

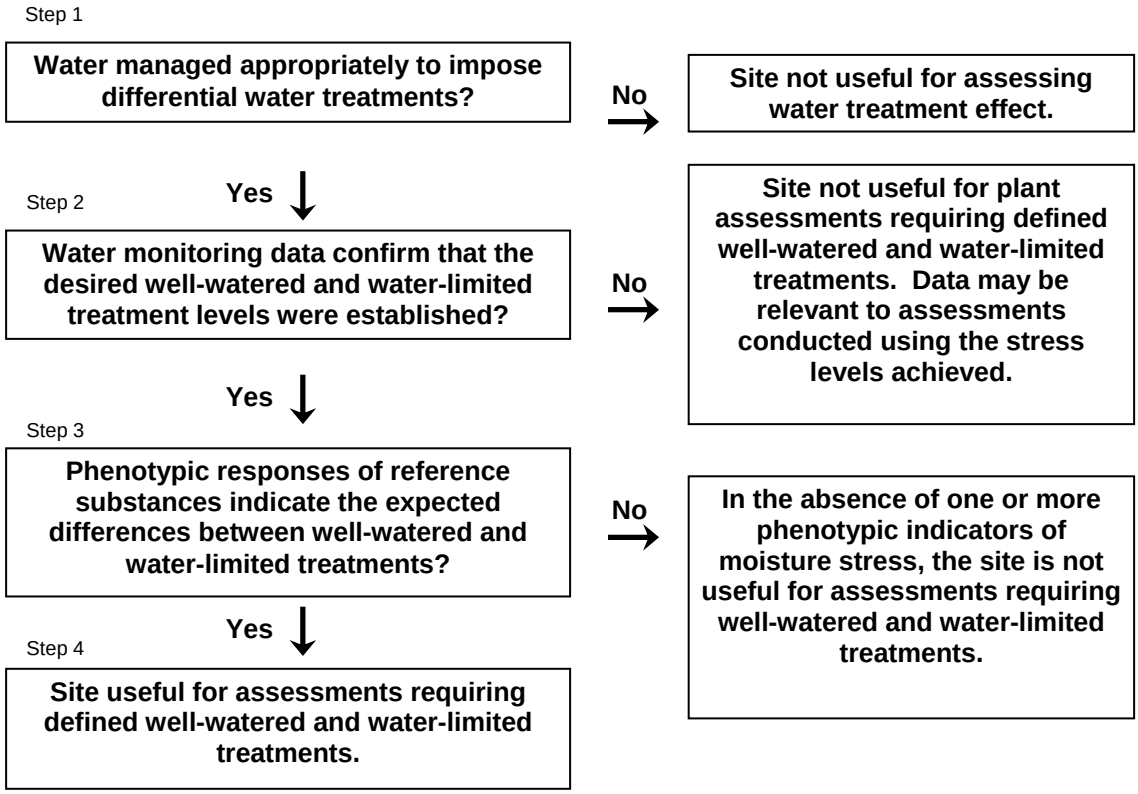
  *Water-limited treatment*: available moisture should be <50% of field capacity during targeted stress treatment (e.g., V10 – R3).

At sites where soil moisture data were not collected, evidence of water treatment effect is necessary.

- **Step 3. Confirm Phenotypic Responses to Water Treatments**. A weight of evidence approach is taken when comparing the references in the well-watered treatment to the references in the water-limited treatment. Indicators of water limitation included yield reduction (minimum of 15%), reduced plant height, reduced ear height, and delayed silking.

  No differences in these phenotypic criteria may indicate that the site is not useful for assessments requiring well-watered and water-limited treatments.

- **Step 4. Confirm Site Meets Inclusion Criteria**. A "yes" answer indicates that a site is applicable for assessments requiring defined well-watered and water-limited treatments. At a minimum, the requirement for a 15% yield reduction in the reference substances due to the imposed water stress treatment must be met for a site to be considered applicable for assessments that require defined well-watered and water-limited treatments. This requirement ensures assessments are conducted under conditions of significant stress as evidenced by yield reduction and irrigation records.




Note: A "no" answer at any step indicates that the site is not useful for assessments requiring well-watered and water-limited treatments. If the answer is "yes" or uncertain, the subsequent step is considered. Other assessments may be appropriate under the treatment levels achieved in the study. All comparisons of plant responses to assess for a water treatment effect at each site are made using the reference substances.

**Figure VIII-2. Decision Diagram for Site Inclusion in Plant Characterization Assessments Requiring Defined Well-Watered and Water-Limited Treatments**

*Note*

The preceding two sections provide an introduction to Monsanto's approach to the plant pest risk assessment of MON 87460 by presenting a list of the characteristics measured (VIII.A) and the methods used to interpret the assessment data (VIII.B). The following sections (VIII.C through VIII.F) present the results of several field, greenhouse, and laboratory studies used as the basis for the assessment of MON 87460

## VIII.C. Phenotypic and Agronomic Assessment

The purpose of these studies was to assess the phenotypic and agronomic characteristics of MON 87460 compared to a conventional control, which had a genetic background similar to MON 87460 but did not possess the drought tolerance trait. In addition, multiple conventional corn hybrids (references) were included in the analysis to establish a range of natural variability for each characteristic. Because MON 87460 reduces yield loss under water-limited conditions, field studies were designed to evaluate the environmental consequences of MON 87460 across a broad range of soil moisture and environmental conditions relevant to where commercial production would be expected. Field studies were established using three different water management regimes: (1) well-watered, (2) well-watered and water-limited treatments in the same field arranged in either a strip- or split-plot design, or (3) water managed according to typical local agronomic practices and water conditions.

*Well-Watered Treatment*

Field studies established under well-watered conditions were intended to provide optimal grain yield and allowed an evaluation of MON 87460 in the absence of trait bias, where no statistical differences were expected between MON 87460 and the control. Field studies managed to impose only well-watered conditions were established at 17 sites in the U.S. during 2006 and 2007 (Table VIII-2). The water treatment in these studies required available soil moisture to be maintained at $\geq$50% of field capacity for the duration of the study to avoid drought stress conditions. Water was provided by natural rainfall and supplemental irrigation as needed.

*Well-Watered and Water-Limited Treatments*

Field studies established under both well-watered and water-limited conditions allowed an evaluation of MON 87460 under conditions where it is expected to reduce yield loss. Field studies managed to impose both well-watered and water-limited conditions were established at nine sites in the U.S. and Chile during 2006 and 2007 (Table VIII-2). The well-watered treatment in these studies required available soil moisture to be maintained at $\geq$50% of field capacity for the duration of the study to avoid drought stress conditions and provide optimal grain yield. The water-limited treatment was managed the same as the well-watered treatment, with the exception that available soil moisture be reduced to <50% of field capacity to impose a moderate drought stress during the late vegetative

through early grain fill growth stages (~V10 – R3) when corn yield potential is most sensitive to stress (Claassen and Shaw, 1970; Boyer and Westgate, 2004). See Section II.D.3 for additional information on the effects of drought stress on corn growth and development. For the nine field sites established with both well-watered and water-limited treatments (Table VIII-2), the inclusion criteria were used to identify sites that were managed appropriately to impose the defined treatment levels (see previous Section VIII.B.2). Due to untimely rainfall during the imposed water-limitation treatments and complications in water management techniques, only six of the nine sites established met the required inclusion criteria of having both well-watered and water-limited treatments for plant characterization assessments requiring both treatments (Table VIII-3). The Chile 2006/2007 study was also used to determine CSPB and NPTII expression levels (Section VI.C) and composition of forage and grain (Section VII).

*Typical Agronomic Practices Treatment*

A field study established under water conditions typical of local agronomic practices allowed an evaluation of MON 87460 under a broad range of environmental conditions relevant to commercial corn production regions. The study was established at six sites in the U.S. during 2006 (Table VIII-2). Each site received water as is typical of the growing area. Four sites were rainfed (IAE, IAW, IL, IN) and two (KS, NE) received normal amounts of supplemental irrigation as needed to produce normal yields. Only five of the sites were useful to collect phenotypic data due to a plot staking error at the KS site in the plots used exclusively for phenotypic data collection. All six sites were used to determine CSPB and NPTII expression levels (Section VI.C) and composition of forage and grain (Section VII) as the staking error did not affect the rows used exclusively for tissue sampling.

*Total of 31 Field Sites for Phenotypic Assessment among the Three Water Regimes*

Phenotypic and agronomic data were collected from 31 field site locations over two years: 13 U.S. sites in 2006, 4 Chilean sites in 2006/2007, and 14 U.S. sites in 2007 (Table VIII-2). As noted previously, phenotypic data were not collected at the KS site in the U.S. 2006 typical agronomic practices study. These field site locations provided a broad range of environmental and agronomic conditions representative of U.S. corn-growing regions. The California and Chile locations selected for a sub-set of these sites provide environments that are well-suited for corn production but typically do not receive any rainfall during the growing season. These environments were used to establish sites with well-watered and water-limited treatments to ensure all water applied to each site occurred through controlled irrigation. Plots were established at each field site as a randomized complete block design with three replications or as strip- or split-plot designs with either three or four replications. Each plot consisted of four to eight rows of corn spaced approximately 30 inches apart and approximately 20 ft in length. Plant growth stage was assessed several times during the growing season.

All combined-site data and sites that met the inclusion criteria for plant characterization assessments requiring both well-watered and water-limited treatments are summarized below in Sections V.C.1 through V.C.3. The following 14 phenotypic and agronomic characteristics were evaluated in each field study: seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight, and yield. The evaluations and timing of plant assessments are described in Table VIII-1. The location of each test site along with year of establishment and water management details are described in Table VIII-2. The phenotypic and agronomic data were analyzed using SAS. Means were calculated across-sites (referred to as combined-site analysis), in which data were pooled from all sites and analyzed statistically, or data are presented for an individual location (referred to as by-site analysis) where appropriate. For each characteristic, MON 87460 was compared to the control. Differences were considered significant at the 5% level ($p \leq 0.05$). Additional details on materials, methods, and results from the individual site analyses, data from sites excluded from analyses, environmental interactions data from individual sites, and water and temperature water data from individual sites are provided in Appendices F, G, H, and I, respectively.

Data collection methods for the agronomic and phenotypic field trials varied by the type of data that were being collected. Seedling vigor, date of 50% pollen shed, date of 50% silking and stay green were reported based on a whole plot visual assessment. Early and final stand counts, dropped ears, stalk lodging and root lodging were reported on a whole plot basis. Plant height and ear height were measured on five representative plants from two rows per plot. Plot weight is a direct measurement of all grain harvested from two rows per plot while grain moisture and test weight are based on a subsample of grain from each plot. Yield is calculated using plot weight, grain moisture and test weight. Abiotic stressors, arthropod damage and disease damage were assessed from two rows on a whole plot basis. Stalk and ear/kernel rot assessments were made on five non-systematically selected plants in two rows from each plot. Arthropods were collected using a sticky trap in the middle of a row in each plot. The sticky traps were left in place for seven days per deployment. European corn borer and corn earworm damage assessments were made on five non-systematically selected plants in three rows from each plot in 2006 and on ten non-systematically selected plants in three rows from each plot in 2007. Tables F-8 through F-13 in Appendix F show the number of replicates planted at each site. For the combined-site analyses, the sample size (n) is the number of replicates multiplied by the number of sites. For the individual site analyses, n is the number of replicates at the site. All sites were planted with three replicates per site except U.S. 2007 Study 1 which had four replicates per site. The standard error values presented in this section and in Appendix F are calculated from the raw data. Thus, each test and control mean has its own individual standard deviation and hence a different standard error.

Regardless of the water regime established, the following studies demonstrate that no phenotypic or agronomic differences were observed for MON 87460 that would lead to a conclusion of increased plant pest potential. MON 87460 is expected to provide reduced

yield loss under water-limited conditions compared to conventional corn. Reduced yield loss is a desirable agronomic characteristic and is not *per se* considered to be associated with plant pest potential.

**Table VIII-2. U.S. and Chile Field Studies Established under Three Different Water Management Regimes for Phenotypic Evaluation of MON 87460 during 2006 and 2007**

| Water Management Treatment / (Experimental Design) | Country/ Year | Location | Site Code |
|---|---|---|---|
| **Well-watered** | U.S. 2006 | Jefferson Co., Iowa | IA1 |
| (Randomized complete block) | | Benton Co., Iowa | IA2 |
| | | Stark Co., Illinois | IL1 |
| | | Warren Co., Illinois | IL2 |
| | | Boone Co., Indiana | IN1 |
| | | Parke Co., Indiana | IN2 |
| | | Pawnee Co., Kansas | KS |
| | | York Co., Nebraska | NE |
| (Randomized complete block) | U.S. 2007 | Jefferson Co., Iowa | IA1 |
| | | Van Horne Co., Iowa | IA2 |
| | | Stark Co., Illinois | IL1 |
| | | Warren Co., Illinois | IL2 |
| | | Clinton Co., Illinois | IL3 |
| | | Boone Co., Indiana | IN |
| | | York Co., Nebraska | NE |
| | | Fayette Co., Ohio | OH |
| | | Berks Co., Pennsylvania | PA |
| **Well-watered and water-limited** | Chile 2006/2007 | Calera de Tango, Chile | CT |
| (Strip-plot design) | | Colina, Chile | CL |
| | | Lumbreras, Chile | LUM |
| | | Quillota, Chile | QUI |
| (Split-plot design) | U.S. 2007 Study-1 | Sutter Co., California | CA |
| | | Carson Co., Texas | TX |
| (Strip-plot design) | U.S. 2007 Study-2 | Pawnee Co., Kansas | KS |
| | | York Co., Nebraska | NE |
| | | Carson Co., Texas | TX |
| **Typical agronomic practices** | U.S. 2006 | Benton Co., Iowa | IAE |
| (Randomized complete block) | | Greene Co., Iowa | IAW |
| | | Stark Co., Illinois | IL |
| | | Parke Co., Indiana | IN |
| | | Pawnee Co., Kansas | KS* |
| | | York Co., Nebraska | NE |

*Phenotypic data were not collected from the KS site in the U.S. 2006 typical agronomic practices study.

**Table VIII-3. Site Inclusion Assessment for U.S. and Chile Field Studies during 2006 and 2007**

| | | Yield ± S.E. (bu/ac) | | | Plant height ± S.E. (in) | | Ear height ± S.E. (in) | | Days to 50% silking ± S.E. | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Study, Location, and Year** | **Site code[1]** | **Well-Watered** | **Water-Limited** | **Reduction (%)[2]** | **Well-Watered** | **Water-Limited** | **Well-Watered** | **Water-Limited** | **Well-Watered** | **Water-Limited** |
| Chile 2006/2007 | CL | 185.5 ± 10.72 | 82.3* ± 11.18 | 56 | 110.7 ± 2.74 | 79.7* ± 7.24 | 63.4 ± 2.38 | 50.9* ± 3.94 | 63.1 ± 0.16 | 63.8 ± 0.21 |
| | CT | 236.5 ± 4.95 | 152.3* ± 9.82 | 36 | 105.9 ± 2.91 | 92.1 ± 2.17 | 55.0 ± 2.08 | 46.0 ± 1.79 | 66.2 ± 0.40 | 67.3 ± 0.16 |
| | LUM | 213.9 ± 4.28 | 94.4* ± 4.81 | 56 | 97.9 ± 1.96 | 75.0* ± 1.22 | 50.4 ± 1.63 | 41.8* ± 0.90 | 70.3 ± 0.36 | 73.7* ± 0.24 |
| | QUI | 203.1 ± 5.27 | 196.3 ± 2.51 | 3 | 112.0 ± 1.72 | 112.8 ± 2.54 | 63.5 ± 2.12 | 63.4 ± 2.06 | 67.7 ± 0.36 | 67.1 ± 0.96 |
| U.S. 2007 Study-1 | CA | 215.9 ±2.01 | 178.8 ±7.91 | 17 | 100.4 ±1.01 | 101.1 ±1.16 | 51.7 ±0.42 | 52.1 ±0.68 | 60.3 ±0.27 | 60.5 ±0.18 |
| | TX | 212.2 ±2.86 | 165.2* ±3.78 | 22 | 72.9 ±0.58 | 62.6* ±0.33 | 32.5 ±0.23 | 29.6* ±0.48 | 57.0 ±0.00 | 57.3* ±0.16 |
| U.S. 2007 Study-2 | KS | 141.0 ± 3.79 | 138.3 ± 7.55 | 2 | 88.1 ± 2.15 | 88.8 ± 2.15 | 42.1 ± 2.29 | 42.5 ± 1.91 | 63.2 ± 0.29 | 62.7 ± 0.24 |
| | NE | 223.0 ± 1.59 | 214.1 ± 1.88 | 4 | 91.0 ± 3.16 | 87.7* ± 3.56 | 41.5 ± 1.71 | 42.1 ± 1.41 | 68.4 ± 0.21 | 68.7 ± 0.14 |
| | TX | 233.7 ± 10.50 | 191.8* ± 8.62 | 18 | 76.1 ± 1.72 | 77.1 ± 2.09 | 27.9 ± 0.67 | 28.2 ± 0.94 | 59.0 ± 0.00 | 59.0 ± 0.00 |

Note: Phenotypic data are mean values for the commercial references planted at each site; S.E. = standard error.

* Indicates statistical difference within site between reference means in the well-watered and water-limited treatments ($p \leq .0.05$) using analysis of variance.

[1]CL = Colina; CT = Calera de Tango; LUM = Lumbreras; QUI = Quillota; CA = California, Study-1; TX = Texas, Study-1 and Study-2; KS = Kansas; NE = Nebraska.

[2]Percent yield reduction calculated as difference between reference mean under well-watered and water-limited conditions, divided by reference mean under well-watered conditions. Sites QUI, KS, and NE did not meet the inclusion criteria of a minimum 15% yield reduction in references as an effect of the water-limited treatment. The lack of differences in plant height, ear height, and days to 50% silking between the water treatments at these sites also confirmed that the plants did not experience meaningful water stress. Consequently, these three sites were not included in assessments that required water-limited conditions.

### VIII.C.1. Field Studies Established under Well-Watered Conditions

Field studies established under well-watered conditions were intended to provide optimal grain yield and allowed an evaluation of MON 87460 in the absence of trait bias, where no statistical differences were expected between MON 87460 and the control.

*U.S. 2006 Well-Watered Assessment*

In 2006, a well-watered field study was established at eight sites (Table VIII-2). Comparative assessments of phenotypic and agronomic characteristics were conducted on MON 87460 and the conventional control. In addition, 19 commercial corn hybrids were included as references. In the combined-site analyses, no differences between MON 87460 and the conventional control were detected for seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ears, stalk lodged plants, final stand count, grain moisture, test weight, or yield (Table VIII-4). The only significant difference detected was an increase in root lodged plants for MON 87460 compared to the control (5.6 vs. 1.5, respectively). Root lodging is a characteristic that may be associated with weediness, because increased lodging can contribute to viable seed being returned to the soil. However, this was not observed consistently for MON 87460. Although the number of root lodged plants was greater for MON 87460 compared to the control, the mean value observed for MON 87460 was within the range of values observed in the commercial references. The difference in root lodged plants does not represent a trend in the data across studies and years and is not considered to be biologically meaningful in terms of increased plant pest potential (Figure VIII-1, Step 3).

*U.S. 2007 Well-Watered Assessment*

In 2007, a well-watered field study was established at nine sites (Table VIII-2). Comparative assessments of phenotypic and agronomic characteristics were conducted on MON 87460 and the conventional control. In addition, 11 commercial corn hybrids were included as references. In the combined-site analysis no differences were detected between MON 87460 and the control for any of the 14 assessed phenotypic and agronomic characteristics (Table VIII-5).

*Summary of U.S. 2006 and 2007 Well-Watered*

The results from these studies support a conclusion of no increased plant pest potential of MON 87460 compared to conventional corn under well-watered conditions.

**Table VIII-4. U.S. 2006: Phenotypic and Agronomic Comparison of MON 87460 to the Control in the Combined-Site Analysis from a 2006 U.S. Field Study Conducted under Well-Watered Conditions**

| | | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| **Phenotypic characteristic** | **Units** | **MON 87460** | **Control** | **Min** | **Max** |
| Seedling vigor | 0-9 scale[2] | 7.2 ± 0.30 | 7.2 ± 0.25 | 6.3 | 8.3 |
| Early stand count | #/plot | 84.8 ± 3.94 | 84.7 ± 4.20 | 71.5 | 115.0 |
| Days to 50% pollen shed | Days | 60.8 ± 0.84 | 60.9 ± 0.82 | 55.3 | 63.8 |
| Days to 50% silking | Days | 60.0 ± 0.65 | 59.8 ± 0.73 | 55.3 | 63.3 |
| Stay green | 0-9 scale[3] | 4.1 ± 0.25 | 4.1 ± 0.22 | 2.7 | 6.5 |
| Ear height | in | 49.6 ± 1.15 | 49.2 ± 1.25 | 31.5 | 53.7 |
| Plant height | in | 97.2 ± 1.84 | 98.8 ± 1.57 | 77.1 | 107.3 |
| Dropped ears | #/plot | 0.4 ± 0.17 | 0.3 ± 0.11 | 0.0 | 2.0 |
| Stalk lodged plants | #/plot | 9.3 ± 3.15 | 8.2 ± 2.36 | 0.3 | 46.0 |
| Root lodged plants | #/plot | 5.6* ± 2.03 | 1.5 ± 0.45 | 0.0 | 30.3 |
| Final stand count | #/plot | 71.8 ± 3.41 | 69.6 ± 3.14 | 52.8 | 96.3 |
| Grain moisture | % | 16.9 ± 0.57 | 17.0 ± 0.60 | 13.3 | 21.7 |
| Test weight | lbs/bu | 57.3 0.61 | 57.7 ± 0.63 | 54.1 | 60.4 |
| Yield | bu/a | 163.9 ± 4.82 | 164.1 ± 5.96 | 151.2 | 209.6 |

S.E. = standard error

***** Indicates a statistically significant difference between the test and control at $p \leq 0.05$.

[1] Reference range = Minimum and maximum mean values among the 19 reference corn hybrids.

[2] Seedling vigor rating scale: 0 = dead and 9 = above average vigor.

[3] Stay green rating scale: 0 = entire plant is dried and 9 = entire plant is green.

**Table VIII-5. U.S. 2007: Phenotypic and Agronomic Comparison of MON 87460 to the Control in the Combined-Site Analysis from a 2007 U.S. Field Study Conducted under Well-Watered Conditions**

| Phenotypic characteristic | Units | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| | | MON 87460 | Control | Min | Max |
| Seedling vigor | 1-9 scale[2] | 3.0 ± 0.34 | 3.0 ± 0.33 | 1.5 | 3.9 |
| Early stand count | #/plot | 87.1 ± 3.87 | 89.4 ± 3.77 | 74.4 | 95.8 |
| Days to 50% pollen shed | Days | 63.6 ± 1.04 | 63.4 ± 1.05 | 60.0 | 68.2 |
| Days to 50% silking | Days | 62.4 ± 1.03 | 62.3 ± 1.01 | 58.6 | 67.0 |
| Stay green | 1-9 scale[3] | 6.0 ± 0.33 | 5.4 ± 0.40 | 4.0 | 5.9 |
| Ear height | in | 46.7 ± 1.47 | 45.2 ± 1.57 | 39.3 | 52.2 |
| Plant height | in | 97.5 ± 2.55 | 96.8 ± 2.76 | 82.2 | 119.4 |
| Dropped ears | #/plot | 0.6 ± 0.16 | 0.5 ± 0.19 | 0.0 | 0.9 |
| Stalk lodged plants | #/plot | 3.5 ± 0.72 | 2.5 ± 0.62 | 0.8 | 5.6 |
| Root lodged plants | #/plot | 0.5 ± 0.25 | 0.5 ± 0.23 | 0.0 | 1.1 |
| Final stand count | #/plot | 71.7 ± 2.78 | 73.1 ± 2.80 | 61.0 | 78.7 |
| Grain moisture | % | 17.3 ± 0.76 | 17.9 ± 0.76 | 15.0 | 23.3 |
| Test weight | lbs/bu | 54.6 ± 0.72 | 55.1 ± 0.43 | 53.1 | 56.9 |
| Yield | bu/a | 162.4 ± 5.82 | 160.6 ± 7.00 | 163.4 | 199.2 |

S.E. = standard error

Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).

[1] Reference range = minimum and maximum values observed among the 11 reference corn hybrids

[2] Seedling vigor rating scale: 1 = above average vigor and 9 = poor.

[3] Stay green rating scale: 1 = 90-100% green and 9 = 0-19% green.

### VIII.C.2. Field Studies Established under Both Well-Watered and Water-Limited Conditions

These field studies were designed to evaluate MON 87460 under water-limited conditions where MON 87460 was expected to reduce yield loss. In addition, the well-watered treatment was included to evaluate whether any potential differences observed between MON 87460 and the control under water-limited conditions (e.g., grain yield) could be attributed to the drought tolerance trait. Data from sites that did not meet the inclusion criteria were not included in statistical analyses, but are provided in Appendix G. Three different studies totaling nine field sites were established in the U.S. and Chile during 2006 and 2007. Due to untimely rainfall during the imposed water-limitation treatments and complications in water management techniques, only six of the nine sites established met the inclusion criteria of having both well-watered and water-limited treatments for plant characterization assessments requiring both treatments.

#### VIII.C.2.1. Chile 2006/2007

*Chile 2006/2007 Well-Watered and Water-Limited*

In 2006/2007, a field study with four sites was established in Chile with well-watered and water-limited treatments (site codes CL, CT, LUM, and QUI) (Table VIII-2).

Comparative assessments of phenotypic and agronomic characteristics were conducted on MON 87460 and a conventional control. In addition, 12 commercial corn hybrids were included as references. Data from three sites met the inclusion criteria and results are presented in Tables VIII-6 and VIII-7. The QUI site was not established with the appropriate water stress treatments; thus, data for this site were not included in the statistical analysis. Data from the QUI site are presented in Appendix G. They appeared consistent with other phenotypic data for MON 87460 and did not impact the conclusions of the plant pest risk assessment

*Assessment*

In the combined-site analysis of the well-watered treatment in Chile, no differences were detected between MON 87460 and the conventional control for any of the 14 assessed phenotypic and agronomic characteristics (Table VIII-6). In the combined-site analysis of the water-limited treatment in Chile, one significant difference was detected between MON 87460 and the control out of the 14 assessed characteristics. As expected, MON 87460 exhibited higher yield (reduced yield loss) ($p \leq 0.05$) than the conventional control (114.5 vs. 86.7 bushels/acre, respectively) under water-limited conditions (Table VIII-7). Reduced yield loss is a desirable agronomic characteristic and is not *per se* considered to be associated with plant pest potential.

*Summary of Chile 2006/2007 Well-Watered and Water-Limited*

The results from this study support the expectation of no statistically significant differences between MON 87460 and the control under well-watered conditions and a yield advantage for MON 87460 over the control under water-limited conditions.

**Table VIII-6. Chile 2006/2007: Phenotypic and Agronomic Comparison of MON 87460 to the Control under Well-Watered Conditions in the Combined-Site Analysis in 2006/2007 Chilean Field Trials Established with Well-Watered and Water-Limited Treatments**

**Well-Watered Treatment**

| Phenotypic characteristic | Units | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| | | MON 87460 | Control | Min | Max |
| Seedling vigor | 0-9 scale[2] | 4.9 ± 0.20 | 4.7 ± 0.17 | 4.3 | 6.0 |
| Early stand count | #/plot | 76.1 ± 1.48 | 73.0 ± 2.53 | 71.0 | 80.0 |
| Days to 50% pollen shed | Days | 66.8 ± 1.35 | 66.7 ± 1.17 | 65.0 | 74.3 |
| Days to 50% silking | Days | 65.2 ± 1.04 | 65.3 ± 0.91 | 62.7 | 71.0 |
| Stay green | 0-9 scale[3] | 2.4 ± 0.50 | 2.9 ± 0.65 | 1.0 | 6.7 |
| Ear height | in | 55.9 ± 2.81 | 52.8 ± 2.03 | 46.1 | 69.1 |
| Plant height | in | 101.1 ± 3.18 | 99.0 ± 2.13 | 94.4 | 116.4 |
| Dropped ears[4] | #/plot | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 |
| Stalk lodged plants[4] | #/plot | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 |
| Root lodged plants[4] | #/plot | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 |
| Final stand count | #/plot | 75.2 ± 1.40 | 74.0 ± 1.95 | 71.3 | 79.3 |
| Grain moisture | % | 14.8 ± 1.00 | 15.2 ± 1.27 | 10.1 | 20.2 |
| Test weight | lbs/bu | 56.4 ± 0.64 | 55.8 ± 0.84 | 54.0 | 61.2 |
| Yield | bu/a | 220.7 ± 7.87 | 220.0 ± 10.19 | 166.7 | 248.4 |

S.E. = standard error

Note: No statistically significant differences between MON 87460 and the control (α=0.05).

[1] Reference range was calculated from the 3 sites exhibiting a water treatment effect among the 12 reference corn hybrids.

[2] Seedling vigor rating scale: 0 = dead and 9 = above average vigor.

[3] Stay green rating scale: 0 = entire plant is dried and 9 = entire plant is green.

[4] No statistical comparisons were made due to lack of variability in the data. The test was considered effectively not different from the control because the test and control mean values were identical.

**Table VIII-7. Chile 2006/2007: Phenotypic and Agronomic Comparison of MON 87460 to the Control under Water-Limited Conditions in the Combined-Site Analysis in 2006/2007 Chilean Field Trials Established with Well-Watered and Water-Limited Treatments**

**Water-Limited Treatment**

| Phenotypic characteristic | Units | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| | | MON 87460 | Control | Min | Max |
| Seedling vigor | 0-9 scale[2] | 5.0 ± 0.29 | 4.8 ± 0.22 | 4.0 | 6.0 |
| Early stand count | #/plot | 76.8 ± 0.81 | 75.7 ± 1.35 | 67.3 | 80.7 |
| Days to 50% pollen shed | Days | 67.4 ± 1.30 | 68.1 ± 1.47 | 65.7 | 75.0 |
| Days to 50% silking | Days | 67.3 ± 1.70 | 66.8 ± 1.75 | 63.3 | 74.3 |
| Stay green | 0-9 scale[3] | 4.3 ± 0.78 | 4.7 ± 0.80 | 1.0 | 7.0 |
| Ear height | in | 48.0 ± 4.86 | 45.1 ± 3.86 | 40.0 | 60.5 |
| Plant height | in | 83.9 ± 5.94 | 78.1 ± 5.44 | 64.9 | 96.8 |
| Dropped ears[4] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| Stalk lodged plants[4] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| Root lodged plants[4] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| Final stand count | #/plot | 76.7 ± 0.91 | 75.1 ± 1.23 | 71.3 | 80.7 |
| Grain moisture | % | 19.5 ± 2.53 | 21.3 ± 3.29 | 9.6 | 25.5 |
| Test weight | lbs/bu | 56.7 ± 1.20 | 56.0 ± 1.33 | 51.3 | 62.2 |
| Yield | bu/a | 114.5* ± 16.04 | 86.7 ± 14.17 | 56.4 | 167.6 |

S.E. = standard error

* Indicates statistical difference between the test and the control ($p \leq 0.05$).

[1] Reference range was calculated from the 3 sites exhibiting a water treatment effect among the 12 reference corn hybrids.

[2] Seedling vigor rating scale: 0 = dead and 9 = above average vigor.

[3] Stay green rating scale: 0 = entire plant is dried and 9 = entire plant is green.

[4] No statistical comparisons were made due to lack of variability in the data. The test was considered effectively not different from the control because the test and control mean values were identical.

### VIII.C.2.2. U.S. 2007

*U.S. 2007 Well-Watered and Water-Limited*

In 2007, two separate field studies were conducted in the U.S. where sites were established with well-watered and water-limited treatments (Table VIII-2). The two trials had different experimental designs which precluded a combined-study analysis.

*U.S. 2007 – Study-1*

In Study-1, two sites were established (site codes CA and TX) with well-watered and water-limited treatments (Table VIII-2). Comparative assessments of phenotypic and agronomic characteristics were conducted on MON 87460 and a conventional control. In addition, seven commercial corn hybrids were included as references. A total of 14 different phenotypic and agronomic characteristics were evaluated. In the combined-site analysis for the well-watered plots, no statistical differences were detected between MON 87460 and the conventional control for any of the assessed phenotypic and agronomic characteristics (Table VIII-8). The results from this study support the expectation of no differences between MON 87460 and the control under well-watered conditions. In the combined-site analysis of the water-limited treatment, no statistical differences were detected between MON 87460 and the conventional control for any of the assessed phenotypic and agronomic characteristics (Table VIII-9).

*U.S. 2007 – Study-2*

In Study-2, three sites were established with well-watered and water-limited treatments (site codes KS, NE, and TX) (Table VIII-2). Comparative assessments of phenotypic and agronomic characteristics were conducted on MON 87460 and a conventional control. In addition, 12 commercial corn hybrids were included as references in the well-watered plots (KS, NE, and TX), and four additional references hybrids were included from the TX water-limited plots. A total of 14 different phenotypic and agronomic characteristics were evaluated. TX was the only site to meet the inclusion criteria for both well-watered and water-limited treatments. Due to rainfall during the imposed water-limitation treatments at the KS and NE sites, the well-watered treatments met the inclusion criteria but the water-limited treatments did not (Figure VIII-2, Step 2). Thus, the water-limited treatment data from KS and NE were not included in the statistical analysis. The KS and NE data excluded from analyses are provided in Appendix G. They appeared consistent with other phenotypic data for MON 87460 and did not impact the conclusions of the plant pest risk assessment.

*Assessment of U.S. 2007 Study-1*

No statistical differences between MON 87460 and the control were observed in the combined-site analysis.

*Assessment of U.S. 2007 Study-2*

In the combined-site analysis for the well-watered plots (KS, NE and TX), no statistical differences were detected between MON 87460 and the conventional control for seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant

height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, and yield (Table VIII-10). The only statistical difference detected was a lower stay green rating (more green tissue) compared to the control (5.8 vs. 6.7, respectively). Stay green is an indication of plant senescence and more green tissue at this late reproductive stage, just prior to harvest, is not a characteristic that is likely associated with an increase in plant pest potential (Radosevich et al., 1997). Although MON 87460 had more green tissue compared to the control, the mean value observed for MON 87460 was within the range of values observed in the commercial references. The difference in stay green rating was small in magnitude, does not represent a trend in the data across studies and years, and is not considered biologically meaningful in terms of increased plant pest potential (Figure VIII-1, Step 3).

In the single-site analysis of the water-limited treatment in TX, no statistical differences were detected between MON 87460 and the conventional control for seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, and yield (Table VIII-11). The only significant difference detected was a lower stay green rating (more green tissue) compared to the control (6.3 vs. 8.3, respectively). More green tissue in MON 87460 is not a characteristic that is interpreted as an increase in plant pest potential. Furthermore, this difference in stay green was not consistently observed in the water-limited treatment at other sites. Thus, the difference detected in stay green at this single site was small in magnitude, does not represent a trend in the data, and is not considered to be biologically meaningful in terms of increased plant pest potential (Figure VIII-1, Step 5).

*Summary of U.S. 2007 Studies 1 and 2 Well-Watered and Water-Limited*

Results of the 2007 studies in the U.S. demonstrate that the observed phenotypic and agronomic characteristics for MON 87460 were within the range of responses expected for corn under well-watered and water-limited conditions. Based on the measured phenotypic and agronomic characteristics, the results support a conclusion of no increased plant pest potential of MON 87460 compared to conventional corn.

**Table VIII-8. U.S. 2007 Study-1: Phenotypic and Agronomic Comparison of MON 87460 to the Control under Well-Watered Conditions in the Combined-Site Analysis in 2007 U.S. Field Trials Established with Well-Watered and Water-Limited Treatments**

**Well-Watered Treatment**

| Phenotypic characteristic | Units | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| | | MON 87460 | Control | Min | Max |
| Seedling vigor | 1-9 scale[2] | 3.0 ± 0.76 | 3.0 ± 0.76 | 1.0 | 5.0 |
| Early stand count | #/plot | 90.4 ± 5.62 | 88.3 ± 7.28 | 74.5 | 98.5 |
| Days to 50% pollen shed | Days | 60.8 ± 1.08 | 60.9 ± 1.22 | 57.8 | 63.5 |
| Days to 50% silking | Days | 58.5 ± 0.57 | 58.4 ± 0.60 | 57.0 | 60.3 |
| Stay green | 1-9 scale[3] | 2.1 0.35 | 3.1 ± 0.30 | 2.5 | 4.5 |
| Ear height | in | 42.3 ± 3.46 | 41.3 ± 2.66 | 32.2 | 52.7 |
| Plant height | in | 85.5 ± 5.84 | 85.9 ± 4.23 | 72.2 | 101.7 |
| Dropped ears | #/plot | 0.0 ± 0.00 | 0.1 ± 0.13 | 0.0 | 0.3 |
| Stalk lodged plants | #/plot | 0.3 ± 0.16 | 0.1 ± 0.13 | 0.0 | 0.6 |
| Root lodged plants | #/plot | 0.3 ± 0.16 | 0.6 ± 0.38 | 0.0 | 7.3 |
| Final stand count | #/plot | 79.8 ± 6.15 | 78.9 ± 6.03 | 62.3 | 93.3 |
| Grain moisture | % | 14.7 ± 0.14 | 14.6 ± 0.23 | 13.5 | 15.4 |
| Test weight[4] | lbs/bu | 58.4 ±0.96 | 57.8 ± 0.63 | 57.6 | 58.8 |
| Yield | bu/a | 227.5 ± 6.41 | 200.2 ± 11.65 | 205.0 | 220.3 |

S.E. = standard error

Note: No statistically significant differences between MON 87460 and the control (α=0.05).

[1] Reference range = Minimum and maximum mean values among the seven references.

[2] Seedling vigor rating scale: 1 = above average vigor and 9 = poor.

[3] Stay green rating scale: 1 = 90-100% green and 9 = 0-19% green

[4]Test weight values reported from the TX site only. Data not provided from the CA site.

**Table VIII-9. U.S. 2007 Study-1: Phenotypic and Agronomic Comparison of MON 87460 to the Control under Water-Limited Conditions in the Combined-Site Analysis in 2007 U.S. Field Trials Established with Well-Watered and Water-Limited Treatments**

**Water-Limited Treatment**

| Phenotypic Characteristic | Units | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| | | MON 87460 | Control | Min | Max |
| Seedling vigor | 1-9 scale[2] | 3.3 ± 0.81 | 3.0 ± 0.76 | 1.0 | 5.0 |
| Early stand count | #/plot | 85.3 ± 5.77 | 90.8 ± 6.53 | 72.5 | 106.5 |
| Days to 50% pollen shed | Days | 60.9 ± 1.26 | 61.4 ± 1.05 | 58.3 | 63.5 |
| Days to 50% silking | Days | 59.1 ± 0.77 | 58.5 ± 0.73 | 57.0 | 60.5 |
| Stay green | 1-9 scale[3] | 3.0 ± 0.53 | 4.1 ± 0.35 | 2.9 | 5.5 |
| Ear height | in | 41.7 ± 4.87 | 40.2 ± 4.06 | 28.7 | 54.1 |
| Plant height | in | 82.5 ± 7.07 | 78.7 ± 6.30 | 61.7 | 103.4 |
| Dropped ears | #/plot | 0.4 ± 0.30 | 0.8 ± 0.37 | 0.0 | 0.8 |
| Stalk lodged plants | #/plot | 0.6 ± 0.43 | 0.8 ± 0.41 | 0.0 | 1.5 |
| Root lodged plants | #/plot | 2.7 ± 2.09 | 0.8 ± 0.49 | 0.0 | 4.3 |
| Final stand count | #/plot | 77.0 ± 6.73 | 79.6 ± 6.20 | 61.8 | 96.0 |
| Grain moisture | % | 14.7 ± 0.35 | 14.5 ± 0.19 | 13.5 | 14.8 |
| Test weight[4] | lbs/bu | 57.6 ± 0.74 | 56.7 ± 0.72 | 56.5 | 57.2 |
| Yield | bu/a | 187.3 ± 16.88 | 160.6 ± 14.10 | 156.5 | 191.0 |

S.E. = standard error

Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).

[1] Reference range = Minimum and maximum mean values among the seven references.

[2] Seedling vigor rating scale: 1 = above average vigor and 9 = poor.

[3] Stay green rating scale: 1 = 90-100% green and 9 = 0-19% green

[4]Test weight values reported from the TX site only. Data not provided from the CA site.

**Table VIII-10. U.S. 2007 Study-2: Phenotypic and Agronomic Comparison of MON 87460 to the Control under Well-Watered Conditions in the Combined-Site Analysis in 2007 U.S. Field Trials Established with Well-Watered and Water-Limited Treatments**

**Well-Watered Treatment**

| | | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| **Phenotypic characteristic** | **Units** | **MON 87460** | **Control** | **Min** | **Max** |
| Seedling vigor | 1-9 scale[2] | 2.3 ± 0.29 | 2.2 ± 0.32 | 1.3 | 2.7 |
| Early stand count | #/plot | 55.8 ± 4.32 | 59.2 ± 2.62 | 52.0 | 68.7 |
| Days to 50% pollen shed[3] | Days | 63.6 ± 0.82 | 63.3 ± 0.87 | 61.0 | 69.0 |
| Days to 50% silking[3] | Days | 62.2 ± 1.02 | 62.3 ± 1.03 | 59.0 | 69.0 |
| Stay green | 1-9 scale[4] | 5.8* ± 0.32 | 6.7 ± 0.67 | 2.0 | 9.0 |
| Ear height | in | 37.9 ± 3.35 | 37.6 ± 2.55 | 26.0 | 45.8 |
| Plant height | in | 84.2 ± 2.73 | 83.9 ± 2.59 | 72.8 | 100.1 |
| Dropped ears | #/plot | 0.4 ± 0.24 | 0.3 ± 0.24 | 0.0 | 1.0 |
| Stalk lodged plants[3] | #/plot | 1.2 ± 0.46 | 1.9 ± 0.77 | 0.0 | 14.3 |
| Root lodged plants | #/plot | 0.9 ± 0.65 | 5.1 ± 4.99 | 0.0 | 9.7 |
| Final stand count | #/plot | 52.7 ± 3.16 | 56.1 ± 1.93 | 52.7 | 63.7 |
| Grain moisture | % | 13.9 ± 0.42 | 13.7 ± 0.53 | 12.3 | 17.3 |
| Test weight | lbs/bu | 60.5 ± 0.27 | 60.7 ± 0.26 | 59.3 | 61.5 |
| Yield | bu/a | 192.3 ± 17.07 | 189.6 ± 23.59 | 133.3 | 261.3 |

S.E. = standard error

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Reference range = minimum and maximum mean values among the 12 references at KS, NE, and TX.

[2] Seedling vigor rating scale: 1 = above average vigor and 9 = below average vigor.

[3] No comparisons were made for Days to 50% pollen shed, Days to 50% silking, and Stalk lodged plants due to a lack of variability.

[4] Stay green rating scale: 1 = 90-100% green and 9 = 0-19% green.

**Table VIII-11. U.S. 2007 Study-2: Phenotypic and Agronomic Comparison of MON 87460 to the Control under Water-Limited Conditions in a 2007 Texas Field Trial Established with Well-Watered and Water-Limited Treatments**

**Water-Limited Treatment**

| | | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| **Phenotypic characteristic** | **Units** | **MON 87460** | **Control** | **Min** | **Max** |
| Seedling vigor | 1-9 scale[2] | 1.7 ± 0.33 | 1.7 ± 0.33 | 1.7 | 2.0 |
| Early stand count | #/plot | 55.3 ± 3.33 | 44.0 ± 7.77 | 51.3 | 59.0 |
| Days to 50% pollen shed[3] | Days | 61.0 ± 0.00 | 61.0 ± 0.00 | 61.0 | 61.0 |
| Days to 50% silking[3] | Days | 59.0 ± 0.00 | 59.0 ± 0.00 | 59.0 | 59.0 |
| Stay green | 1-9 scale[4] | 6.3* ± 0.33 | 8.3 ± 0.33 | 8.3 | 9.0 |
| Ear height | in | 25.5 ± 2.02 | 27.9 ± 2.51 | 26.6 | 30.3 |
| Plant height | in | 73.5 ± 3.18 | 75.6 ± 1.63 | 74.1 | 83.2 |
| Dropped ears | #/plot | 0.0 ± 0.00 | 0.7 ± 0.67 | 0.0 | 0.7 |
| Stalk lodged plants[3] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| Root lodged plants | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.3 |
| Final stand count | #/plot | 54.7 ± 2.67 | 44.0 ± 7.77 | 49.3 | 58.0 |
| Grain moisture | % | 13.0 ± 0.07 | 12.7 ± 0.18 | 12.3 | 12.8 |
| Test weight | lbs/bu | 59.7 ± 0.26 | 59.7 ± 0.52 | 59.6 | 60.4 |
| Yield | bu/a | 228.3 ± 11.53 | 168.8 ± 27.78 | 173.8 | 208.1 |

S.E. = standard error

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Reference range = minimum and maximum mean values among the four references at TX.

[2] Seedling vigor rating scale: 1 = above average vigor and 9 = below average vigor.

[3] No comparisons were made for Days to 50% pollen shed, Days to 50% silking, and Stalk lodged plants due to a lack of variability.

[4] Stay green rating scale: 1 = 90-100% green and 9 = 0-19% green.

## VIII.C.3. Field Studies Established under Water Conditions Typical for Local Agronomic Practice

This field study was designed to evaluate MON 87460 under a broad range of environmental conditions relevant to commercial corn production. No differences were expected between MON 87460 and the control with the exception of reduced yield loss for MON 87460 if soil moisture became limiting.

*Assessment*

In 2006, a field study with water managed according to local practices was established at five sites (Table VIII-2). Comparative assessments of phenotypic and agronomic characteristics were conducted on MON 87460 and a conventional control. In addition, 15 commercial corn hybrids were included as references. In the combined-site analysis, no statistical differences were detected between MON 87460 and the control for any of the 14 assessed phenotypic and agronomic characteristics (Table VIII-12).

*Summary of U.S. 2006 under Conditions Typical for Local Agronomic Practice*

The results from these studies support a conclusion of no increased plant pest potential of MON 87460 compared to conventional corn under conditions where water was managed according to typical local agronomic practices.

**Table VIII-12. Phenotypic and Agronomic Comparison of MON 87460 to the Control in the Combined-Site Analysis from a 2006 U.S. Field Study Conducted under Typical Agronomic Conditions**

| Phenotypic characteristic | Units | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|---|
| | | MON 87460 | Control | Min | Max |
| Seedling vigor | 0-9 scale[2] | 7.6 ± 0.13 | 7.8 ± 0.14 | 7.0 | 8.0 |
| Early stand count | #/plot | 64.5 ± 0.67 | 62.3 ± 1.21 | 58.7 | 72.3 |
| Days to 50% pollen shed | Days | 63.1 ± 0.55 | 63.1 ± 0.53 | 59.0 | 65.0 |
| Days to 50% silking | Days | 62.2 ± 0.71 | 61.7 ± 0.69 | 57.3 | 64.7 |
| Stay green | 0-9 scale[3] | 3.7 ± 0.52 | 3.7± 0.55 | 1.0 | 6.7 |
| Ear height | in | 45.6 ± 1.54 | 44.9 ± 1.53 | 31.9 | 51.2 |
| Plant height | in | 97.8 ± 1.74 | 97.2 ± 1.72 | 84.9 | 108.3 |
| Dropped ears | #/plot | 0.1 ± 0.13 | 0.1 ± 0.09 | 0.0 | 0.7 |
| Stalk lodged plants | #/plot | 5.5 ± 1.03 | 5.1 ± 1.55 | 0.3 | 7.7 |
| Root lodged plants | #/plot | 2.1 ± 0.64 | 1.1 ± 0.51 | 0.0 | 5.7 |
| Final stand count | #/plot | 57.9 ± 0.82 | 57.4 ± 0.96 | 53.5 | 58.7 |
| Grain moisture | % | 17.5 ± 0.52 | 17.7 ± 0.63 | 15.5 | 22.6 |
| Test weight | lbs/bu | 55.3 ± 0.73 | 54.8 ± 0.95 | 49.7 | 60.3 |
| Yield | bu/a | 170.2 ± 6.26 | 165.3 ± 7.41 | 143.6 | 213.4 |

S.E. = standard error
Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).
[1] Reference range = Minimum and maximum mean values among the 15 reference corn hybrids.
[2] Seedling vigor rating scale: 0 = dead and 9 = above average vigor.
[3] Stay green rating scale: 0 = entire plant is dried and 9 = entire plant is green.

## VIII.D. Seed Germination and Dormancy Assessment

APHIS considers the potential for weediness to constitute a plant pest factor. (Section IX). As mentioned in Section VIII.A, several weediness indicators (lodging, ear drop) were included in the testing described in Section VIII.C and no biologically meaningful differences were shown between MON 87460 and conventional corn. Other indicators, specifically seed germination and dormancy, are also relevant to weediness determinations, and these were tested separately as described below.

The purpose of this study was to assess seed germination and dormancy characteristics of MON 87460 compared to a conventional control, which had a genetic background similar to MON 87460 but did not possess the drought tolerance trait. In addition, seed from multiple conventional corn hybrids (references) were included in the analysis to establish a range of natural variability for each characteristic. Seed germination and dormancy mechanisms vary with species and their genetic basis tends to be complex. Seed dormancy (e.g., hard seed) is an important characteristic that is often associated with plants that are considered as weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003). Information on germination and dormancy is therefore useful when assessing a plant for

increased weediness potential. For corn, the potential for hard seed (dormant) is negligible or nonexistent. Standardized germination assays are available and routinely used to measure the germination characteristics of corn seed. The Association of Official Seed Analysts (AOSA), an internationally recognized seed testing organization, recommends a temperature range of 20-30°C as optimal for germination of corn (AOSA, 2006). The results of this study, in particular the absence of hard seed, support a conclusion of no increased weediness potential of MON 87460 compared to conventional corn.

Seed of MON 87460, the conventional control, and three commercial corn hybrids (references; nine total, three per location) were produced in 2006 at three locations (Greene County, IA; Stark County, IL; and Pawnee County, KS) with variable environmental conditions representative of corn producing regions in the U.S. The conventional control corn had a genetic background similar to MON 87460.

The germination characteristics, temperature regimes and the evaluation descriptions are presented in Table VIII-13. The tests were conducted in temperature-controlled growth chambers using the rolled towel test method. Four replicates of the seed materials from each location were tested in seven growth chambers, each maintained in the dark under one of the following temperature regimes: constant target temperature of approximately 5, 10, 20, or 30°C; and alternating target temperatures of approximately 10/20, 10/30, or 20/30°C (Table VIII-13). In the alternating temperature regimes, the lower temperature was maintained for 16 h and the higher temperature for 8 h. Seeds were evaluated four and seven days after planting in the AOSA-recommended temperature regime (20/30°C) and at four, seven, and twelve days after planting in the additional temperature regimes.

A statistical comparison between MON 87460 and the control was conducted using SAS (SAS Release 9.1.3 (TS1M3). 2002-2003). Statistical significance was set at $p \leq 0.05$. A summary of the results is provided in Table VIII-14, while the details of the materials, methods, and results from individual sites analysis are provided in Appendix J.

In the initial analysis, no production site × seed substance interactions were detected for any combination except percent germinated and percent viable firm swollen seed at 10°C. These two characteristics were analyzed separately by site to account for the interaction. In the combined site analysis, no statistical differences were detected ($\alpha = 0.05$) between MON 87460 and the control in any temperature regime for any characteristic (27 comparisons, Table VIII-14).

As noted above, significant production site × seed substance type interactions were detected in percent germinated and percent viable firm swollen seed at 10°C. Analyses were therefore conducted on an individual site basis for these variables at 10°C. No differences were detected between MON 87460 and the control for seed from the IL or KS production sites. For the IA site, MON 87460 had greater percent germination and lower percent viable firm swollen seed than the control (91.5 vs. 87.0; 6.5 vs. 11.2, respectively). However, no differences were detected at other sites, and for the IA site, the values for MON 87460 and the control at 10°C were within the reference range for

both percent germination and percent viable firm swollen seed. Thus, the difference is not expected to constitute a meaningful biological change for the crop in terms of weediness potential (Figure VIII-1, Step 3).

The biological characteristics evaluated in this study were used to characterize MON 87460 in the context of plant pest risk assessment. The results of this study, in particular the absence of hard seed, support a conclusion of no increased weediness potential of MON 87460 compared to conventional corn.

**Table VIII-13. Seed Germination and Dormancy Parameters Evaluated**

| Germination characteristic | Temperature regime (°C) | Evaluation description |
|---|---|---|
| Normally Germinated | 20/30 | Seedlings that exhibited normal developmental characteristics and possessed both a root and a shoot. |
| Abnormally Germinated | 20/30 | Germinated, but with insufficient root or shoot development, may have possessed a hollow coleoptile, or exhibited mechanical damage. |
| Total Germinated | 5, 10, 20, 30, 10/20, 10/30 | Seedlings that had germinated. |
| Dead | 5, 10, 20, 30, 10/20, 10/30, 20/30 | Seeds that had visibly deteriorated and had become soft to the touch. |
| Viable Hard | 5, 10, 20, 30, 10/20, 10/30, 20/30 | Seeds that did not imbibe water and remained hard to the touch. |
| Viable Firm Swollen | 5, 10, 20, 30, 10/20, 10/30, 20/30 | Seeds that had visibly swollen (imbibed water) and were firm to the touch but lacked any evidence of growth. |

Note: Germination tests were conducted in temperature-controlled growth chambers using the rolled towel test method to measure the described characteristics. Four replicates of MON 87460, the control, and the nine references were tested in seven growth chambers, each maintained in the dark under one of the following temperature regimes: constant target temperature of approximately 5, 10, 20, or 30°C; and alternating target temperatures of approximately 10/20, 10/30, or 20/30°C. In these alternating temperature regimes, the lower temperature was maintained for 16 h and the higher temperature for 8 h. Counts for characteristics were made four and seven days after planting for seed placed in the AOSA–recommended temperature of 20/30°C; counts were made on four, seven, and twelve days after planting for seed in the additional temperature regimes.

**Table VIII-14. Germination of MON 87460 and a Conventional Control Corn across Sites[1]**

| | | Mean % ± S.E. | | Reference Range | |
|---|---|---|---|---|---|
| **Temperature** | **Characteristic** | **MON 8746** | **Control** | **Min** | **Max** |
| 5°C | Dead | 3.9 ± 0.90 | 4.5 ± 0.93 | 4.0 | 11.0 |
| | Germinated | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.3 |
| | Viable firm swollen | 96.1 ± 0.90 | 95.5 ± 0.93 | 89.0 | 96.0 |
| | Viable hard[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| 10°C | Dead | 1.0 ± 0.30 | 1.0 ± 0.32 | 0.8 | 6.0 |
| | Germinated[3] | - | - | - | - |
| | Viable firm swollen[3] | - | - | - | - |
| | Viable hard[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| 20°C | Dead | 0.6 ± 0.23 | 0.3 ± 0.18 | 0.5 | 5.8 |
| | Germinated | 99.4 ± 0.23 | 99.8 ± 0.18 | 94.3 | 99.5 |
| | Viable firm swollen[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| | Viable hard[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| 30°C | Dead | 1.2 ± 0.55 | 1.7 ± 0.36 | 0.5 | 6.0 |
| | Germinated | 98.8 ± 0.55 | 98.3 ± 0.36 | 94.0 | 99.5 |
| | Viable firm swollen[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| | Viable hard[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| 20 / 30°C | Dead | 0.5 ± 0.19 | 0.9 ± 0.23 | 0.3 | 4.3 |
| (AOSA) | Normal germinated | 98.7 ± 0.38 | 98.4 ± 0.26 | 93.3 | 98.0 |
| | Abnormal | 0.8 ± 0.30 | 0.7 ± 0.22 | 1.0 | 4.3 |
| | Viable firm swollen[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| | Viable hard[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| 10 / 20°C | Dead | 1.0 ± 0.39 | 1.7 ± 0.34 | 0.3 | 5.0 |
| | Germinated | 99.0 ± 0.39 | 98.3 ± 0.34 | 95.0 | 99.8 |
| | Viable firm swollen[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| | Viable hard[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| 10 / 30°C | Dead | 1.5 ± 0.34 | 1.3 ± 0.37 | 0.3 | 5.0 |
| | Germinated | 98.5 ± 0.34 | 98.8 ± 0.37 | 95.0 | 99.8 |
| | Viable firm swollen[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |
| | Viable hard[2] | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 | 0.0 |

S.E. = standard error; n = 12 for MON 87460 and control.

Note: No statistically significant differences between MON 87460 and the control (α=0.05).

[1] Seed was produced at Greene County, IA, Stark County, IL, and Pawnee County, KS, in the U.S.

[2] Analysis could not be conducted due to lack of variability in the data. For these data the values were all zero, indicating no biological differences.

[3] Across-site analyses could not be run due to site × substance interactions.

The data in this table are the combined-site results of the seeds from the three production sites. The results for the individual site analysis where the site × substance interaction occurred are included in Appendix J.

## VIII.E. Pollen Morphology and Viability Assessment

APHIS also considers the potential for gene flow to, and introgression of the biotechnology-derived trait into, other corn varieties to determine the potential of increased weedy or invasive characteristics in other plant species. Therefore, pollen morphology and viability of MON 87460 were also assessed.

The purpose of this study was to assess whether the introduction of the drought tolerance trait and the expression of the CSPB protein altered the pollen characteristics of MON 87460 compared to a conventional control, which had a genetic background similar to MON 87460 but did not possess the drought tolerance trait. In addition, pollen from multiple conventional corn hybrids (references) was included in the analysis to establish a range of natural variability for each characteristic. Pollen was collected from four replications of MON 87460, the control, and four commercial corn references grown in California during 2007 under a split-plot design, under well-watered and water-limited conditions (Table VIII-2). Pollen samples from three plants per plot were fixed and stained with 1:5 diluted Alexander stain (Alexander, 1980) and evaluated for viability and general morphology. Data analysis compared the mean of MON 87460 to the mean of the control corn for average pollen diameter and percent viable pollen. Additional materials and methods are provided in Appendix K.

No differences were detected ($\alpha = 0.05$) between MON 87460 and the control for pollen diameter or viability in either the well-watered or the water-limited treatment (Tables VIII-15 and VIII-16). No visual differences in general pollen morphology were observed between MON 87460 and the control. These results demonstrate that the introduction of the drought tolerance trait and the expression of the CSPB protein did not alter the overall morphology or viability of pollen from MON 87460 compared to the control under well-watered or water-limited conditions. The lack of differences between the pollen collected from MON 87460 compared to the conventional control for the assessed characteristics demonstrate that the observed values were within the range of responses expected for corn.

**Table VIII-15. Viability and Diameter of Pollen Collected from MON 87460 and a Conventional Control Corn under Well-Watered Conditions in 2007**

| | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|
| **Characteristic** | **MON 87460** | **Control** | **Min** | **Max** |
| Viability (%) | 98.4 ± 0.50 | 98.8 ± 0.44 | 97.4 | 98.6 |
| Diameter (μm) | 86.3 ± 1.35 | 86.5 ± 1.20 | 85.5 | 88.6 |

S.E. = standard error; n = 4 for MON 87460 and control.
No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).
[1] Minimum and maximum mean values of reference substances. References were four commercial hybrids.

**Table VIII-16. Viability and Diameter of Pollen Collected from MON 87460 and a Conventional Control Corn under Watered-Limited Conditions in 2007**

| | Mean ± S.E. | | Reference Range[1] | |
|---|---|---|---|---|
| **Characteristic** | **MON 87460** | **Control** | **Min** | **Max** |
| Viability (%) | 96.9 ± 0.64 | 97.2 ± 0.67 | 96.3 | 98.5 |
| Diameter (μm) | 86.9 ± 1.01 | 85.6 ± 0.73 | 86.2 | 89.7 |

S.E. = standard error; n = 4 for MON 87460 and control.
No statistically significant differences between MON 87460 and the control (α=0.05).
[1] Minimum and maximum mean values of reference substances. References were four commercial hybrids.

## VIII.F. Environmental Interactions Assessment

Environmental interactions evaluations were conducted as part of the plant characterization studies for MON 87460. These data are used to evaluate plant pest potential for MON 87460 compared to a conventional control. In addition, multiple conventional corn hybrids (references) were included in the analysis to establish a range of natural variability for each characteristic. The environmental interactions evaluation included data collected in the phenotypic studies (plant-insect, plant-disease, and plant-environment interactions) and studies on abiotic stress tolerance, potential for the crop to volunteer, and survival outside of cultivation.

These environmental interactions characteristics are all useful to assess plant pest potential of MON 87460. In addition, certain characteristics, including volunteer potential and survival outside cultivation, can be used for an assessment of weediness, an element of plant pest potential. Abiotic stress tolerance was evaluated qualitatively from natural occurrences in the phenotypic field studies and also quantitatively under controlled conditions in greenhouse and growth chamber studies. Studies to assess tolerance to drought, cold, heat, and salt stress at sensitive young growth stages under controlled conditions are useful for characterizing the extent of stress tolerance imparted by the insertion of the *cspB* gene. Such a characterization is useful to identify any potential changes in tolerance that warrant further investigation of plant pest risk under stress conditions. If no changes in tolerance are observed, further investigation is not warranted because the characteristics of the biotechnology-derived plant are not meaningfully different from the control under stress conditions.

Results of the environmental interactions assessment show the drought tolerance trait did not unexpectedly alter MON 87460 compared to conventional corn. The lack of differences in plant response to abiotic stressors (excluding drought stress), disease damage, arthropod damage, and arthropod pest and beneficial abundance indicate that the introduction of the drought tolerance trait is unlikely to be biologically meaningful in terms of increased plant pest potential. Finally, arthropod (pest and beneficial) abundance indicates no significant impact on non-target organisms for purposes of NEPA.

### VIII.F.1. Insect, Disease, and Abiotic Stress Interactions Assessment in Field Studies

In the two years of field studies conducted for evaluation of phenotypic and agronomic characteristics of MON 87460, observational data on the presence of and differential response to biotic (insects, diseases) and abiotic (e.g., drought, wind, nutrient deficiency) stressors were also collected to examine the environmental interactions of MON 87460 compared with those of the conventional control corn. The observed stressors were "natural" (i.e., no artificial infestation or interference was used). Therefore, the same stressors were not necessarily observed at each field site.

In these studies, environmental interactions were assessed qualitatively in all 31 sites and quantitatively for insect interactions in six selected sites. Observation of plant interactions with insect pests and diseases, and plant responses to abiotic stressors were collected from each of the 31 field sites in 2006 and 2007. The purpose of these evaluations was to assess plant-insect or plant-disease interactions, or plant response to abiotic stressors of MON 87460 compared to the control. For the plant-insect interactions, plant-disease interactions, and plant responses to abiotic stressors, the reported values represent the range of ratings observed across the three or four replications at each site. MON 87460 and the control were considered qualitatively different in response to a stressor if the ratings between MON 87460 and the control corn did not overlap across all replications for that particular stressor (e.g. "none" rating vs. "slight-moderate" rating). The ratings observed among the commercial reference hybrids provide qualitative assessment data common to the crop for each stressor assessed.

*Qualitative Assessments*

Across all 31 sites, approximately 21 arthropods (species or group), 19 disease categories (species or group) and 15 abiotic stressors were evaluated. No qualitative differences were observed in MON 87460 compared to the control for disease, damage or plant response to abiotic stressors (other than water stress) evaluated. For pest and beneficial arthropod evaluations, two differences were observed out of approximately 388 comparisons. Grasshopper damage was lower for MON 87460 compared to the control (none vs. slight) at the IAE site at Observation 3 (Appendix H, Table H-17). The difference in grasshopper damage was not considered biologically meaningful because the difference detected at the IAE site was within the range of the references (none-slight). Additionally, no differences were detected between MON 87460 and the control for grasshopper damage for Observations 2 and 4 at the IAE site, or at any observation time at the other sites (Appendix H, Table H-17). At the IA1 site, European corn borer damage was higher for MON 87460 compared to the control (moderate vs. slight; Appendix H, Table H-10). However, the observed incidence of European corn borer damage was not detected at other sites or in additional observation times at this site. Thus, the difference in European corn borer damage does not represent a trend in the data and is not considered biologically meaningful.

*Quantitative Assessments*

*European Corn Borer and Corn Earworm Damage*

Specific quantitative assessments for corn earworm and European corn borer damage were conducted at three well-watered sites in 2006 (Appendix H, Tables H-4 and H-5, at three well-watered sites in 2007 (Appendix H, Tables H-11 and H-12), and a single site at TX in U.S. 2007 Study-1, with well-watered and water-limited treatments (Appendix H, Table H-24). In combined-site analyses across all years and sites, and for the TX site in Study-1, no statistical differences were detected between MON 87460 and control for corn earworm damage or for European corn borer damage.

*Arthropod Abundance*

Arthropod collections were conducted at three well-watered sites in 2006 (Appendix H, Tables H-6 and H-7), at three well-watered sites in 2007 (Appendix H, Tables H-13 and H-14) and a single site at TX in U.S. 2007 Study-1, with well-watered and water-limited treatments (Appendix H, Tables H-25 and H-26). In an assessment of pest and beneficial arthropod abundance across all sites, a total of eight differences were detected out of 326 comparisons (163 pest and 163 beneficial arthropod comparisons) between MON 87460 and the control. For three of the eight detected differences, the mean abundance values from MON 87460 were within the respective reference ranges. For the remaining differences, the mean abundance value from MON 87460 was higher (two comparisons) or lower (three comparisons) than the reference range. Furthermore, each detected difference was observed at a single observation time point. This suggests the detected differences were not indicative of a consistent response in the data associated with the trait and are unlikely to be biologically meaningful in terms of plant pest potential of MON 87460 compared to the control.

*Summary of Insect, Disease, and Abiotic Stressor Interactions Assessment in Field Studies*

The results of the environmental interactions evaluation for MON 87460 supports the conclusion that the introduction of the drought tolerance trait did not unexpectedly alter MON 87460 compared to conventional corn. The lack of differences in plant response to abiotic stressors (excluding moisture deficit), disease damage, and arthropod damage indicates that the introduction of the drought tolerance trait is unlikely to be biologically meaningful in terms of increased pest potential. Finally, arthropod (pest and beneficial) abundance indicates no significant impact on non-target organisms for purposes of NEPA.

**VIII.F.2. Abiotic Stress Tolerance Assessment in Controlled Environment Studies**

Abiotic stress tolerance was evaluated qualitatively from natural occurrences in the phenotypic field studies (Section VIII.F.1) and also quantitatively under controlled environment conditions in greenhouse and growth chamber studies as presented in this

section. Studies to assess tolerance to drought, cold, heat, and salt stress at sensitive young growth stages under controlled conditions are useful for characterizing the extent of stress tolerance imparted by the inserted stress tolerance protein (CSPB) (see mode of action Section I.D). Although drought, cold, heat or salt tolerance could be associated with some plants that are weeds, a change in these factors in corn would not necessarily be correlated with increased weediness. This is because of the inherent non-weedy nature of corn exemplified by a lack of dispersal mechanisms, the lack of ability to survive in highly competitive plant communities without the intervention of man, a lack of dormancy mechanism that allows long term survival in the soil, and lack of other factors that would be needed for corn to function as a weed. In all studies, MON 87460 was compared to a conventional control from planting through study completion, which in no cases lasted past the vegetative growth stages, so that yield was not an observed factor. Results from these studies show that MON 87460 is susceptible to drought, cold, heat, and salt stress and support the conclusion that the abiotic stress tolerance of MON 87460 during young plant growth stages is not meaningfully different compared to conventional corn.

### VIII.F.2.1. Drought Tolerance Assessment

The purpose of this study was to assess the effect of various levels of drought stress on the growth and development of plants of MON 87460 compared to a conventional control in a greenhouse environment. The test substance, MON 87460, and a conventional corn control were established in pots in a greenhouse in excess of what was needed for the study. At the V4 growth stage, 80 test plants and 80 control plants were selected for uniformity and arranged in a complete factorial treatment structure (8 treatment combinations) in a randomized complete block design with 20 replications. The factors were drought level (well-watered, mild, moderate, or severe) and plant substance type (test or control).

Drought stress treatments were initiated at the V4 growth stage and continued for 15 days. Pots in each treatment were irrigated to maintain target pot weights that were intended to provide a range of growth reductions as follows:

| Treatment | Pot weight target for irrigation (g) |
|---|---|
| Well-watered | 4700 - 4800 |
| Mild | 3500 - 3900 |
| Moderate | 2700 - 2900 |
| Severe | 2160 - 2400 |

Plants were evaluated prior to drought initiation and at 7 and 15 days after drought stress treatment (DAT). Plants were evaluated for plant height, growth stage, chlorophyll content, and leaf rolling score. Fresh and dry weights of above-ground biomass were measured at the conclusion of the experiment.

Prior to the imposition of the drought stress treatments, no statistical differences were detected ($\alpha = 0.05$) between MON 87460 and the control for chlorophyll content. No numeric differences were detected between MON 87460 and the control for growth stage, where statistical comparisons could not be made due to lack of variability (Table VIII-17). MON 87460 exhibited shorter plant height than the control (88.0 vs. 89.6 cm, respectively). This difference is small in magnitude, does not represent a consistent trend across the other treatments in this study, and likely represents biological variation in MON 87460 and the control.

Well-watered treatment

Plants in the well-watered treatment were included to provide plant characterization data common to MON 87460 and the control in the absence of drought stress. In the well-watered treatment, no significant differences were detected between MON 87460 and control for plant height, chlorophyll content and leaf rolling. Three differences were observed in plants that received the well-watered treatment after drought treatments were imposed. MON 87460 had fewer leaves than the control (growth stage 9.6 vs. 9.9, respectively) and had lower fresh and dry weight (620.6 vs. 659.4 g and 65.5 vs. 70.5 g, respectively). (Table VIII-17).

Mild drought treatment

In the mild drought treatment, no significant differences were detected between MON 87460 and control for any of the measured characteristics (Table VIII-17).

Moderate drought treatment

In the moderate drought treatment, no significant differences were detected between MON 87460 and control for chlorophyll content and for biomass (fresh weight and dry weight). Four differences were detected between MON 87460 and the control in the moderate drought treatment. MON 87460 had less plant height than the control 14 DAT (127.0 vs. 130.2 cm, respectively), had fewer leaves at 7 DAT and 14 DAT (growth stage 5.4 vs. 5.8 and 7.3 vs. 7.7, respectively) and MON 87460 had a higher leaf rolling score 7 DAT than the control (2.4 vs. 2.1, respectively, Table VIII-17).

Severe drought treatment

In the severe drought treatment, no significant differences were detected between MON 87460 and control for plant height, growth stage, chlorophyll content, or biomass (fresh weight and dry weight). One difference was detected between MON 87460 and the control for plants in the severe drought treatment. MON 87460 had a lower leaf rolling score than the control (2.6 vs. 3.0, respectively, Table VIII-17).

*Summary of Drought Tolerance Assessment*

The biological endpoints measured in this study were used to assess drought stress on the growth and development of the biotechnology-derived crop compared to a conventional control. Compared to test and control plants in the well-watered treatment, test and control plants in the mild, moderate, and severe treatments exhibited a dose-dependent pattern of lower plant height, growth stage, fresh weight, and dry weight with increasing

water stress. Furthermore, the differences observed in plant height, growth stage, and leaf rolling were not consistent across treatments, do not indicate a competitive advantage for MON 87460, and do not represent a meaningful trend. Therefore, based on the characteristics measured to assess drought tolerance, the results support a conclusion that MON 87460 is still sensitive to drought stress and does not differ from conventional corn after exposure to a range of stress levels imposed during early vegetative growth stages.

**Table VIII-17. Comparison of MON 87460 to the Control Prior to Drought Treatment and at 7 and 15 Days after Treatment**

| Evaluation timing | Characteristic | Well-watered Mean ± S.E. | | Mild Mean ± S.E. | | Moderate Mean ± S.E. | | Severe Mean ± S.E. | |
|---|---|---|---|---|---|---|---|---|---|
| | | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| Prior to treatment [1] | Plant height (cm) | 88.0* ± 0.41 | 89.6 ± 0.52 | - | - | - | - | - | - |
| | Growth stage [2] | 4.0[3] ± 0.0 | 4.0[3] ± 0.0 | - | - | - | - | - | - |
| | Chlorophyll [4] | 51.4 ± 0.35 | 50.9 ± 0.46 | - | - | - | - | - | - |
| Post-treatment (7 DAT) | Plant height (cm) | 126.3 ± 0.86 | 127.9 ± 1.09 | 119.1 ± 1.23 | 119.8 ± 1.07 | 112.2 ± 0.92 | 114.3 ± 0.70 | 112.3 ± 0.74 | 112.4 ± 0.83 |
| | Growth stage | 6.2 ± 0.08 | 6.1 ± 0.1 | 5.9 ± 0.07 | 6.0 ± 0.05 | 5.4* ± 0.11 | 5.8 ± 0.1 | 5.4 ± 0.11 | 5.6 ± 0.11 |
| | Chlorophyll | 54.4 ± 0.53 | 53.7 ± 0.8 | 55.1 ± 0.71 | 53.9 ± 0.52 | 53.4 ± 1.09 | 53.7 ± 0.48 | 54.0 ± 0.65 | 52.4 ± 0.71 |
| | Leaf roll [5] | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 2.4* ± 0.18 | 2.1 ± 0.14 | 2.6* ± 0.18 | 3.0 ± 0.17 |
| Post-treatment (15 DAT) | Plant height (cm) | 175.3 ± 0.97 | 178.0 ± 0.88 | 152.1 ± 1.25 | 151.0 ± 1.75 | 127.0* ± 0.93 | 130.2 ± 1.0 | 119.1 ± 0.68 | 120.3 ± 0.97 |
| | Growth stage | 9.6* ± 0.11 | 9.9 ± 0.07 | 9.0 ± 0.0 | 8.9 ± 0.08 | 7.3* ± 0.1 | 7.7 ± 0.11 | 6.1 ± 0.07 | 6.3 ± 0.11 |
| | Chlorophyll | 53.4 ± 0.49 | 53.8 ± 0.43 | 53.6 ± 0.64 | 52.8 ± 0.5 | 56.0 ± 0.76 | 56.2 ± 0.64 | 55.6 ± 0.72 | 54.7 ± 0.56 |
| | Leaf roll | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.1 ± 0.05 | 1.0 ± 0.0 | 2.2 ± 0.12 | 2.1 ± 0.11 | 3.8 ± 0.1 | 3.7 ± 0.11 |
| | Fresh weight (g) | 620.6* ± 14.73 | 659.4 ± 9.24 | 457.6 ± 14.64 | 430.9 ± 14.60 | 230.8 ± 5.5 | 249.1 ± 6.22 | 174.1 ± 4.53 | 179.9 ± 4.05 |
| | Dry weight (g) | 65.5* ± 1.51 | 70.5 ± 0.93 | 51.4 ± 1.32 | 49.1 ± 1.36 | 32.4 ± 0.65 | 35.1 ± 0.72 | 26.5 ± 0.67 | 26.8 ± 0.59 |

S.E. = standard error; n=20 for test and control plants in each treatment. *Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$) provided by t-test comparisons. [1] Due to the randomization of the substances and drought treatments to the plants within a replication before drought treatments were applied, the pre-treatment measurements for each substance consist of four sub-samples (n=20). [2] Growth stage was determined by the number of leaves with a visible leaf collar. [3] Data could not be analyzed due to lack of variability. [4] Chlorophyll - relative chlorophyll content was an average of readings at the base, midpoint and tip of the fourth leaf measured with a Single Photon Avalanche Diode-containing (SPAD) meter. [5] Leaf roll was rated on a scale of 1 to 5 (1=no rolling, 2=slight rolling upper leaves (younger), 3=more rolling upper leaves and slight rolling lower leaves, 4=all leaves show signs of rolling and 5=severe leaf rolling with little or no top leaf visible).

### VIII.F.2.2. Cold Tolerance Assessment

The purpose of this study was to assess the effect of cold temperature-induced stress on the growth and development of plants of MON 87460 compared to a conventional corn control. The test substance, MON 87460, and a conventional control were established in pots, in excess of what was needed for the study, in growth chambers programmed for optimal conditions (30/22 °C). At the V3 growth stage, uniform plants were selected and transferred to growth chambers with optimal (30/22° C), mild (20/15 °C), moderate (15/10 °C), and severe (4/4 °C) cold treatments for eight days. In each chamber, 20 replicates (one plant per pot) each of MON 87460 and the control were arranged in a completely randomized design with a photoperiod of 16 hours. Each treatment was considered to be a separate experiment. All plants were watered as needed for the duration of the experiment.

Plants were evaluated for plant height, growth stage, chlorophyll content, and vigor three times during the experiment: prior to treatment and at 4 and 8 DAT. Fresh and dry weights of above-ground biomass were measured at the conclusion of the experiment. No significant differences were observed in plants prior to temperature treatments (Table VIII-18).

Optimal temperature treatment (30/22° C)

Plants in the optimal temperature treatment were included to provide plant characterization data for MON 87460 and the control in the absence of cold stress and to validate that the cold temperature treatments affected corn growth as expected. In the optimal temperature treatment, no significant differences were detected between MON 87460 and the control for plant height, chlorophyll content, vigor, or fresh weight. Three differences were detected for plants in the optimal chamber. MON 87460 had more leaves than the control 4 and 8 DAT (growth stage 3.6 vs. 3.3 and 4.5 vs. 4.1, respectively) and MON 87460 had greater dry weight than the control (2.8 g vs. 2.4 g, respectively). (Table VIII-18).

Mild temperature treatment (20/15° C)

In the 20/15° C treatment, no differences were detected between MON 87460 and the control for any of the measured characteristics (Table VIII-18).

Moderate temperature treatment (15/10° C)

In the 15/10° C treatment, no differences were detected between MON 87460 and the control for any of the measured characteristics (Table VIII-18).

Severe temperature treatment (4/4° C)

In the 4/4° C treatment, no differences were detected between MON 87460 and the control for any of the measured characteristics (Table VIII-18).

*Summary of Cold Tolerance Assessment*

The biological endpoints measured in this study were used to assess the effects of cold temperatures on the growth and development of the biotechnology-derived crop compared to a conventional control. Compared to test and control plants in the optimal

temperature group, test and control plants in the mild, moderate and severe treatments exhibited a dose-dependent pattern of lower plant height, growth stage, vigor, fresh weight and dry weight. No significant differences were observed between MON 87460 and the control in the mild, moderate, or severe cold treatments. Based on the characteristics measured to assess cold tolerance, the results support a conclusion that MON 87460 is still sensitive to cold stress and any differences from conventional corn in response to cold stress are not related to the gene of interest.

**Table VIII-18. Comparison of MON 87460 to the Control Prior to Cold Treatment and at 4 and 8 Days after Treatment**

| | | Optimal Mean ± S.E. (30/22°C) | | Mild Mean ± S.E. (20/15°C) | | Moderate Mean ± S.E. (15/10°C) | | Severe Mean ± S.E. (4/4°C) | |
|---|---|---|---|---|---|---|---|---|---|
| **Evaluation Timing** | **Characteristic** | **MON 87460** | **Control** | **MON 87460** | **Control** | **MON 87460** | **Control** | **MON 87460** | **Control** |
| Prior to treatment | Plant height (cm) | 43.1 ± 0.47 | 42.3 ± 0.33 | 43.2 ± 0.45 | 42.4 ± 0.39 | 43.9 ± 0.42 | 42.8 ± 0.44 | 44.3 ± 0.35 | 44.1 ± 0.56 |
| | Growth stage[1] | 3.0 ± 0.0 | 3.0 ± 0.0 | 3.0 ± 0.0 | 3.0 ± 0.0 | 3.0 ± 0.0 | 3.0 ± 0.0 | 3.0 ± 0.0 | 3.0 ± 0.0 |
| | Chlorophyll[2] | 40.3 ± 0.79 | 39.5 ± 0.65 | 41.3 ± 0.54 | 39.9 ± 0.82 | 39.8 ± 0.74 | 40.2 ± 0.88 | 43.6 ± 0.65 | 42.9 ± 0.75 |
| | Vigor[3,4] | 2 ± 0.0 | 2 ± 0.0 | 2 ± 0.0 | 2 ± 0.0 | 2 ± 0.0 | 2 ± 0.0 | 2 ± 0.0 | 2 ± 0.0 |
| Post-treatment (4 DAT) | Plant height (cm) | 62.6 ± 0.41 | 62.7 ± 0.46 | 54.5 ± 0.83 | 53.4 ± 1.01 | 48.8 ± 0.66 | 47.6 ± 0.93 | 44.4 ± 0.5 | 44.4 ± 0.75 |
| | Growth stage | 3.6* ± 0.11 | 3.3 ± 0.1 | 3.0[4] ± 0.0 | 3.0[4] ± 0.0 | 3.0[4] ± 0.0 | 3.0[4] ± 0.0 | 3.0[4] ± 0.0 | 3.0[4] ± 0.0 |
| | Chlorophyll | 45.1 ± 0.83 | 44.1 ± 0.67 | 43.9 ± 0.57 | 43.3 ± 1.03 | 39.2 ± 0.77 | 40.2 ± 0.75 | 44.8 ± 0.66 | 44.4 ± 0.6 |
| | Vigor[4] | 2 ± 0.0 | 2 ± 0.0 | 3 ± 0.0 | 3 ± 0.0 | 3 ± 0.0 | 3 ± 0.0 | 4 ± 0.0 | 4 ± 0.0 |
| Post-treatment (8 DAT) | Plant height (cm) | 80.2 ± 0.77 | 81.7 ± 0.95 | 64.3 ± 0.74 | 62.6 ± 0.76 | 54.0 ± 0.61 | 53.5 ± 0.74 | 44.3 ± 0.52 | 44.5 ± 0.8 |
| | Growth stage | 4.5* ± 0.11 | 4.1 ± 0.07 | 3.9 ± 0.08 | 4.0 ± 0.05 | 3.0[4] ± 0.0 | 3.0[4] ± 0.0 | 3.0[4] ± 0.0 | 3.0[4] ± 0.0 |
| | Chlorophyll | 41.5 ± 0.82 | 41.5 ± 0.68 | 45.0 ± 0.84 | 43.2 ± 1.02 | 38.4 ± 0.7 | 39.2 ± 0.79 | 44.7 ± 0.41 | 44.5 ± 0.84 |
| | Vigor[4] | 2 ± 0.0 | 2 ± 0.0 | 3 ± 0.0 | 3 ± 0.0 | 5 ± 0.0 | 5 ± 0.0 | 6 ± 0.0 | 6 ± 0.0 |
| | Fresh weight (g) | 26.2 ± 1.37 | 23.4 ± 1.06 | 16.5 ± 0.65 | 15.8 ± 0.89 | 9.4 ± 0.37 | 8.9 ± 0.4 | 4.5 ± 0.27 | 4.4 ± 0.28 |
| | Dry weight (g) | 2.8* ± 0.14 | 2.4 ± 0.09 | 1.3 ± 0.06 | 1.3 ± 0.07 | 1.3 ± 0.05 | 1.2 ± 0.05 | 0.5 ± 0.03 | 0.6 ± 0.04 |

S.E. = standard error; n=20 for MON 87460 and control in each temperature treatment. *Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$) provided by t-test comparisons. [1] Number of leaves with a visible leaf collar. [2] Relative chlorophyll content was an average of readings at the base, midpoint, and tip of the leaf measured with a Single Photon Avalanche Diode (SPAD)-containing meter. [3] Plant vigor was rated using a scale of 1 – 9, where 1 = good and 9 = poor. [4] Not statistically analyzed due to lack of variation.

### VIII.F.2.3. Heat Tolerance Assessment

The purpose of this study was to assess the effect of heat-induced stress on the growth and development of plants of MON 87460 compared to a conventional control. The test substance, MON 87460, and a conventional control were established in pots, in excess of what was needed for the study, in growth chambers programmed for optimal conditions (30/22 °C). At the V3 growth stage, uniform plants were selected and transferred to growth chambers with optimal (30/22°C), mild (40/35°C), moderate (43/35°C) and severe (47/35°C) heat treatments (day/night) for five days. In each chamber, 20 replicates (one plant per pot) each of MON 87460 and the control were arranged in a completely randomized design with a photoperiod of 16 hours. Each treatment was considered to be a separate experiment. All plants were watered as needed for the duration of the experiment.

Plants were evaluated for plant height, growth stage, chlorophyll content, vigor and necrosis prior to heat treatment and at 3 and 5 DAT. Fresh and dry weights of above-ground biomass were measured at the conclusion of the experiment.

Prior to imposition of the heat treatments, no numeric differences were detected between MON 87460 and the control for growth stage, vigor, and necrosis, where statistical comparisons could not be made due to a lack of variability. MON 87460 was taller (44.8 vs. 42.7 cm) and had less chlorophyll content (41.0 vs. 43.3 SPAD units [Single Photon Avalanche Diode-containing meter]) than the control in plants selected for the optimal treatment. MON 87460 was shorter than the control (43.9 vs. 45.2 cm) in plants selected for the severe treatment. These differences are small in magnitude, do not represent a consistent trend across the other treatments in this study, and likely represent biological variation in MON 87460 and the control (Table VIII-19).

Optimal temperature treatment (30/22°C)

Plants in the optimal temperature treatment were included to provide plant characterization data for MON 87460 and the control in the absence of heat stress and to validate that high temperatures affected corn growth as expected. In the optimal temperature treatment no significant differences were detected between MON 87460 and the control for plant height, vigor and necrosis at 3 and 5 DAT. Five differences were observed in plants grown under optimal conditions. MON 87460 had less chlorophyll content compared to the control 3 and 5 DAT (45.9 vs. 47.7 and 45.6 vs. 47.0 SPAD units, respectively). MON 87460 had more leaves than the control 3 DAT (growth stage 3.6 vs. 3.2, respectively). At the conclusion of the experiment, MON 87460 had greater fresh and dry weight than the control (14.5 vs. 12.5 g and 1.1 vs. 1.0 g, respectively) (Table VIII-19).

Mild temperature treatment (40/35°C)

In the 40/35 °C treatment, no differences were detected between MON 87460 and the control for plant height, growth stage, vigor, necrosis, and plant biomass (fresh weight and dry weight). One difference was detected between MON 87460 and the control for plants in the mild treatment. MON 87460 had lower chlorophyll content than the control 3 DAT (42.3 vs. 43.8, respectively, Table VIII-19).

Moderate temperature treatment (43/35°C)

In the 43/35 °C treatment, no significant differences were detected between MON 87460 and the control for any of the measured characteristics (Table VIII-19).

Severe temperature treatment (47/35°C)

In the 47/35 °C treatment, no significant differences were detected between MON 87460 and the control for any measured characteristics, with the exception of vigor. MON 87460 had less vigor than the control 5 DAT (8.9 vs. 8.6, respectively, Table VIII-19).

*Summary of Heat Tolerance Assessment*

The biological endpoints measured in this study were used to assess heat stress on the growth and development of the biotechnology-derived crop compared to a conventional control. Compared to test and control plants in the optimal temperature group, test and control plants in the mild, moderate and severe treatments exhibited a dose-dependent pattern of lower plant height, growth stage, vigor, fresh weight and dry weight with increasing temperature. Two differences were observed for plants exposed to heat stress. A reduction in chlorophyll content in the mild treatment 3 DAT and reduced vigor observed in the severe treatment 5 DAT are not indicative of heat tolerance in MON 87460. The differences in chlorophyll content and vigor were not observed across treatments, and therefore, did not represent consistent trends in the data. Based on the characteristics measured to assess heat tolerance, the results support a conclusion that MON 87460 is still sensitive to heat stress and any differences from conventional corn in response to heat stress are not related to the gene of interest.

**Table VIII-19. Comparison of MON 87460 to the Control Prior to Heat Treatment and at 3 and 5 Days after Treatment**

| | | Optimal Mean ± S.E. 30/22°C | | Mild Mean ± S.E. 40/35°C | | Moderate Mean ± S.E. 43/35°C | | Severe Mean ± S.E. 47/35°C | |
|---|---|---|---|---|---|---|---|---|---|
| **Evaluation timing** | **Characteristic** | **MON 87460** | **Control** | **MON 87460** | **Control** | **MON 87460** | **Control** | **MON 87460** | **Control** |
| Prior to treatment | Plant height (cm) | 44.8* ± 0.42 | 42.7 ± 0.4 | 43.6 ± 0.5 | 43.3 ± 0.59 | 43.9 ± 0.34 | 43.4 ± 0.59 | 43.9* ± 0.4 | 45.2 ± 0.42 |
| | Growth stage [1] | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 |
| | Chlorophyll [2] | 41.0* ± 0.71 | 43.3 ± 0.43 | 44.3 ± 0.43 | 45.8 ± 0.62 | 44.4 ± 0.54 | 44.4 ± 0.52 | 45.2 ± 0.37 | 44.2 ± 0.64 |
| | Vigor [3] | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 |
| | Necrosis[4] | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 |
| Post-treatment (3 DAT) | Plant height (cm) | 64.0 ± 0.37 | 63.4 ± 0.51 | 57.9 ± 0.5 | 56.8 ± 0.69 | 52.1 ± 0.71 | 50.3 ± 0.89 | 44.7 ± 0.48 | 45.8 ± 0.4 |
| | Growth stage | 3.6* ± 0.11 | 3.2 ± 0.08 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 |
| | Chlorophyll | 45.9* ± 0.48 | 47.7 ± 0.43 | 42.3* ± 0.48 | 43.8 ± 0.58 | 38.6 ± 0.62 | 37.6 ± 0.62 | 20.0 ± 1.9 | 22.1 ± 2.19 |
| | Vigor | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.1 ± 0.07 | 1.1 ± 0.05 | 1.6 ± 0.25 | 1.5 ± 0.2 | 8.8 ± 0.09 | 8.7 ± 0.11 |
| | Necrosis | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 4.4 ± 0.17 | 4.2 ± 0.2 |
| Post-treatment (5 DAT) | Plant height (cm) | 71.7 ± 0.65 | 71.7 ± 0.7 | 60.1 ± 0.51 | 58.8 ± 0.65 | 54.8 ± 0.6 | 52.7 ± 0.91 | 44.6 ± 0.46 | 45.8 ± 0.42 |
| | Growth stage | 4.0[5] ± 0.0 | 4.0[5] ± 0.0 | 3.4 ± 0.11 | 3.5 ± 0.11 | 3.0 ± 0.0 | 3.0 ± 0.0 | 3.0[5] ± 0.0 | 3.0[5] ± 0.0 |
| | Chlorophyll | 45.6* ± 0.5 | 47.0 ± 0.43 | 40.1 ± 0.51 | 40.9 ± 0.54 | 32.6 ± 0.94 | 33.2 ± 0.88 | 8.9 ± 0.73 | 8.1 ± 0.82 |
| | Vigor | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.5 ± 0.11 | 1.8 ± 0.1 | 5.1 ± 0.1 | 5.0 ± 0.11 | 8.9* ± 0.07 | 8.6 ± 0.15 |
| | Necrosis | 1.0[5] ± 0.0 | 1.0[5] ± 0.0 | 1.0[5] ±0.0 | 1.0[5] ± 0.0 | 1.1 ± 0.05 | 1.0 ± 0.0 | 4.8 ± 0.12 | 4.6 ± 0.15 |
| | Fresh weight (g) | 14.5* ± 0.38 | 12.5 ± 0.3 | 9.8 ± 0.24 | 9.8 ± 0.29 | 7.2 ± 0.24 | 6.9 ± 0.29 | 1.2 ± 0.12 | 1.5 ± 0.19 |
| | Dry weight (g) | 1.1* ± 0.03 | 1.0 ± 0.03 | 1.0 ± 0.03 | 1.0 ± 0.03 | 0.7 ± 0.04 | 0.7 ± 0.03 | 0.4 ± 0.01 | 0.4 ± 0.01 |

S.E. = standard error; n = 20 for MON 87460 and the control in each temperature treatment. *Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$) provided by t-test comparisons. [1] Number of leaves with a visible leaf collar. [2] Relative chlorophyll content was an average of readings at the base, midpoint and tip of the fourth leaf measured with a Single Photon Avalanche Diode-containing (SPAD) meter. [3] Plant vigor was rated using a scale of 1-9, where 1=good and 9=poor. [4] Necrosis was rated using a scale of 1-5, where 1=good and 5=poor. [5] Not statistically analyzed due to lack of variation.

### VIII.F.2.4. Salt Tolerance Assessment

The purpose of this study was to assess the effect of various salt treatments on the growth and development of plants of MON 87460 compared to a conventional control. The test substance, MON 87460, and a conventional corn control were established in pots in a greenhouse in excess of what was needed for the study. Fourteen days after planting, 80 test plants and 80 control plants were selected for uniformity and placed in a complete factorial treatment structure (8 treatment combinations) in a randomized complete block design with 20 replications. The factors were salt level (no salt, mild, moderate, or severe) and plant substance type (test or control).

Beginning 17 days after planting and continuing for 12 days, pots in each salt treatment were irrigated with either reverse osmosis water or varying concentrations of a NaCl-$CaCl_2$ solution (up to 600 mM) to achieve different soil electrical conductivity (EC) levels for each salt treatment as follows:

| Treatment | Target EC (dS/m) | Average EC (dS/m) | Total salt per pot (g) |
|---|---|---|---|
| No Salt | < 1 | 0.5 | 0.0 |
| Mild | approx. 2 – 4 | 2.6 | 14.4 |
| Moderate | approx. 5 – 7 | 4.6 | 40.2 |
| Severe | approx. 8 – 10 | 7.6 | 58.4 |

Plants were evaluated for plant height, growth stage, chlorophyll content, and vigor three times during the experiment: prior to treatment and at 9 and 12 DAT. Fresh and dry weights of above-ground biomass were measured at the conclusion of the experiment.

Prior to the imposition of the salt treatments, no statistical differences were detected ($\alpha = 0.05$) between MON 87460 and the control for plant height or vigor (Table VIII-20). No numeric differences were detected between MON 87460 and the control for growth stage, where statistical comparisons could not be made due to lack of variability. MON 87460 exhibited greater chlorophyll content than the control (50.2 vs. 48.4, respectively) but this difference diminished over time in untreated plants.

No Salt Treatment

Plants that did not receive a salt treatment were included to provide plant characterization data for MON 87460 and the control in the absence of salt stress and to validate that the salt treatments affected corn growth as expected. Within the untreated group, no significant differences were detected between MON 87460 and the control for any measured characteristic at 9 and 12 DAT (Table VIII-20).

Mild Salt Treatment

In the mild salt treatment, no differences were detected between MON 87460 and the control for any measured characteristics, with the exception of dry weight. MON 87460 exhibited lower dry weight than the control (11.3 vs. 11.7 g, respectively; Table VIII-20).

Moderate Salt Treatment

In the moderate salt treatment, no differences were detected between MON 87460 and the control for plant height, growth stage, and biomass (fresh weight and dry weight). Two differences were detected between MON 87460 and the control for plants in the moderate treatment. MON 87460 had increased chlorophyll content 9 DAT (48.4 vs. 46.1 SPAD units, respectively) and had increased vigor over the control 12 DAT (6.2 vs. 6.6, respectively, Table VIII-20).

Severe Salt Treatment

In the severe salt treatment, no differences were detected between MON 87460 and the control for growth stage, chlorophyll content, and biomass (fresh weight and dry weight). Two differences were detected between MON 87460 and the control for plants in the severe treatment. MON 87460 was shorter than the control 9 DAT (73.2 vs. 75.6 cm, respectively) and had decreased vigor 12 DAT (7.9 vs. 7.5 respectively, Table VIII-20).

*Summary of Salt Tolerance Assessment*

The biological endpoints measured in this study were used to assess various salt levels on the growth and development of the biotechnology-derived crop compared to a conventional control. Compared to test and control plants in the untreated group, test and control plants in the mild, moderate and severe salt treatments exhibited a dose-dependent pattern of lower plant height, growth stage, vigor, fresh weight and dry weight. Across salt treatments, a total of five differences were detected between MON 87460 and the control. Reduced dry weight for MON 87460 compared to the control in the mild treatment was not indicative of salt tolerance. The increased chlorophyll content and improved vigor in the moderate salt treatment were not observed in the mild or severe salt treatments. The differences detected between MON 87460 and the control were not consistent across treatments and did not represent a trend in the data. Based on the characteristics measured to assess salt tolerance, the results support a conclusion that MON 87460 is still sensitive to salt stress and any differences from conventional corn in response to salt stress are not related to the gene of interest.

**Table VIII-20. Comparison of MON 87460 to the Control Prior to Salt Treatment and at 9 and 12 Days after Treatment**

| | | Untreated Mean ± S.E. | | Mild Mean ± S.E. | | Moderate Mean ± S.E. | | Severe Mean ± S.E. | |
|---|---|---|---|---|---|---|---|---|---|
| **Evaluation timing** | **Characteristic** | **MON 87460** | **Control** | **MON 87460** | **Control** | **MON 87460** | **Control** | **MON 87460** | **Control** |
| Prior to treatment[1] | Plant height (cm) | 54.2 ± 0.41 | 54.8 ± 0.41 | - | - | - | - | - | - |
| | Growth stage[2] | 4.0[3] ± 0.00 | 4.0[3] ± 0.00 | - | - | - | - | - | - |
| | Chlorophyll[4] | 50.2* ± 0.35 | 48.4 ± 0.36 | - | - | - | - | - | - |
| | Vigor[5] | 2.1 ± 0.04 | 2.1 ± 0.06 | - | - | - | - | - | - |
| Post-treatment (9 DAT) | Plant height (cm) | 107.8 ± 1.01 | 109.5 ± 1.20 | 85.7 ± 0.57 | 87.5 ± 0.72 | 76.4 ± 0.63 | 77.3 ± 0.9 | 73.2* ± 0.75 | 75.6 ± 0.66 |
| | Growth stage | 7.2 ± 0.11 | 7.1 ± 0.07 | 6.5 ± 0.11 | 6.5 ± 0.11 | 5.7 ± 0.13 | 5.8 ± 0.12 | 5.5 ± 0.11 | 5.6 ± 0.11 |
| | Chlorophyll | 48.6 ± 0.68 | 47.4 ± 1.06 | 52.1 ± 0.56 | 52.5 ± 0.76 | 48.4* ± 0.76 | 46.1 ± 0.67 | 32.4 ± 2.02 | 34.9 ± 1.29 |
| | Vigor | 2.0 ± 0.07 | 2.1 ± 0.05 | 3.7 ± 0.11 | 3.5 ± 0.14 | 5.6 ± 0.11 | 5.7 ± 0.11 | 6.6 ± 0.11 | 6.4 ± 0.11 |
| Post-treatment (12 DAT) | Plant height (cm) | 125.2 ± 1.98 | 127.4 ± 1.13 | 94.4 ± 0.70 | 96.2 ± 1.04 | 77.1 ± 0.82 | 77.4 ± 0.67 | 73.4 ± 0.77 | 75.9 ± 0.63 |
| | Growth stage | 8.0 ± 0.05 | 8.0 ± 0.00 | 6.9 ± 0.07 | 7.0 ± 0.07 | 5.8 ± 0.09 | 5.9 ± 0.07 | 5.5 ± 0.11 | 5.7 ± 0.11 |
| | Chlorophyll | 43.5 ± 0.99 | 42.9 ± 1.01 | 47.8 ± 0.77 | 48.3 ± 0.56 | 42.8 ± 0.63 | 42.9 ± 0.54 | 24.1 ± 1.55 | 25.9 ± 1.48 |
| | Vigor | 2.0 ± 0.07 | 1.9 ± 0.08 | 4.0 ± 0.07 | 3.9 ± 0.14 | 6.2* ± 0.09 | 6.6 ± 0.14 | 7.9* ± 0.07 | 7.5 ± 0.11 |
| | Fresh weight (g) | 217.9 ± 3.22 | 218.8 ± 3.05 | 108.8 ± 2.10 | 111.1 ± 1.62 | 53.0 ± 1.01 | 53.8 ± 0.92 | 42.7 ± 0.79 | 46.1 ± 0.80 |
| | Dry weight (g) | 19.4 ± 0.23 | 19.8 ± 0.40 | 11.3* ± 0.18 | 11.7 ± 0.13 | 6.8 ± 0.17 | 6.8 ± 0.14 | 5.6 ± 0.08 | 5.9 ± 0.10 |

S.E. = standard error; n = 20 for MON 87460 and control for each treatment. *Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$) provided by t-test comparisons. [1] Due to the randomization of the substances and treatments within a replication before the treatments were applied, the pre-treatment measurements consist of four sub-samples (n=20). [2] Number of leaves with a visible leaf collar. [3] Data could not be analyzed due to lack of variability. [4] Relative chlorophyll content was an average of readings at the base, midpoint and tip of the third (pre-treatment) or fourth (post-treatment) leaf measured with a Single Photon Avalanche Diode-containing (SPAD) meter. [5] Plant vigor was rated using a scale of 1 – 9, where 1 = good and 9 = poor.

### VIII.F.3. Volunteer Potential Assessment

Volunteer potential can also play a role in determining whether a regulated article has increased weediness potential. The purpose of this study was to assess the volunteer potential of MON 87460 compared to a conventional control. In some crops, seed remaining in the field after harvest have the potential to over-winter and volunteer in the subsequent cropping season. In the fall of 2006, field trials were established at three locations to assess volunteer potential. Comparative assessments were conducted on MON 87460 and a conventional control. In addition, six commercial corn hybrids were included as references. Normal seed germination rates were confirmed for MON 87460, control, and reference starting seeds by standard viability testing. The trials were established at each location as a randomized complete block design with three replications. Each plot was 20 ft long by 5 ft wide and was hand-seeded by uniformly scattering approximately 200 seed on the soil surface. Seed were then incorporated with a disk or field cultivator. Plots were assessed for volunteer plants in the fall of 2006 and spring of 2007. Additional materials and methods are provided in Appendix L.

Agronomic practices used to prepare and maintain each study site were characteristic of each respective region. No irrigation was applied to the study areas and no plot management was required after the seed were incorporated into the soil. Volunteer plant counts were taken after planting in fall 2006 until soil temperatures dropped below 50°F and re-commenced in spring 2007 approximately a week prior to the average local planting date for each field site. Volunteer plant counts were taken approximately every two weeks thereafter until mid-June, for a total of six to seven observations at each site.

No volunteer plants were observed at any site or observation time during the fall or spring (Table VIII-21). The fact that no plants of any study substance emerged and survived as volunteer plants supports a conclusion that the introduction of the drought tolerance trait did not alter the volunteer potential of MON 87460 compared to conventional corn. Furthermore, these results demonstrate that the drought tolerance trait in MON 87460 confers no biologically meaningful change to the invasiveness or potential for corn to persist in the environment.

**Table VIII-21. Observed Volunteer Corn Plants of MON 87460 Compared to the Control and References in a 2006/2007 U.S. Field Trial**

| Site[1] | Season | MON 87460 | Control | References[2] |
|---|---|---|---|---|
| BG | Fall[3] | - | - | - |
| | Spring | 0 | 0 | 0 |
| RL | Fall | 0 | 0 | 0 |
| | Spring | 0 | 0 | 0 |
| RV | Fall | 0 | 0 | 0 |
| | Spring | 0 | 0 | 0 |

N = 3 for MON 87460 and control at each site.
[1] Site code: BG = Guthrie County, IA; RL = Jefferson County, IA; RV = Parke County, IN.
[2] Minimum and maximum values for the reference range could not be calculated because no volunteer plants were observed.
[3]No fall observations were made at BG because soil temperature was <50°F at the first observation timepoint.

### VIII.F.4. Survival Outside of Cultivation Assessment

Weediness or invasiveness may also be indicated if corn exhibited an increased rate of survival outside of cultivation. The purpose of this study was to assess the ability of MON 87460 to establish and persist in areas that are not cultivated for agricultural production. Four sites were established in 2007. Each site was unmanaged and received no agricultural inputs allowing MON 87460, the conventional control, and the reference corn hybrids (three per site) to compete with existing vegetation and abiotic and biotic stressors present in each environment. Additional materials and methods are provided in Appendix M.

Phenotypic and agronomic characteristics encompassing plant growth, development, and yield were assessed for MON 87460, the control, and the references in unmanaged environments. The experiment was established at each of four sites in a randomized complete block design with three replications. Early stand count, final stand count, vegetative plant height, plant height at maturity, number of ears produced per plot, number of ears produced per plant, and seed produced per plot were evaluated (Table VIII-22).

Additionally, replacement values were calculated for each environment. Each replacement value is the ratio of the number of seeds produced to the number of seeds sown, represented by a number equal to or greater than zero. A replacement value less than one means that fewer seeds were produced than were sown. This is interpreted to mean that the population is not replacing itself and is declining. For corn, this would mean that it is not exhibiting a trend towards increased weediness.

No differences were observed between MON 87460 and the control for vegetative plant height, plant height at maturity, number of ears produced per plot, number of ears produced per plant, and seed produced per plot at any of the four sites (Table VIII-22). Two differences were observed between MON 87460 and the control at the MO site.

Early stand count was greater for MON 87460 when compared to the control (41.3 vs. 24.0 plants per plot, respectively) and final stand count was greater for MON 87460 when compared to the control (38.3 vs. 21.0 plants per plot, respectively). It is noteworthy that MO was the only site of the four planted sites where seeds were produced; however, the replacement values for MON 87460 and the control were much less than one (0.15 and 0.03, respectively). This means that the populations for MON 87460 and the control are declining and that MON 87460 is not exhibiting increased weediness. Thus, MON 87460 is not likely to persist in any of the four unmanaged environments and did not demonstrate a competitive advantage in this study compared to conventional corn. As such, the drought tolerance trait in MON 87460 confers no biologically meaningful change to the fitness, invasiveness, or potential for corn to persist outside of managed agricultural environments.

**Table VIII-22. Survival Outside of Cultivation Phenotypic Comparison of MON 87460 to the Control in 2007 U.S. Field Trials**

| Site[1] | Early Stand Count (plants/plot) Mean ± S.E. | | | Late Vegetative Plant Height (inches) Mean ± S.E. | | | Final Stand Count (plants/plot) Mean ± S.E. | | |
|---|---|---|---|---|---|---|---|---|---|
| | MON 87460 | Control | Reference | MON 87460 | Control | Reference | MON 87460 | Control | Reference |
| IL | 53.0 ± 2.08 | 57.3 ± 5.24 | 30.7-48.3 | 8.1 ± 1.53 | 8.8 ± 0.87 | 7.6-10.4 | 16.7 ± 1.45 | 22.0 ± 1.15 | 9.3-19.3 |
| MO | 41.3* ± 1.76 | 24.0 ± 3.06 | 21.3-30.3 | 22.0 ± 1.60 | 23.6 ± 2.41 | 22.9-31.3 | 38.3* ± 1.67 | 21.0 ± 2.31 | 19.3-28.0 |
| NE | 2.3 ± 2.33 | 0.3 ± 0.33 | 0.0-1.0 | 17.8 | 16.0 | 19.5-28.0 | 1.0 ± 1.00 | 0.0 ± 0.00 | 0.0-0.3 |
| TX | - | - | - | - | - | - | - | - | - |

| Site | Plant Height at Maturity (inches) Mean ± S.E. | | | # Ears Produced (ears/plot) Mean ± S.E. | | | # Seed Produced (seed/plot) Mean ± S.E. | | |
|---|---|---|---|---|---|---|---|---|---|
| | MON 87460 | Control | Reference | MON 87460 | Control | Reference | MON 87460 | Control | Reference |
| IL | 6.9 ± 1.00 | 8.1 ± 1.11 | 6.9-8.9 | - | - | - | - | - | - |
| MO | 27.9 ± 1.33 | 29.8 ± 3.31 | 29.1-38.9 | 8.7 ± 3.18 | 3.7 ± 1.45 | 3.0-10.7 | 7.7 ± 1.86 | 1.3 ± 0.88 | 2.3-28.3 |
| NE | 20.7 | - | 30.0-30.0 | - | - | - | - | - | - |
| TX | - | - | - | - | - | - | - | - | - |

| Site | Average # Ears (ears/plant) Mean ± S.E. | | | Replacement Value[2] | | |
|---|---|---|---|---|---|---|
| | MON 87460 | Control | Reference | MON 87460 | Control | Reference |
| IL | - | - | - | 0.0 | 0.0 | 0.0-0.0 |
| MO | 0.2 ± 0.08 | 0.2 ± 0.05 | 0.2-0.4 | 0.15 | 0.03 | 0.05-0.57 |
| NE | - | - | - | 0.0 | 0.0 | 0.0-0.0 |
| TX | - | - | - | 0.0 | 0.0 | 0.0-0.0 |

S.E. = standard error; n = 3 for MON 87460 and control at each site. *Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$). Dash (-) indicates no data available. Mean values without S.E. values are from a sample size of one and S.E. cannot be calculated.

[1] Site codes: IL = Effingham County, IL; MO = Shelby County, MO; NE = York County, NE; TX = Carson County, TX

[2] Due to seed counting errors, the exact number of seed sown in each plot varied. Therefore, replacement values at the MO site were conservatively based on 50 seeds sown per plot using the formula, Replacement Value = [Number of Seed Produced] / 50. Replacement values were not statistically analyzed. Replacement values < 1.0 indicate the seed population is declining.

## VIII.G. Overall Conclusions of Phenotypic, Agronomic, and Environmental Interactions Assessment of MON 87460

An extensive and robust set of information and data were used to assess whether the introduction of the drought tolerance trait and the expression of the CSPB and NPTII proteins altered the plant pest potential of MON 87460 compared to a control, which had a genetic background similar to MON 87460 but did not possess the drought tolerance trait. The assessment was based on thorough phenotypic, agronomic, and environmental interactions characterization, and comparison of MON 87460 to control and conventional reference corn hybrids. Data were collected for seed dormancy and germination parameters, phenotypic and agronomic characteristics during plant growth and development, pollen characteristics, observations for plant-insect, plant disease and plant-abiotic stressor interactions, abiotic stress tolerance, volunteer potential, and survival outside cultivation.

Results from the phenotypic and agronomic assessments indicate that MON 87460 does not possess characteristics that would confer a plant pest risk compared to conventional corn. Data indicate that MON 87460 does not confer any increased susceptibility or tolerance to specific insect, disease, or abiotic stressors, with the exception of drought. MON 87460 is expected to provide reduced yield loss under water-limited conditions compared to conventional corn. This yield response is a desirable agronomic characteristic and is not considered to be associated with plant pest potential. Taken together, these data support a conclusion that MON 87460 poses no increased plant pest potential, including weediness potential, and no adverse environmental impact compared to conventional corn.

## VIII.H. References


Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology. 55:13-18.

Anderson, W.P. 1996. Weed Science Principles and Applications, 3rd ed. West Publishing Company, St. Paul, MN. pp 27-37.

AOSA. 2006. Association of Official Seed Analysts. Rules for testing seeds. AOSA, Lincoln, NE.

Barker, T., H. Campos, M. Cooper, D. Dolan, G. Edmeades, J. Habben, J. Schlusser, D. Wright, and C. Zinselmeier. 2005. Improving Drought Tolerance in Maize. Pages 173-253 in Plant Breeding Reviews. Vol 25, J. Janick, (ed.) John Wiley and Sons, Inc., Hoboken, New Jersey.

Black, R.D. and D.H. Rogers. 1989. Soil water measurements: An aid to irrigation water management. Cooperative Extension Service, Kansas State University. Manhattan, KS.

Boyer, J. S. and M. E. Westgate. 2004. Grain yields with limited water. Journal of Experimental Botany. 55:2385-2394.

Campos, H., M. Cooper, G.O. Edmeades, C. Loffler, J.R. Schussler, and M. Ibanez. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. Maydica. 51:369-381.

Claassen, M. M. and R. H. Shaw. 1970. Water deficit effects on corn. II. Grain components. Agronomy Journal. 62: 652-655.

Lingenfelter, D.D. and N.L. Hartwig. 2003. Introduction to Weeds and Herbicides. Agricultural Research and Cooperative Extension CAT UC175. The Pennsylvania State University, University Park, PA.

OECD. 1993. Safety considerations for biotechnology: Scale-up of crop plants. Organization for Economic Co-operation and Development, Paris, France http://www.biosafety.be/CU/BSL_Ressources/PDF/M00034525.pdf Accessed January 9, 2009.

Radosevich, S., J. Holt, and C. Ghersa. 1997. Weed Ecology: Implications for Management. Second ed. Pages 3-6. John Wiley & Sons, Inc., New York, NY.

Yuan, S. and Lin, H.H. 2008. Role of salicylic acid in plant abiotic stress. Zeitschrift Fur Naturforschung Section C-a Journal of Bioscience. 63:313-320.

## IX. Environmental Consequences and Impact on Agronomic Practices

This section provides a brief review and assessment of the plant pest potential of MON 87460 and its impact on agronomic practices. USDA-AHPIS has responsibility, under the Plant Protection Act (PPA) (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

The definition of plant pest in the PPA is broad and includes living organisms that could directly or indirectly injure, damage, or cause disease in any plant or plant product [7 U.S.C. § 7702(14)]. This section summarizes the plant pest risk for MON 87460 based on the information presented in the previous sections of the petition. Information related to plant pest risk characteristics include disease and pest susceptibilities, expression of the gene product (CSPB, NPTII), changes to plant metabolism, weediness of the regulated article, any impacts on the weediness of any other plant with which it can interbreed, and the transfer of genetic information to organisms with which it cannot interbreed. Issues related to agricultural or cultivation practices are considered in the Appendix O of this petition.

The regulatory end-point for "GE plants" is not zero risk but rather a determination that deregulation of the regulated article is not likely to pose a plant pest risk. As part of its plant pest risk assessment, the genetic construct inserted into MON 87460 was evaluated to determine if those sequences cause plant disease. Morphological characteristics of MON 87460 were analyzed to determine if it will become weedy or invasive. The potential for gene flow and introgression of the genetic construct into other plant varieties or wild relatives are also evaluated to determine the potential of increased weedy or invasive characteristics in other plant species. Finally, the propensity of MON 87460 to become a greater reservoir of plant pests (insects or pathogens) compared to conventional plants and the potential for horizontal gene transfer was evaluated. Using this risk assessment process, the data and analysis presented in this petition leads to a conclusion that MON 87460 is unlikely to be a plant pest and therefore should no longer be subject to regulation under 7 CFR § 340.

The plant pest risk assessment of MON 87460 was based primarily on eight lines of evidence: (1) modern corn has inherently low plant pest potential, (2) insertion of a single functional copy of the inserted *cspB* and *nptII* expression cassettes, (3) characterization of the CSPB and NPTII proteins expressed in MON 87460, (4) lack of allergenicity and toxicity of the CSPB and NPTII proteins, (5) compositional and nutritional equivalence of forage and grain as compared to conventional corn, (6) phenotypic and agronomic characteristics demonstrating no increased plant pest potential, (7) negligible risk to NTOs and threatened or endangered species, and (8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects than conventionally bred drought tolerant corn.

APHIS has recently proposed to amend 7 CFR Part 340 to include its noxious weed authority. Because the data show that MON 87460 has no potential to cause injury, damage or disease to any protected interest, MON 87460 would also not be considered a "noxious weed" as defined by the Plant Protection Act.

## IX.A. Characterization of MON 87460 and the expressed CSPB and NPTII Proteins

### IX.A.1. Mode of Action

MON 87460 reduces yield loss under water-limited conditions compared to conventional corn. Under well-watered conditions, grain yield for MON 87460 is not different from conventional corn. Like conventional corn, MON 87460 is still subject to yield loss under water-limited conditions, particularly during flowering and grainfill periods when corn yield potential is most sensitive to stress by disrupting kernel development.

Efficacy in MON 87460 is derived by expression of the inserted *Bacillus subtilis* cold shock protein B (CSPB). A detailed description of the mode of action for CSPB and its drought tolerance trait is provided in Section I.D. CSPB is an extensively studied, stress-inducible protein known to facilitate adaptation to environmental stresses in bacteria. CSPB acts by interacting with RNA and unfolding secondary structures in RNA molecules, which is known to have an effect on RNA stability and on the ability of the cell to translate those RNA molecules, thus helping to preserve normal cellular functions (Schindler et al., 1999; Graumann et al., 1997). The CSPB protein in MON 87460 binds RNA and helps to maintain plant cellular functions in rapidly growing and reproductive organs under water-limited conditions. Our data suggest that CSPB in MON 87460 works by minimizing the effect of water limitation on photosynthesis, stomatal conductance, and carbon fixation, and ultimately improves corn grain yield, primarily through increased kernel number per ear. The *nptII* gene was inserted to facilitate selection of plants containing *cspB* during early product development and does not pose any safety concerns (EFSA, 2007; FDA 1994 and 1998; Flavell et al., 1992; Fuchs et al., 1993a and 1993b; and Nap et al., 1992).

Knowing that stress response proteins allow organisms to survive in adverse environments, it was hypothesized that inserting a stress response protein into plants could impart a desirable phenotype. Using a high through-put screening biotechnology approach, Castiglioni et al. (2008) demonstrated that bacterial cold shock proteins (CSPs) can confer improved stress adaptation to multiple plant species. Twenty-two events expressing the cold shock protein B (CSPB) were evaluated in water-limited field trials using commercial grade corn in environments that received no rainfall during the 10 to 14 days immediately prior to flowering. The water-limited treatment resulted in an average reduction in growth rates of 50% of the well-watered rate. Analyzing data from all events, the CSPB containing events demonstrated a 3.6% - 24% increase in leaf extension rates relative to non-transgenic controls, improvements in chlorophyll content (2.5% - 4.4%), and photosynthetic rates (3.6% - 8.5%). These measures of vegetative performance indicated that the CSPB protein has a positive impact on overall plant productivity and, therefore, yield potential. When plants were grown under well-watered conditions in both the greenhouse and field, no appreciable difference between CSPB-

expressing lines and the control were detected. MON 87460 was chosen for further development based on its yield performance under water-limited conditions compared to the control and its favorable agronomic performance.

As with bacterial and other plant cold shock domain (CSD) containing proteins, the CSPB protein from *B. subtilis*, expressed in MON 87460 accumulates in actively growing tissues where it binds to RNA and facilitates the unfolding of RNA secondary structures that are formed due to stress leading to more normal translation under stress conditions (Schindler et al., 1999; Graumann et al., 1997). Data from *in vitro* and *in vivo* experiments indicate that CSPB preferentially binds plant RNA. CSPB was also effective in unfolding secondary RNA structures *in vitro,* while variants of the CSPB protein with impaired RNA binding functions were unable to bind and unfold RNA. *In vitro* co-immunoprecipitation experiments with CSPB, CSPB with an impaired binding function and RNA from MON 87460 further confirm that CSPB interacts with RNA while the variant lacking a functional RNA binding site will not interact with RNA *in vitro*.

Water-limited conditions during the growing season can diminish corn productivity and yield, particularly during flowering and grainfill periods when corn yield potential is most sensitive to stress by disrupting fertilization and kernel development (Claassen and Shaw, 1970; Boyer and Westgate, 2004; Campos et al., 2006). In field trials, improvements in MON 87460 yield and yield components under water-limited conditions were demonstrated in multiple years. Results from these studies demonstrate that the major component contributing to the improved yield of MON 87460 under water-limited conditions is the increased number of kernels per ear, which is consistent with our current understanding of the effect of drought stress on corn yield potential (Westgate et al., 2004; Campos et al., 2006; Welcker et al., 2007).

### IX.A.2. CSPB and NPTII Protein Safety

The safety assessment of the CSPB protein included extensive protein characterization demonstrating the lack of similarity to known allergens and toxins and the long history of safe consumption of similar proteins (Section VI). CSPB protein does not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which have adverse effects on mammals. This has been shown by extensive assessments with bioinformatics tools, such as FASTA sequence alignment search and an eight-amino acid sliding window search (Section VI.D). Digestive fate experiments conducted with the CSPB protein demonstrated that the full-length protein is rapidly digested in simulated gastric fluid (SGF), a characteristic shared among many proteins with a history of safe consumption. A small transiently stable CSPB protein fragment was very quickly degraded during short exposure to simulated intestinal fluid (SIF). Rapid digestion of the full-length CSPB protein in SGF and SIF, together with rapid degradation of the small transiently stable fragment in SIF, indicates that it is highly unlikely that the CSPB protein and its fragment will reach absorptive cells of the intestinal mucosa (Section VI.D). Proteins that are rapidly digestible in mammalian gastrointestinal systems are unlikely to be allergens when consumed. Finally, the CSPB protein represents no more than 0.00007% of the total protein in the grain of MON 87460

(Section VI.D). Acute oral toxicity studies with mice demonstrated that CSPB protein is not acutely toxic and does not cause any adverse effects even at the highest dose levels tested, which was 4.7 mg/kg body weight. The dietary safety assessment of CSPB based on the acute toxicity data and corn product dietary pattern establishes that the margin of exposure (MOE) for the overall U.S. population is 26,700. For children aged 1-6 years old, an age group with the highest corn consumption on a body weight basis, the MOE was greater than or equal to 11,400 for CSPB. Dietary exposure in animals will also be low with chickens, swine, and dairy cows consuming only nanogram quantities of each protein per kilogram of body weight (Section VI.D). Collectively, these data establish the dietary safety of the CSPB protein. Because NTOs, including humans, will primarily come in contact with CSPB through dietary exposure it can be concluded with reasonable certainty that the CSPB protein has no meaningful toxic potential to exposed organisms in the environment.

The dietary safety of NPTII has been extensively evaluated through several lines of experimental evidence, and several products containing NPTII have been approved by regulatory agencies on a global basis. NPTII is the most commonly used antibiotic resistance marker in several commercially grown biotechnology-derived crops including YieldGard® Rootworm corn (MON 863), Bollgard® cotton (MON 531), Bollgard®II cotton (MON 15985), and Roundup Ready cotton (MON 1445). The safety of NPTII has been addressed in multiple publications (Flavell et al., 1992; Nap et al., 1992; Fuchs et al., 1993a and 1993b; EFSA, 2009). FDA evaluated NPTII as part of a petition for FLAVR SAVR® tomatoes and approved its use as a food additive. Additionally, EPA established an exemption from the requirement of a tolerance for NPTII for use as a selectable marker in raw agricultural commodities (40 CFR Part 180.1134). In 2007, the European Food Safety Authority (EFSA) affirmed its conclusion that the presence of *nptII* does not pose a threat to human health or the environment. Moreover, the USDA previously evaluated the safety of NPTII in other biotechnology-derived products, including corn. Similar to these products, there is negligible risk for the production of NPTII in MON 87460 to result in a plant pest risk. A published assessment of the ecological impact of NPTII in crops reported that the amount of free kanamycin accumulating in soils, through the action of microorganisms or animal feces, is restricted by absorption to soil components so that no direct selection pressure for kanamycin resistant plants can occur (Nap et al., 1992). Also, enhanced physiological fitness resulting from potential pleiotropic effects of *nptII* gene expression is not likely to occur (Nap et al., 1992; EFSA, 2009). Thus, based on all the available evidence, it can be concluded that the NPTII protein is safe for use as a selectable marker in biotechnology-derived plants and it has no meaningful toxic potential to exposed organisms in the environment.

### IX.A.3. Composition and Nutrition of Forage and Grain

Compositional equivalence between corn improved through biotechnology-derived traits and conventional hybrids provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 1998). Compositional analyses of forage and grain tissues from MON 87460 were conducted to assess the levels of key nutrients, anti-nutrients, and key secondary metabolites for comparison to conventional

corn. These results, based on evaluation of 77 different components (9 in forage and 68 in grain) confirmed that the corn grain and forage derived from MON 87460, and the intended foods and feeds derived from MON 87460, can be considered compositionally and nutritionally equivalent to conventional corn hybrids that have a history of safe consumption that are currently in commerce (Section VII).

### IX.A.4. Phenotypic and Agronomic Characteristics

Phenotypic and agronomic characteristics of MON 87460 were evaluated in field studies conducted during 2006 and 2007 in the major corn production regions of the U.S. and Chile (Section VIII). Because MON 87460 reduces yield loss under water-limited conditions, field studies were designed to evaluate the relevant characteristics of MON 87460 across a broad range of soil moisture and environmental conditions relevant to where commercial production would be expected. Six field studies totaling 31 sites were established using three water management regimes: (1) well-watered (17 sites), (2) well-watered and water-limited treatments (9 sites), and (3) water managed according to typical local agronomic practices and water conditions (5 sites). Phenotypic and agronomic characteristics and environmental interactions were assessed under these water management regimes. Characteristics evaluated include seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight and yield. For each characteristic, MON 87460 was compared to the control and means were calculated across sites (referred to as combined-site analysis, in which data were pooled from all sites and analyzed statistically).

Results from the combined-site analyses within each of the six studies detected only four instances of a difference between MON 87460 and the control. For the well-watered regime, two separate studies totaling 17 sites were established in the U.S. during 2006 (8 sites) and 2007 (9 sites). In the combined-site analyses of these data, no differences were detected between MON 87460 and the control in the 2007 study. In the 2006 study, an increase in root lodged plants was detected for MON 87460 compared to the control (5.6 vs. 1.5, respectively). For the well-watered and water-limited regime, three different studies totaling nine field sites (six sites included in combined-site analyses) were established in Chile (1 study, 3 sites in 2006/2007) and the U.S. (2 studies, 3 sites in 2007). In the Chile 2006/2007 study, no differences were detected with the exception of reduced yield loss for MON 87460 compared to the control under water-limited conditions (114.5 vs. 86.7 bushels/acre, respectively). In the U.S. 2007 Study-1, no differences were detected in either the well-watered or water-limited treatments. In the U.S. 2007 Study-2, stay green rating was lower (more green tissue) for MON 87460 compared to the control in both the well-watered (5.8 vs. 6.7) and water-limited treatments (6.3 vs. 8.3), respectively. For the typical agronomic practices regime, one study with five sites was established in the U.S. during 2006, and no differences between MON 87460 and the control were detected in this study. In summary of the phenotypic data, the results support the conclusion that MON 87460 possesses no characteristics that would indicate an increased plant pest risk compared to conventional corn. The magnitude of the detected differences was small, the differences did not represent a trend in the data across studies and years, and the mean values of MON 87460 were within the

ranges of values observed for the commercial references. In no instance did the field studies suggest that MON 87460 would cause damage, injury, or disease to plants.

Efficacy of MON 87460 can be demonstrated by directly comparing its grain yield to a non-drought tolerant, conventional control under water-limited conditions. The well-watered and water-limited field trials described above are relevant to demonstrate the efficacy of MON 87460. A comparison of the average percent reduced yield loss of MON 87460 compared to the control under both field trial designs demonstrates the efficacy of MON 87460 under water-limited conditions (Section I.B.2). As expected, MON 87460 provided reduced yield loss compared to the control under water-limited conditions and equivalent yield to the control under well-watered conditions. Reduced yield loss values for MON 87460 in the water-limited treatment were highly variable, but are representative of the natural variation expected for corn grown under sub-optimal soil moisture conditions.

### IX.A.5. Potential for Risks to Non-target Organisms

Evaluation of the potential risks to non-target organisms (NTOs) may be considered a component of a plant pest risk assessment, or could be considered separately as part of a NEPA environmental assessment. The nature of MON 87460 as a product with no pesticidal activity can lead to a conclusion that all exposed organisms are considered to be NTOs. During the U.S. and Chile phenotypic field studies at 31 locations in 2006 and 2007, each field site was rated at four time intervals during the season for specific insects (pest and non-pests), and diseases (Section VIII.F.1). The purpose of these observations was to assess whether the plant-disease or plant-insect interactions of MON 87460 were altered compared to commercial corn. Twenty-one pest and non-pest arthropod categories (species or group) and 19 disease categories were evaluated.

Of the more than 388 pest and non-pest arthropod evaluations, only two differences were observed between MON 87460 and the control. Grasshopper damage was lower for MON 87460 compared to the control (none vs. slight) and European corn borer damage was higher for MON 87460 compared to the control (moderate vs. slight) (Section VIII.F.1). The differences detected were either within the range of the references, or were isolated to a single study-site location. The few differences detected were small in magnitude, did not represent a trend in the data, and are not considered to be biologically meaningful in terms of increased plant pest potential. Out of the more than 425 disease stressor observations, no differences were detected between MON 87460 and the control. These results support the conclusion that MON 87460 does not have altered environmental interactions relative to other corn and expression of the CSPB protein show no apparent direct impact on arthropods or diseases of corn.

In addition, compositional analyses of forage and grain (Section VII) confirm that the levels of key nutrients, anti-nutrients, and secondary metabolites in MON 87460 are comparable to those in conventional corn and that the forage and grain derived from MON 87460 are compositionally equivalent to conventional corn. In total, these studies demonstrate no biologically meaningful interactions with the species exposed to MON 87460.

## IX.B. Weediness Potential

In the U.S., corn is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1979; Muenscher, 1980), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). Modern corn cannot survive as a weed due to intense selection for domestication purposes during the evolution of corn. During domestication of corn, traits often associated with weediness such as seed dormancy, a dispersal mechanism, or the ability to form reproducing populations outside of cultivation, have not been selected. For example, the corn ear is enclosed with husks. Consequently, seed dispersal of individual kernels is limited. Even if individual kernels of corn were distributed within a field or along transportation routes from the fields to storage or processing facilities, sustainable volunteer corn populations are not found growing in fence rows, ditches, and road sides. Corn is poorly suited to survive without human assistance and is not capable of surviving as a weed (Baker, 1965; Keeler, 1989; Galinat, 1988). Although corn seed can overwinter and emerge as volunteer plants in rotational crops, the populations do not persist, and agronomic management practices, including mechanical and chemical measures, can be used to control the volunteer plants.

In comparative studies conducted between MON 87460 and a non-drought-tolerant, conventional control, dormancy and germination, growth and development, and reproductive characteristics were evaluated for changes that would impact plant pest potential, and in particular, plant weediness potential. No meaningful differences from conventional corn were observed (Section VIII). The introduction of the drought tolerance trait did not unexpectedly alter the assessed characteristics compared to the control. Thus, the results support a conclusion of no increased weediness potential of MON 87460 compared to conventional corn. Furthermore, extensive post-harvest monitoring of field trial plots planted with MON 87460 under USDA-APHIS notifications did not reveal any differences in survivability or persistence relative to other corn. A complete list of USDA notifications approved for MON 87460 is presented in Appendix N**.** These data suggest that MON 87460 is no more likely to become a weed than conventional corn.

## IX.C. Gene Flow

Gene flow (often used synonymously with the term “outcrossing” or “cross pollination”) is a natural biological process that occurs in most crop species, including corn. Pollen-mediated gene flow is a term used to describe the movement of plant genes from one plant to another via pollen. The rate of pollen-mediated gene flow depends on biotic and abiotic factors such as plant biology, pollen biology and volume, plant phenotype, overlap of flowering times, proximity of the pollen source and sink, ambient conditions such as temperature and humidity, and field architecture.

## IX.C.1. Vertical Gene Flow – Transfer of Genetic Information among Species with which Corn Can Interbreed

Corn morphology fosters cross-pollination; therefore, high levels of pollen-mediated gene flow can occur in this species. Researchers also recognize that: (1) the amount of gene flow that occurs can be high because of open-pollination, (2) the percent gene flow will vary by population, hybrid or inbred, (3) the level of gene flow decreases with greater distance between the source and recipient plants; (4) environmental factors affect the level of gene flow, (5) corn pollen is viable for a short period of time under field conditions, (6) corn produces ample pollen over an extended period of time, and (7) there are no purposeful insect pollinators of corn (pollinating insects, especially bees, are occasional visitors to the tassels but rarely visit silks of corn).

For gene flow to occur by normal sexual transmission, certain conditions must exist: (1) the two parents must be sexually compatible; (2) there must be overlapping phenology; and (3) a suitable factor (such as wind or insects) must be present and capable of transferring pollen between the two parents.

Several studies have been conducted on the extent of pollen-mediated gene flow in corn. As expected, results were found to vary depending on the experimental design, environmental conditions and detection method. In general, percent gene flow was found to diminish with increasing distance from the source field. It was previously reported that corn cross-pollination rate fell below 1% at distances >200 m (Jemison and Vayda, 2000; Luna et al., 2001). A more recent study showed that corn cross pollination rate decreased below 0.9% beyond 15 m (Bannert, 2006).

As discussed in Sections II.C and II.D.5, corn and annual teosinte are genetically compatible, wind-pollinated and may hybridize when in close proximity to each other e.g., in areas of Mexico and Guatemala. Corn easily crosses with teosinte; however, teosinte is not present in the U.S. other than as an occasional botanical garden specimen or small feral populations of *Zea mexicana* in Florida, Alabama and Maryland and *Zea perennis* in South Carolina (http://plants.usda.gov). These specimens would only flower at the same time as corn if they were subject to artificial day length shortening for several weeks at a time (Wilkes, 1967). Differences in factors such as flowering time, geographical separation, and development factors make natural crosses in the U.S. highly unlikely. Additionally these states are not part of the targeted geographic area in which MON 87460 is expected to have the greatest benefit, the U.S. Great Plains where corn production is subject to frequent drought stress.

In contrast with corn and teosinte, which easily hybridize under certain conditions, it is only with extreme difficulty and special techniques that corn and another closely related species, *Tripsacum* (gamma grass) hybridize. Furthermore, the offspring of the cross show varying levels of sterility (Galinat, 1988; Mangelsdorf, 1974; Russell and Hallauer, 1980) (Section II.D.5). A single species, *Tripsacum floridanum*, found in the extreme southern Florida counties of Miami-Dade, Collier and Monroe has been categorized as a threatened species by the state of Florida and listed on the USDA's Natural Resources Conservation Service Database (http://plants.usda.gov/java/profile?symbol=TRFL4).

However, given the level of difficulty for natural hybridization between species of *Tripsacum* and *Zea*, and the occurrence of *T. floridanum* primarily in both highly urbanized and non-agricultural, swampy areas of the state, it is very unlikely there would be any impact on this species due to the introduction of MON 87460.

### IX.C.2. Horizontal Gene Flow – Transfer of Genetic Information to Species with which Corn Cannot Interbreed

Monsanto is aware of no reports of the transfer of genetic material from corn to other species with which corn cannot sexually interbreed. Southern blots examining multiple generations of MON 87460 demonstrate that the *cspB* and *nptII* expression cassettes are stably inherited with patterns typical of Mendelian genetics (Section V.D.1). It is therefore not expected that these genes would have any altered potential to transfer to other organisms. The probability for horizontal gene flow to occur is judged to be exceedingly small. Even if it were to occur, the consequences would be negligible since the genes introduced into MON 87460 are of bacterial origin and the two proteins produced have no meaningful toxicity to humans and other NTOs under the conditions of use. In addition, the *nptII* gene is not expected to pose any additional risk of antibiotic resistance when MON 87460 is cultivated. A study of corn expressing an antibiotic resistance marker gene demonstrated that the presence of such crops does not affect the frequency of antibiotic resistance in soil bacteria (Demanèche et al., 2008).

## IX.D. Corn Production, Current Agronomic Practices, and Land Use

This section provides a review of corn grain and seed production, agronomic and land use practices for corn, and any anticipated environmental consequences from the commercialization of MON 87460.

Areas such as the Midwestern U.S. Corn Belt have sufficient precipitation to routinely support high levels of corn production. As shown in Sections VII and VIII, no phenotypic, compositional, or environmental differences between MON 87460 and conventional corn have been observed under well-watered conditions. Therefore, it is not anticipated that adoption of MON 87460 in Midwestern regions of the U.S. will have any significant impact on cultural practices, including tillage and pest management. In the Great Plains, there is often insufficient precipitation for corn production resulting in water-limited conditions. Under these conditions, some potential impacts to irrigation practices and dryland acreage are foreseeable, and are discussed in more detail below. However the introduction of MON 87460 is no more likely to impact irrigation practices and dryland acreage in the Great Plains than the use of conventionally bred drought tolerant corn.

### IX.D.1. Overview of U.S. Corn Production

#### IX.D.1.1. Grain Production

Corn is the largest U.S. crop in terms of acreage planted and net crop value. In the past 10 years (1997-2007), total annual corn acreage planted varied from approximately 76 to 93 million acres (http://www.nass.usda.gov). Total annual production ranged from 9 to

13.1 billion bushels, and total annual value fluctuated from 17 to 52 billion dollars depending on production output and commodity prices (Table IX-1).

Corn is planted in almost every state in the continental U.S. The two largest corn producing regions are the Midwest, comprising eight states (Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio and Wisconsin) contributing 65% to the national corn production total, and the Great Plains, as defined by Riebsame (1990), including portions of ten states (Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas and Wyoming) contributing 26% to the national corn production total (Table IX-2).

Yields vary considerably from region to region because of the diversity in rainfall and irrigation, climatic conditions, and soil productivity. A comparison of the Midwest and the Great Plains (Table IX-3) illustrates that while average bushel/acre yields are not very dissimilar, the acreage routinely devoted to corn production in the Great Plains is approximately 44% of that utilized in the Midwest. This is due to a number of factors including significantly lower rainfall, with the Great Plains having approximately a 20 inch (50 cm) annual average while the Midwest has a 34 inch (86 cm) annual average (http://www.met.utah.edu/jhorel/html/wx/climate/normrain.html).

**Table IX-1. Field Corn Production in the U.S. (1996 – 2008)**

| Year | Acres planted (x 1000) | Acres harvested (x 1000) | Yield (bu/acre) | Production (x 1000 bu) | Price ($/bu) | Value ($ billion) |
|---|---|---|---|---|---|---|
| 1996 | 79,229 | 72,644 | 127.1 | 9,232,557 | 2.71 | 25.15 |
| 1997 | 79,537 | 72,671 | 126.7 | 9,206,832 | 2.43 | 22.35 |
| 1998 | 80,165 | 72,589 | 134.4 | 9,758,685 | 1.94 | 18.92 |
| 1999 | 77,386 | 70,487 | 133.8 | 9,430,612 | 1.82 | 17.10 |
| 2000 | 79,551 | 72,440 | 136.9 | 9,915,051 | 1.85 | 18.50 |
| 2001 | 75,702 | 68,768 | 138.2 | 9,502,580 | 1.97 | 18.88 |
| 2002 | 78,894 | 69,330 | 129.3 | 8,966,787 | 2.32 | 20.88 |
| 2003 | 78,603 | 70,944 | 142.2 | 10,087,292 | 2.42 | 24.48 |
| 2004 | 80,929 | 73,631 | 160.3 | 11,805,581 | 2.06 | 24.38 |
| 2005 | 81,779 | 75,117 | 147.9 | 11,112,187 | 2.00 | 22.20 |
| 2006 | 78,327 | 70,638 | 149.1 | 10,531,123 | 3.04 | 32.09 |
| 2007 | 93,527 | 86,520 | 150.7 | 13,037,875 | 4.00 | 52.09 |
| 2008 | 85,982 | 78,640 | 153.9 | 12,101,238 | NA* | NA* |

Source: http://www.nass.usda.gov
*Data currently not available

**Table IX-2. Field Corn Production by Regions and States in the U.S. (2008)**

| State | Acres planted (x 1000) | Acres harvested (x1000) | Yield (bu/acre) | Production (x1000 bu) | % to national total production |
|---|---|---|---|---|---|
| **Midwest Region** | | | | | |
| Illinois | 12,100 | 11,900 | 179 | 2,130,100 | |
| Indiana | 5,700 | 5,460 | 160 | 873,600 | |
| Iowa | 13,300 | 12,800 | 171 | 2,188,800 | |
| Michigan | 2,400 | 2,140 | 138 | 295,320 | |
| Minnesota | 7,700 | 7,200 | 164 | 1,180,800 | |
| Missouri | 2,800 | 2,650 | 144 | 381,600 | |
| Ohio | 3,300 | 3,120 | 135 | 421,200 | |
| Wisconsin | 3,800 | 2,880 | 137 | 394,560 | |
| **Regional total** | **51,100** | **48,150** | **154(avg)** | **7,865,980** | **65.00** |
| **Northeast Region** | | | | | |
| New York | 1,090 | 640 | 144 | 92,160 | |
| Pennsylvania | 1,350 | 880 | 133 | 117,040 | |
| Connecticut | 27 | | | | |
| Maine | 29 | | | | |
| Massachusetts | 19 | | | | |
| New Hampshire | 15 | | | | |
| Rhode Island | 2 | | | | |
| Vermont | 94 | | | | |
| **Regional total** | **2,626** | **1,520** | **139(avg)** | **209,200** | **1.70** |
| **Mid-Atlantic Region** | | | | | |
| Delaware | 160 | 152 | 125 | 19,000 | |
| Maryland | 460 | 400 | 121 | 48,400 | |
| New Jersey | 85 | 74 | 116 | 8,584 | |
| Virginia | 470 | 340 | 108 | 36,720 | |
| West Virginia | 43 | 26 | 130 | 3,380 | |
| **Regional total** | **1,218** | **992** | **120(avg)** | **116,084** | **0.96** |
| **Southeast Region** | | | | | |
| Alabama | 260 | 235 | 104 | 24,440 | |
| Arkansas | 440 | 430 | 155 | 66,650 | |

**Table IX-2 (cont.). Field Corn Production by Regions and States in the U.S. (2008)**

| State | Acres planted (x 1000) | Acres harvested (x1000) | Yield (bu/acre) | Production (x1000 bu) | % to national total production |
|---|---|---|---|---|---|
| Florida | 70 | 35 | 105 | 3,675 | |
| Georgia | 370 | 310 | 140 | 43,400 | |
| Louisiana | 520 | 510 | 144 | 73,440 | |
| Kentucky | 1,210 | 1,120 | 136 | 152,320 | |
| Mississippi | 720 | 700 | 140 | 98,000 | |
| North Carolina | 900 | 830 | 78 | 64,740 | |
| South Carolina | 355 | 315 | 65 | 20,475 | |
| Tennessee | 690 | 630 | 118 | 74,340 | |
| **Regional total** | **5,524** | **5,115** | **119 (avg)** | **621,480** | **5.15** |
| | | | | | |
| **Great Plains Region** | | | | | |
| Colorado | 1,250 | 1,080 | 137 | 147,960 | |
| Kansas | 3,850 | 3,630 | 134 | 486,420 | |
| Montana | 78 | 35 | 136 | 4,760 | |
| Nebraska | 8,800 | 8,550 | 163 | 1,393,650 | |
| New Mexico | 140 | 55 | 180 | 9,900 | |
| North Dakota | 2,550 | 2,300 | 124 | 285,200 | |
| Oklahoma | 370 | 320 | 115 | 36,800 | |
| South Dakota | 4,750 | 4,400 | 133 | 585,200 | |
| Texas | 2,300 | 2,030 | 125 | 253,750 | |
| Wyoming | 95 | 52 | 134 | 6,968 | |
| **Regional total** | **24,183** | **22,452** | **138 (avg)** | **3,210,608** | **26.53** |
| | | | | | |
| **Northwest Region** | | | | | |
| Washington | 165 | 90 | 205 | 18,450 | |
| Oregon | 60 | 33 | 200 | 6,600 | |
| Idaho | 300 | 80 | 170 | 13,600 | |
| **Regional total** | **525** | **203** | **192 (avg)** | **38,650** | **0.33** |
| | | | | | |
| **Southwest Region** | | | | | |
| Arizona | 50 | 15 | 165 | 2,475 | |
| California | 670 | 170 | 195 | 33,150 | |
| Nevada | 5 | | | | |
| Utah | 70 | 23 | 157 | 3,611 | |
| **Regional total** | **795** | **208** | **172 (avg)** | **39,236** | **0.33** |
| **United States** | **85,982** | **78,640** | **154 (avg)** | **12,101,238** | |

Source: http://www.nass.usda.gov

**Table IX-3. Comparison of Field Corn Production in the Midwest vs. the Great Plains**

| 2007 Corn Yield Measurements and Average Precipitation | Midwest | Great Plains |
|---|---|---|
| Total Acres Planted | 56,300,000 | 24,834,000 |
| Avg. Bushels/Acre | 150 | 132 |
| Total Bushel Yield | 8,515,450,000 | 3,315,645,000 |
| Avg. Precipitation (inches)* | 34.45 | 20.38 |

*Thirty year (1961-1990) annual average precipitation
Source: http://www.met.utah.edu/jhorel/html/wx/climate/normrain.html

### IX.D.1.2. Seed Production

Standardized seed production practices are responsible for maintaining high quality seed stocks, an essential basis for U.S. agriculture. By the early 20th century, agronomists learned how to develop specific plant varieties with desirable traits. In the U.S., state agricultural experiment stations developed many seed varieties which were distributed to farmers for use. As seeds were saved by farmers and later sold to neighbors however, the desirable traits of the varieties often were lost through random genetic changes and contamination with other crop and weed seeds (Sundstrom et al., 2002). The value of seed quality (including genetic purity, vigor, weed seed presence, seed borne diseases and inert materials, such as dirt) was quickly identified as a major factor in crop yields. States developed seed laws and certification agencies to ensure that purchasers who received certified seed could be assured that the seed met established seed quality standards (Bradford, 2006). The federal government passed the U.S. Federal Seed Act of 1939 to recognize seed certification and official certifying agencies. Regulations first adopted in 1969 under the Federal Seed Act recognize land history, field isolation, and varietal purity standards for foundation, registered, and certified seed. Under international agreements such as the Organization for Economic Co-Operation and Development (OECD) scheme, the U.S. and other countries mutually recognize minimum seed quality standards (Bradford, 2006). The Association of Official Seed Certifying Agencies (AOSCA) represents state and private seed certification in the U.S., and includes international member countries in North and South America, as well as Australia and New Zealand.

Seed certification is based on varietal lineage, as well as quality production and processing standards. Seeds produced for sale to a crop grower (certified seeds) are a limited number of generations from a verified seed stock of the specified variety (Bradford, 2006). Breeder seed is generally produced under the strictest standards and under the supervision of the breeder. Breeder seed is used to produce foundation seed, which is used to produce registered seed, which is then used to produce certified seed that is sold for commercial planting (Bradford, 2006). In addition to documenting the pedigree of the seed, certification programs also monitor crop rotations, previous crops and weeds in the field, as well as isolation of the field from other varieties of the same

genus or species (Bradford, 2006). Inspectors walk the fields to note the occurrence of off-type plants, other crop plants, weeds, or disease. After seed harvesting and cleaning, the seed is later tested for germination capacity, and analyzed for the presence of seed of other varieties or other crops, weed seeds and inert matter (e.g., dirt or stones) to assure high quality before the seed bags are tagged as "certified" (Bradford, 2006). Within a seed crop, the main sources of off-types, or seed from another plant, result from "volunteers," or seed from crops grown in the field at an earlier date, pollen transfer and mixing that occurs during harvesting and handling (Bradford, 2006). Seed producers can and do take steps, such as cleaning equipment, appropriate crop rotation and other stewardship measures, to control for these factors.

Seed producers also learned over the years to account for pollen flow between nearby fields, and seed certifying agencies adopted spatial isolation requirements (codified in federal regulations for certain crops) that must be followed to produce certified seed that will guarantee the purchaser receives the intended seed variety. The isolation required for a particular crop depends on its flowering characteristics (including timing), sexual compatibility with neighboring crops, pollen quantity and viability, and the mode of pollen dissemination (Sundstrom et al., 2002).

The standards put in place in the first half of the 20th century were based on physical appearance and performance determined by field inspections. Those standards are still in place today for the vast majority of crops produced. These standards have never required 100% varietal purity. For example, AOSCA stated that the maximum limits for seed of other varieties or off-types in foundation seed lots range from 0 to 0.2% among different species, while the limits for certified seed range from 0.1 to 2% by weight. This also can be demonstrated by stating that the maximum number of seeds of other varieties of the same crop permitted in one pound of certified seed is one seed for cotton and wheat, two for watermelons, four for rice, and six for sunflowers (Bradford, 2006; AOSCA, 2004; CCIA, 2005). The standards that have served society well for at least half a century were adopted to reflect a balance between the level of purity required to meet market needs and prevent consumer fraud and the cost of achieving that purity standard. While it is possible to achieve higher levels of purity, this involves higher production costs, often prohibitively higher. A recent study found that relative to standard corn seed production practices, the costs to achieve higher levels of genetic purity (in this case, a 0.3% biotech threshold) would be approximately 35% higher (Bradford, 2006; Kalaitzandonakes and Magnier, 2004). A non-biotechnology-derived corn seed standard has been established by AOSCA (http://www.identitypreserved.com/handbook/aosca-nongmocorn.htm) requiring 660 feet of isolation distance, field inspections and purity tests using AOSCA approved laboratories. This standard allows up to a 1% presence of biotechnology-derived seed in corn seed sold as "non-GMO." This AOSCA standard has been in place for a number of years, coexistent with widescale planting of biotechnology-derived corn. MON 87460 will have no greater impact on this or any other seed purity standard than any other corn hybrid, conventional or biotechnology-derived.

Almost all seed corn currently utilized in the U.S. is produced through hybridization. In this process one variety of corn produces pollen to fertilize a second variety, which has

been de-tasseled (the pollen producing male organs are removed) so it cannot fertilize itself. Because so much grain corn is grown in the continental U.S., hybrid seed production and bulk up is often done elsewhere (South America and Hawaii are commonly used) and in the off-season. Overall, three types of isolation practices (i.e., spatial, temporal, and physical barriers) help reduce the frequency of unwanted gene flow into seed corn via pollen transport. Most seed companies meet or exceed all national and international standards with regard to isolation and quality production practices.

In addition to instituting their own requirements, seed companies are working together regionally, nationally and internationally to establish better guidelines for biotechnology-derived seed production. Several groups set standards for production and monitor the sale and transportation of seed. Among these organizations, various standards act to guide the production of high-quality commercial seed including the certifications specified below.

Certification by the OECD is applied to varieties that meet established conditions of identity, uniformity, and stability. OECD certified varieties have an added economic value and are published in official OECD lists. The OECD helps ensure the varietal identity and quality of seed by setting appropriate requirements and controls throughout production, processing and labeling. Certified seeds are produced and officially controlled according to common harmonized procedures. OECD certification provides official worldwide recognition of "quality-guaranteed" seed, facilitating international trade and contributing to removal of technical trade barriers.

The AOSCA is dedicated to assisting companies in the production, identification, distribution and promotion of certified classes of seed. Established in 1919 in the United States, the organization has grown to include members from around the world. AOSCA establishes minimum standards for quality and identity. Its goal is to standardize certification regulations and procedures internationally so companies compete under one set of standards. The association cooperates with the OECD and other international organizations to develop standards, regulations, procedures, and policies to expedite movement of seed and encourage international commerce in improved varieties.

It is anticipated that MON 87460 seed will be produced and marketed in accordance with OECD and AOSCA standards and the U.S. Federal Seed Act and will have no adverse impact on seed production practices.

### IX.D.2. U.S. Corn Agronomic Practices

MON 87460 reduces yield loss under water-limited conditions. The targeted geographic area where MON 87460 is expected to have the greatest impact is the Great Plains, an area of significant corn production that is prone to frequent drought stress. Approximately 24 M acres in the Great Plains were planted to corn in 2008 resulting in 26% of the national corn production total. Key considerations for corn production in the Great Plains include climate and environment, soil quality and fertility, tillage and crop rotation practices, hybrid selection, nutrients, moisture, irrigation, and the management of

insects, weeds (including volunteer corn), and diseases. A summary of the corn cultivation and management practices in the Great Plains is provided below.

### IX.D.2.1. Climate and Environment

The climate of the Great Plains is characterized by two primary gradients which define the area and determine its environment; these are precipitation (decreasing east to west) and temperature (increasing north to south). Total annual precipitation ranges from 30 inches in the east to less than 15 inches in the west (Hubbard, 1997, Parton et al., 1994). The growing season varies from 110 days in the north to approximately 300 days in the south (Skold, 1997). Lack of moisture has resulted in a steppe or semi-arid natural grassland ecosystem with plant growth limited by precipitation and nutrient availability (Parton et al., 1994). Drought is a factor in this system with the degree and timing controlled by temperature, precipitation and the ratio of precipitation to potential evapotranspiration (Parton et al., 1994). Crops grown vary according to the climatic gradients of rainfall and temperature. Rain fed corn and soybean are produced in the east, dryland wheat and sorghum in the west. However, irrigation practices in the western sections of the Great Plains have supported significant corn production. It is in this type of climate that MON 87460 is expected to provide benefit and reduce grower risk due to drought.

### IX.D.2.2. Soil Quality and Fertility

Corn is grown in a variety of soils in the U.S. ranging from the sand hills of Nebraska and Colorado to the clays of delta regions, from strongly acidic to strongly alkaline soils, and from shallow soils on residual material to deep soils in loess, till or alluvium. The primary types of soils found in the Great Plains are classified as Mollisols (http://soils.usda.gov/technical/classification/taxonomy) and, due to their fertility and distribution throughout the world, are considered agriculturally and economically important. Mollisols form in semi-arid to semi-humid areas, typically under a grassland cover. They have a high organic matter, nutrient-enriched surface soil, typically between 60-80 cm thick, clay subsurface, and a soft, granular soil structure. This fertile surface soil layer results from the long-term addition of organic materials derived from plants. The combination of high organic matter and clay subsurface soil, in conjunction with good structural properties, facilitates water and nutrient storage and a degree of permeability favorable to water intake and air exchange. Corn hybrids perform well in mollisols unless environmental conditions such as drought intervene, causing yield loss. It is anticipated that MON 87460, which has agronomic and phenotypic characteristics equivalent to conventional corn (Section VIII), will also grow well in mollisol soils.

### IX.D.2.3. Tillage Practices

There are three main tillage practices employed in all corn production areas: conventional tillage, reduced tillage, and conservation tillage. Conventional tillage practices leave <15% crop residue cover after planting and involve the use of a moldboard plow or other intensive tillage procedure. Reduced tillage practices leave between 15-30% crop residue cover after planting and exclude the use of a moldboard plow or other intensive tillage procedures. Conservation tillage is a system that covers 30% or more of the soil surface

with crop residue after planting to reduce soil erosion by water and consists of three subtypes: no-till, ridge-till and mulch-till. These subtypes differ in the timing of cultivation of the seedbed and type of equipment used. Over the last two decades there has been a trend toward the increased use of conservation tillage practices with most of the growth coming from the expanded adoption of no-till. Studies in Great Plains states Nebraska, Montana and Texas (Smika and Wicks, 1968; Tanaka and Aase, 1987; Unger and Wiese, 1979) indicate that soil precipitation storage efficiencies increase when tillage is minimized or eliminated thus reducing the number of times moist soil is brought to the surface. Crop residues on the surface trap snow, absorb raindrop impact, slow runoff and minimize evaporation and wind velocities as well as aid in weed control. Considering the moisture retention benefits of minimal tillage, it is therefore not surprising that in 2004, four of the top five states with significant increases in no-till corn were from the Great Plains Table IX-4 (Peterson, 2005).

**Table IX-4. Greatest Increases in No-Till Corn in 2004**

| Rank | State | Increases (acres) |
|---|---|---|
| 1 | South Dakota | >253,000 |
| 2 | Colorado | >224,000 |
| 3 | North Dakota | >201,000 |
| 4 | Kansas | >191,000 |
| 5 | Wisconsin | >121,000 |

Source: Peterson, 2005

Coupled with public awareness of environmental issues, the trend toward increased conservation tillage continues particularly in the Mississippi watershed (Figure IX-1) whose tributaries, the Missouri, the Platte, the Arkansas and Oklahoma Rivers, drain the states of both the Midwest and the Great Plains. Given that, with the exception of yield under water-limited conditions, MON 87460 is agronomically and phenotypically equivalent to conventional corn, it is not anticipated that the introduction of MON 87460 will have any impact on current tillage practices or on the trend towards increasing adoption of conservation tillage by growers.

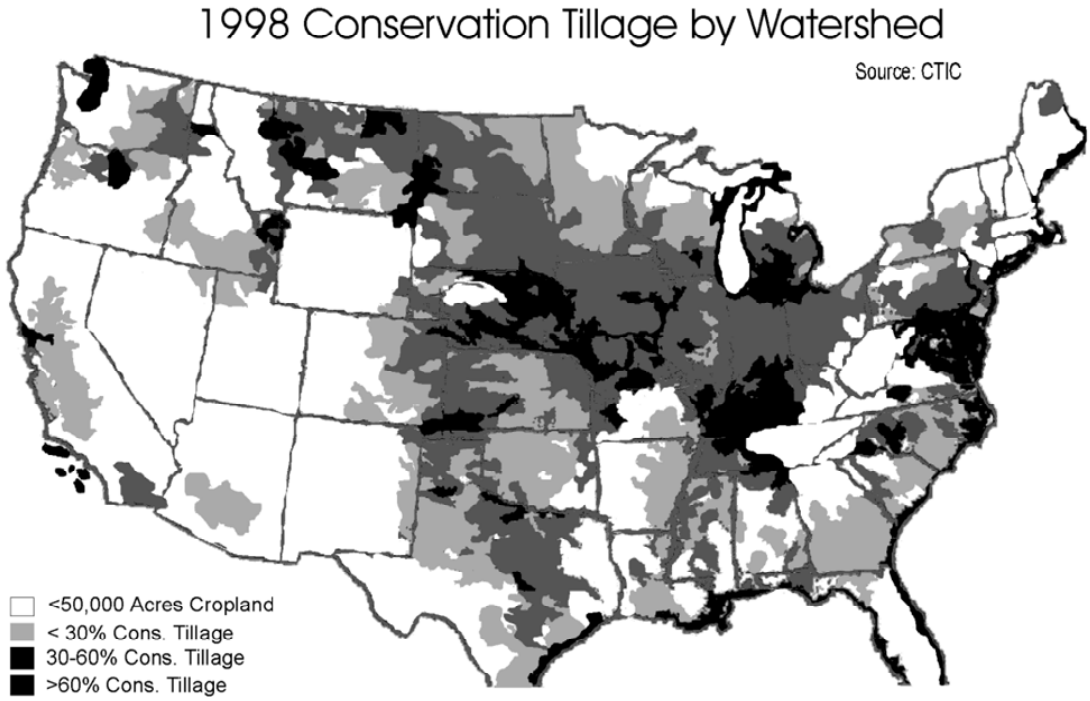



**Figure IX-1. U.S. Conservation Tillage Acreage by Watershed**

Source: http://agecon.okstate.edu/isct/labranza/walters/conservation.doc

### IX.D.2.4. Corn Hybrids

In the U.S. hundreds of corn hybrids are marketed by seed companies. Their selection is primarily based on yield potential, climatic environment, and disease/pest resistance for that locale.

Due to the extensive north-south orientation of the Great Plains, one of the most variable factors is the length and warmth of the growing season. Corn planting can begin in March in the Southern areas of the Great Plains while significant planting in the cooler Northern Great Plains typically does not begin until May. Harvest may occur as early as August in Texas or as late as October in North Dakota. Planting date and hybrid selection are managed to avoid occurrence of the critical corn pollination stage during the hottest, most stressful times of midsummer. Additionally growers in the Southern Great Plains can grow hybrids with a much longer maturity cycle than is possible in the Northern Great Plains (Nielson and Wishart, 2004). Planting densities in dryland areas is a subject of debate with ranges of 11,000 to 24,000 kernels of corn per acre being claimed to produce optimal yield results (Blumenthal et al., 2003; Norwood, 2001).

Hybrid characteristics that contribute to drought tolerance are complex and may not be readily apparent. The best indicator of drought tolerance is corn grain yield under moisture stress. Over the last several decades, breeding programs have been particularly successful at increasing the ability of corn to withstand drought occurring at flowering as demonstrated by decreases in anthesis-silking interval, and increases in ears per plant and kernels per ear, all of which result in yield increases (Campos et al., 2006). These breeding efforts have resulted in hybrids that are characterized as having excellent to very good drought tolerance and are available from major seed companies:

http://www.asgrowanddekalb.com/seedresourceguide/search/seeds
http://www.pioneer.com/web/site/portal/menuitem.b98005ee4490c1a5d6c1f492d10093a0/

Seed companies provide recommendations to growers for seed selection based on geographic location. Approximately 25% of the conventionally bred hybrids offered for planting in the dryer western areas of the Great Plains were rated as having excellent drought tolerance and were recommended for fields that regularly experience drought stress; 50-65% of the hybrids offered for planting in these areas were rated as having very good performance in fields that regularly experience some drought stress, but were not recommended for fields routinely experiencing extreme drought stress.

If approved for use, it is highly likely that biotechnology-derived traits conferring drought tolerance will become part of breeding programs. This additional tool for breeders has the potential to result in an expanded selection of hybrids for growers to choose from as they consider their individual growing needs and the likelihood of water-limited conditions during the growing season. It is not anticipated that addition of the MON 87460 drought tolerance trait in future breeding programs will result in a reduction in the selection of hybrids currently available to growers or in the introduction of an enhanced susceptibility to insects or disease, based on the mode of action and field observations (Sections I.D and VIII).

### IX.D.2.5. Nutritional and Moisture (Including Irrigation) Requirements of Corn

Corn, like all higher plants, requires at least thirteen elements from the soil for growth and development (Olson and Sander, 1988). The thirteen elements include the primary elements (nitrogen, phosphorus, and potassium), secondary elements (calcium, magnesium, sulfur), and micronutrient elements (iron, manganese, zinc, copper, boron, molybdenum and chlorine). By far the most important are the primary elements, which are depleted in the soil as the corn plant develops. Whereas nitrogen and phosphate uptake continues until maturity, potassium absorption is largely completed by the silking stage. This is why fertilization of corn fields is essential to ensure production and profitability. In 2005, nitrogen was applied to 96% of the planted corn acreage at an average usage rate of 138 pounds of nitrogen per acre per crop year. Phosphate was applied to 81% of the corn acreage at an average rate of 58 pounds per acre per crop year. Potassium, applied at 84 pounds per acre per crop year, was applied to 65% of the acreage planted to corn (USDA-NASS, 2006a).

Corn is extremely sensitive to soil moisture deficits. As stated previously total annual precipitation in the Great Plains ranges from 30 inches in the east to less than 15 inches in the west (Hubbard, 1997; Parton et. al., 1994). The additional moisture requirement can be supplemented by irrigation, which is practiced on 10-11 million corn acres or approximately 15% of the total U.S. corn acreage (USDA-ERS, 2002). The impact of irrigation on corn yields for the area is dramatic; an average 157 bushels/acre from irrigated production vs. an average 75 bushels/acre, from dryland production (Nielson and Wishart, 2004).

Water supply sources in the Great Plains include rain, surface water in rivers, streams, and lakes primarily from snowmelt, and groundwater in aquifers. Agriculture is the main demand for water use (Skold, 1997) and depletion of existing water basins and aquifers is associated with the increased use of irrigation. Irrigated land for all crops in the Great Plains increased more than fourfold from 3.5M acres in 1950 to 15M acres in 1990 (Opie, 1996) while total water applied increased by only 11% (USDA-ERS, 2003). Adoption of more water conserving practices and production shifts of some commodities to more humid and cooler areas is largely responsible for more efficient water use. Most agricultural freshwater withdrawals from surface and ground water for corn production occur in Nebraska, South Dakota, western Kansas, eastern Colorado and northern Texas where irrigated acres are concentrated (Figure IX-2).

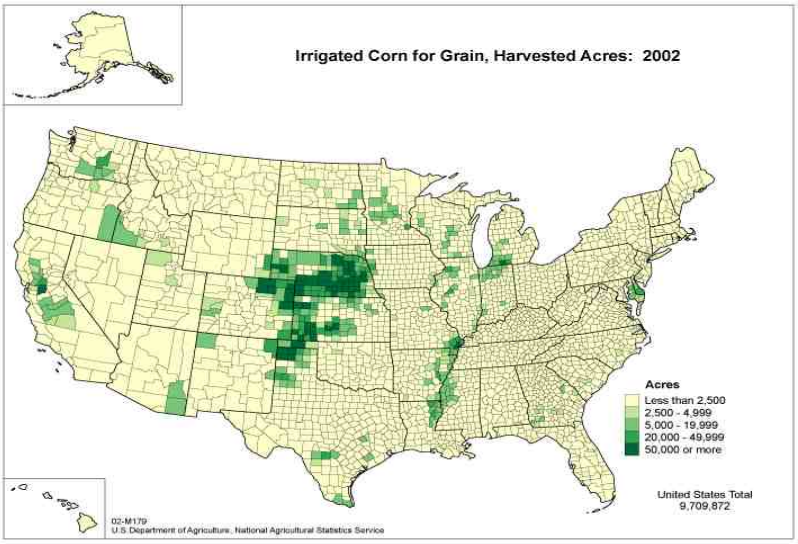



**Figure IX-2. U.S. Acres of Irrigated Corn Harvested for Grain**

Source: http://www.nass.usda.gov/research/2002mapgallery/fieldcrops.html

The shaded area of the Great Plains (Figure IX-2) correlates with the location of the Ogallala aquifer which was tapped for irrigation ground water in the 1950s resulting in depletion of the aquifer (McGuire, 2007). With the exception of New Mexico and Colorado, total irrigated acres planted to corn in Great Plains states increased from 1992 to 2007 (Table IX-5). Of the states with more than 1M acres under irrigation, only Kansas experienced a significant decline in the percent total corn acres under irrigation and a return to dryland production while Nebraska, South Dakota and Texas numbers remained relatively the same. According to Mapp (1988) the primary historic causes for a decline in irrigated acres are increases in pumping costs for pressurized groundwater irrigation systems and the low profitability of irrigated crops relative to dryland crops. Costs of supplying irrigation water vary widely reflecting different combinations of water sources, suppliers, distribution systems and other factors such as field proximity to water, topography, aquifer conditions and energy sources. Ground water is usually pumped on-farm with higher energy costs than surface water which is usually supplied from off-farm sources through storage and canal systems. In 2003, the average cost nationwide for ground water application was $40 per acre or ~$1.2B total. The average cost nationwide for surface water application was $26 per acre or ~$0.9B total (USDA-ERS, 2003). However, in recent years the growing biofuels industry and worldwide concern over food shortages have caused commodity prices to increase significantly. This may result in the continued high levels of irrigation use despite the higher costs.

Given the above, it is foreseeable that growers may choose to determine whether MON 87460 can be used in dryland production without benefit of irrigation. This is a decision driven by economic considerations, and is not anticipated to have a significant impact on irrigation practices.

**Table IX-5. Changes to Irrigated Corn Acreage in the Great Plains**

| State | 1992 | | | 1997 | | | 2002 Acres | | | 2007 Acres | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Total Acres* | *Irrigated Acres* | *% Irg.* | *Total Acres* | *Irrigated Acres* | *% Irg.* | *Total Acres* | *Irrigated Acres* | *% Irg.* | *Total Acres* | *Irrigated Acres* | *% Irg.* |
| Colorado | 891,720 | 808,351 | 92 | 919,784 | 769,567 | 84 | 708,197 | 634,015 | 90 | 1,060,000 | 700,000 | 66 |
| Kansas | 1,748,802 | 1,154,370 | 66 | 2,497,516 | 1,465,920 | 59 | 2,494,179 | 1,346,807 | 54 | 3,700,000 | 1,569,000 | 42 |
| Montana | 18,240 | 17,323 | 95 | 12,925 | 12,263 | 95 | 11,642 | 11,642 | 100 | 38,000 | 38,000 | 100 |
| Nebraska | 7,235,528 | 4,703,268 | 65 | 8,279,499 | 4,966,816 | 60 | 7,344,715 | 4,505,579 | 61 | 9,200,000 | 5,725,000 | 62 |
| New Mexico | 72,348 | 70,194 | 97 | 80,122 | 75,870 | 95 | 48,096 | 47,904 | 100 | 55,00 | 55,000 | 100 |
| North Dakota | 595,347 | 53,836 | 9 | 578,953 | 46,654 | 8 | 991,390 | 54,445 | 5 | 2,350,000 | 106,900 | 5 |
| Oklahoma | 123,567 | 69,104 | 56 | 150,404 | 85,678 | 57 | 182,777 | 99,457 | 54 | 270,000 | 140,000 | 52 |
| South Dakota | 3,097,251 | 172,233 | 6 | 3,175,113 | 148,001 | 5 | 3,165,190 | 123,229 | 4 | 4,500,000 | 201,000 | 5 |
| Texas | 1,549,680 | 740,431 | 48 | 1,656,229 | 871,364 | 53 | 1,815,560 | 658,177 | 36 | 2,000,000 | 975,000 | 49 |
| Wyoming | 54,341 | 52,767 | 97 | 49,717 | 47,931 | 96 | 34,095 | 33,507 | 98 | 60,000 | 57,500 | 96 |

Source: http://www.agcensus.usda.gov/index.asp

### IX.D.2.6. Crop Rotation

Growers make crop rotation decisions based on several factors, but a large consideration is the economic return resulting from differences in input costs, crop yield potential, and commodity prices. Crop rotations in the Western Dryland region of the Great Plains have relied on wheat and sorghum due to their ability to tolerate moderate water stress and produce acceptable yields. Changes in tillage management in the Great Plains cited previously (Section IX.D.2.3) have allowed farmers to change from the traditional wheat-fallow rotation to more intensive rotations such as wheat-corn-fallow. Intensive cropping systems typically have higher precipitation use efficiency, thus increasing crop yield per inch of rain. Dryland producers in Colorado have been adopting more intensive cropping systems, including dryland corn in rotation with wheat, at an increasing rate since 1990. The area planted to corn in northeastern Colorado, typically a wheat-fallow area, increased from 20,000 acres per year in years previous to 1990 to 220,000 acres in 1999 (Davis and Peterson, 2002). In addition to enhanced moisture conservation, a fourteen-year study of no-till intensive dryland rotation management showed increases in soil carbon, nitrogen, and organic matter and aggregate stability (USDA-ARS, 2007). These increases in soil quality measurements were correlated with greater rotation intensity and with less fallow frequency.

Depending on commodity prices, growers may continue to choose to increase the amount of corn in their rotations. Given that MON 87460 is agronomically and phenotypically equivalent to conventional corn, it is anticipated that it will readily become part of any dryland crop rotation system that includes corn. Corn hybrids with enhanced drought tolerance, either biotechnology-derived or conventional, may provide additional economic benefit in place of crops more commonly grown in these drier agricultural areas. If so, this decision will be driven largely by grower's economic concerns. In this scenario, MON 87460 is no more likely to displace wheat or sorghum than conventional drought tolerant corn hybrids already available to the grower.

### IX.D.2.7. Management of Diseases and Insects

MON 87460 was evaluated to determine whether the presence of the drought tolerance trait would have an impact on disease and insect management. Management of diseases and insects during corn growth and development is essential for protecting the yield of the harvested grain. Estimates for annual yield losses because of diseases have ranged from 7 to 17% (Shurtleff, 1980). Incidence of disease infestation is highly variable and depends on many factors such as location, climate, and other environmental factors. Most corn hybrids on the market today have acceptable levels of resistance to common diseases. The diseases found to occur in corn grown in the U.S. are summarized in Table IX-6. In addition, several nematode species have been known to cause diseases in corn (Dicke and Guthrie, 1988). The use of fungicides in corn is limited because the incidence and severity of most diseases tends to be low and quite variable. The fungicides currently used on corn plants in the U.S. include azoxystrobin, chlorothalonil, propiconazole (USDA-NASS, 2006a).

The corn crop is subject to attack by a complex of insects from the time it is planted until it is used as food and feed. The economically important insect pests in North America

include wireworms, the black cutworm, European corn borer, Southwestern corn borer, the corn rootworms, grasshoppers, fall armyworm, and corn earworm. Table IX-7 lists the insect pests in corn grown in the U.S. Approximately 27 active pesticidal ingredients are registered for use in corn for the control of insect pests. In its annual survey of agricultural chemical usage, USDA determined that 23% of the corn acreage was treated with insecticides in 2005 (USDA-NASS, 2006a). Tefluthrin, cyfluthrin, and tebupirimphos were the most widely applied insecticides, at 7, 7, and 6%, respectively, to the corn acres. Chlorpyrifos was only applied to 2% of the acres, but total quantity applied is more than 3 times greater than next highest insecticide at 2.0 million pounds.

The introduction of biotechnology-derived insect protected corn has offered growers an alternative and effective solution for the control of major insect pests in corn and in 2006 approximately 40% of the total corn acreage in the U.S. was planted with hybrids possessing insect protection traits (USDA-NASS, 2006b).

MON 87460 has no pesticidal activity. As demonstrated by environmental observations among numerous field studies (Section VIII.F.1), it has no apparent impact on arthropods and diseases of corn. Therefore, no changes to current disease and insect management practices, including pesticide use, conventional breeding selection for resistance, or adoption of biotechnology-derived traits are anticipated from the introduction of MON 87460.

### IX.D.2.8. Weed Management

MON 87460 was evaluated to determine whether the presence of the drought tolerance trait would have an impact on weed management. Weeds cause significant losses and require careful management by growers because they interfere with corn plants by competing for available resources including water, nutrients and light. Economically damaging weeds in corn include annuals and perennials, grasses, broadleaf and sedge species. Some weeds can tolerate cold, wet conditions better than corn, and can get a head start prior to planting. Fields infested with perennial weeds present special problems for corn growers. Like annual weeds, perennials can reproduce by seeds, but they also regrow and spread vegetatively. Their rhizomes propagate new shoots, usually soon after corn is planted. Unless effectively controlled, perennial weeds can quickly gain a season-long advantage over the corn crop.

Corn yield loss is generally proportional to the amount of weeds present. While the ratio is not always one-to-one, some studies suggest that for every pound of weed dry matter, there is a reduction of approximately one pound of corn plant dry matter (Gianessi et al., 2002). Competition for light, nutrients, and moisture resources by the crop and weeds can lead to proportional reductions in yield (Knake et al., 1990). Numerous studies have shown that weed control early in the growing season is necessary to reduce yield losses in corn. Weed species such as giant foxtail, barnyardgrass and pigweed can reduce corn yields by up to 13, 35 and 50% respectively (Bosnic and Swanton, 1997; Fausay et al., 1997; Knake and Slife, 1965). In a study of mixed weed populations competing with corn, corn yields were reduced by up to 20% when the weed plants reached a height of eight inches (Carey and Kells, 1995).

Corn is typically planted in wide rows (30 inches) and has an upright leaf orientation. As a result, corn is not successful in competing with weeds early in the growing season. Corn is also usually planted early when soil temperature and weather conditions favor weed over corn growth. A survey of Extension Service weed scientists solicited estimates of the percent of corn acreage infested with individual weed species by state or region, as well as the potential impact on corn yields if the species were left uncontrolled. In this survey, 12 annual broadleaf, nine annual grass, and seven perennial species were identified as troublesome weeds (Table IX-8) (Gianessi et al., 2002). Estimates of yield loss ranged from a low of 15% due to wirestem muhly and sandburs to a high of 48% from burcucumber.

Until the early 1950s, tillage and cultivation practices were primarily used for weed control in corn, but since then they have been largely replaced by the use of herbicides. Herbicide use in corn became widespread by the end of the 1970s. In 2005, herbicides were applied to 97% of the corn planted acreage (USDA-NASS 2006a). Atrazine continues to be the most widely applied herbicide with 66% of the planted acreage being treated. It was applied at an average rate of 1.133 pounds per acre. Glyphosate isopropylamine salt was applied to 31% of planted acres, up from 19% in 2003, at an average rate of 0.963 pounds per acre. In terms of area applied, that was followed closely by S-metolachlor and acetochlor, at 23% of the planted corn acreage treated. In 2001, the EPA identified glyphosate as the most widely used conventional agricultural pesticide in the U.S. (Kiely et al., 2004). The addition of herbicide-tolerant corn to an integrated weed management program offers multiple benefits to growers including a broader spectrum of weeds controlled, reduced crop injury, and less herbicide carry-over. Roundup Ready Corn 2 developed by Monsanto and Liberty Link corn developed by Bayer provide glyphosate-tolerance and glufosinate-tolerance, respectively. These herbicide-tolerance technologies are easy to use and both herbicides are more environmentally friendly than other herbicide options (Knezevic and Cassman, 2003).

Volunteer corn commonly occurs in rotational crops in the season following corn cultivation regardless of whether or not the corn was conventional or biotechnology-derived. When corn is grown for silage, on approximately 9% of the U.S. corn acres, volunteer corn plants typically do not occur in rotational crops since corn harvested for silage does not produce viable seed. In the warmer climates of the Southeast and Southwest, the occurrence of volunteer corn is rare because any corn grain remaining after harvest is likely to germinate in the fall and the resulting plants can usually be controlled by tillage or by freezing temperatures in the winter. In the Northern corn-growing regions, volunteer corn does not always occur in the rotational crop because of seed decomposition over the winter, efficient harvest procedures, and tillage prior to planting rotational crops.

The first step to manage volunteer corn in rotational crops is to minimize or reduce the potential for volunteers. The following practices are implemented to reduce volunteer corn in rotational crops: (1) adjust harvest equipment to minimize the amount of corn grain lost in the field, (2) plant corn hybrids that reduce the extent of ear drop, (3) choose

corn hybrids with superior stalk strength and reduced lodging, and (4) practice no-till production to significantly reduce the potential for volunteer growth in the rotational crop. If volunteer corn does occur in subsequent crops, pre-plant tillage or in-crop cultivation are very effective management tools. Selective herbicides labeled for the control of volunteer corn in the particular rotational crop are available. Assure II® (quizalofop), Fusilade® DX (fluazifop), Fusion® (fluazifop + fenoxaprop), Poast® (sethoxydim), and Select® 2EC (clethodim) provide effective postemergence control of volunteer corn in labeled crops. These products are labeled for use in eight field crops, including soybean, cotton, sugar beet and alfalfa, and eleven vegetable rotation crops.

MON 87460 will likely be combined through conventional breeding with deregulated herbicide tolerance traits, and consequently, growers will be able to achieve the same high level of weed control as they have with other biotechnology-derived herbicide tolerant corn hybrids. Additionally, because MON 87460 is agronomically and phenotypically equivalent to conventional corn, it is not anticipated that MON 87460 will respond differently to commonly used herbicides, or that its introduction will have any impact on weed management practices in the U.S. compared to current production practices that include biotechnology-derived or conventional corn hybrids, including drought tolerant varieties.

® Assure II is a trademark of E.I. DuPont de Nemours, Inc.
® Fusilade and Fusion are trademarks of Syngenta Group Company.
® Poast is a trademark of BASF Corporation.
® Select is a trademark of Valent U.S.A. Corporation.

**Table IX-6. Diseases of Corn**

| Common Name | Causative Agent [transmittal agent] |
|---|---|
| ***Seed rots and seedling blights*** | *Fusarium moniliform*, *Pythium* spp. |
| ***Foliar Diseases*** | |
| Bacterial leaf blight and stalk rot | *Pseudomonas avenae* |
| Bacterial stripe | *Pseudomonas andropogonis* |
| Stewart's wilt | *Erwinia stewartii* |
| Chocolate spot | *Pseudomonas coronafaciens* |
| Goss's wilt | *Clavibacter michiganense* |
| Holcus spot | *Pseudomonas syringe* |
| Anthracnose | *Colletotrichum graminicola* |
| Eyespot | *Kabatiella zeae* |
| Gray leaf spot | *Cercospora zeae-maydis* |
| Northern leaf spot | *Bipolaris zeicola* |
| Northern corn leaf blight | *Exserohilum turcicum* |
| Physoderma brown spot | *Physoderma maydis* |
| Southern corn leaf blight | *Bipolaris maydis* |
| Yellow leaf blight | *Phyllosticta maydis* |
| Common rust | *Puccinia sorghi* |
| Southern corn rust | *Puccinia polysora* |
| Common corn smut | *Ustilago maydis* |
| ***Systemic Diseases*** | |
| Head smut | *Sphacelotheca reiliana* |
| Crazy top | *Sclerophthora macrospora* |
| Sorghum downy mildew | *Peronosclerospora sorghi* |
| Maize dwarf mosaic virus | [aphids] |
| Maize chlorotic dwarf virus | [leafhoppers] |
| Corn lethal necrosis | [chrysomelid beetles] |
| Maize white line mosaic virus | [not identified] |
| Corn stunt | [leafhoppers] |
| Maize bushy stunt | [leafhoppers] |
| ***Stalk and root rots*** | |
| Gibberella stalk rot | *Gibberella zeae* |
| Diplodia stalk rot | *Stenocarpella maydis* |
| Anthracnose stalk rot | *Colletotrichum graminicola* |
| Charcoal rot | *Macrophomina phaseolina* |
| Fusarium stalk rot | *Fusarium moniliforme* |
| Pythium stalk rot | *Pythium aphanidermatum* |
| Bacterial stalk rot diseases | *Erwinia chrysanthemi* |
| Root rots | *Pythium* spp. |
| ***Ear rots and storage molds*** | |
| Fusarium ear rot | *Fusarium moniliforme* |
| Gibberella ear rot | *Gibberella zeae* |
| Diplodia ear rot | *Diplodia maydis* |
| Aspergillus ear and kernel rot | *Aspergillus flavus* |
| ***Storage molds*** | *Penicillium* spp., *Aspergillus* spp. |

Source: Smith and White, 1988

**Table IX-7. Insect Pests of Corn**

| Common Name | Latin name |
|---|---|
| ***Soil Insects*** | |
| Northern corn rootworm | *Diabrotica barberi* |
| Western corn rootworm | *Diabrotica virgifera virgifera* |
| Southern corn rootworm | *Diabrotica undecimpunctata* |
| Black cutworm | *Agrotis ipsilon* |
| Wireworms | *A. mancus, Horistonotus uhlerii, Melanotus cribulosus,* others |
| Billbugs | *Sphenophorus* spp. |
| White grubs | *Phyllophaga* spp. |
| Corn root aphid | *Anuraphis maidiradicis* |
| Seedcorn maggot | *Delia platura* |
| Grape colaspis | *Colaspis brunnea* |
| Seed corn beetle | *Stenolophus lecontei* |
| | |
| ***Insects attacking the leaf, stalk, and ear*** | |
| Corn earworm | *Helicoverpa zea* |
| European corn borer | *Ostrinia nubilalis* |
| Corn leaf aphid | *Rhopalosiphum maidis* |
| Fall armyworm | *Spodoptera frugiperda* |
| Stalk borers | *Diatraea spp.* |
| Armyworm | *Pseudaletia unipuncta* |
| Lesser stalk borer | *Elasmopalpus lignosellus* |
| Chinch bug | *Blissus leucopterus leucopterus* |
| Grasshoppers | *Melanoplus differentialis* |
| Corn flea beetle | *Chaetocnema pulicaria* |
| Japanese beetle | *Popillia japonica* |
| | |
| ***Other insects*** | |
| Thrips | *Anaphothrips* spp., *Frankliniella* spp. |
| Leafhoppers | *Trigonotylus brevipes*, others |
| Western bean cutworm | *Striacosta albicosta* |
| Corn blotch leaf miner | *Agromyza parvicornis* |
| Spider mites | *Oligonychus* spp., *Tetranychus* spp. |
| Pink scavenger caterpillar | *Pyroderces rileyi* |
| Garden symphlan | *Scuttigerella immaculata* |
| Hop-vine borer | *Hydraecia immanis* |
| Sod webworms | Subfamily Cramdinae |
| Leaf rollers | |
| Stink bugs | |
| | |
| ***Insect disease vectors*** | Several |
| | |

Sources: Dicke and Guthrie, 1988; and University of Missouri, 1998.

**Table IX-8. Troublesome Weeds in U.S. Corn Fields**

| Weed Species | Area Infested [1] | Acreage Infested (%) | Potential Yield Loss (%) |
|---|---|---|---|
| **Annuals** | | | |
| ***Broadleaves*** | | | |
| Burcucumber | PA/OH/TN/SE | 5-10 | 48 |
| Cocklebur | MW/NP/SE | 20-60 | 33 |
| Jimsonweed | MW/CO | 5-20 | 17 |
| Kochia | NP/NW | 10-70 | 33 |
| Lambsquarters | MW/SE/NE/CA | 15-80 | 33 |
| Morningglory | MW/SE/SP | 20-75 | 33 |
| Nightshade | MW/NP/CA | 25-50 | 26 |
| Pigweeds/Waterhemp | US | 30-90 | 36 |
| Ragweed, Common | MW/SE/NE | 20-70 | 30 |
| Ragweed, Giant | MW/NP | 10-45 | 28 |
| Smartweeds | MW/SD/NE/SE | 30-70 | 22 |
| Velvetleaf | MW/NE/NP | 25-70 | 28 |
| | | | |
| ***Grasses*** | | | |
| Barnyardgrass | SP/NW/CA | 80-90 | 23 |
| Bermudagrass | MD/SE/UT/CA | 10-20 | 47 |
| Crabgrass spp. | MW/SE/NE | 20-80 | 29 |
| Cupgrass, Woolly | IA/WI | 15-20 | 29 |
| Foxtail spp. | MW/NE/NP | 50-90 | 31 |
| Millet, Wild-Proso | UT/WY/CO/ID | 15-40 | 31 |
| Panicum, Fall | MW/SE/NE/NP | 15-80 | 30 |
| Sandburs | NP/UT/WY | 5-30 | 15 |
| Shattercane | MW/SP | 5-40 | 33 |
| | | | |
| **Perennials** | | | |
| Bindweed, Field | ND/SW/CA | 40-80 | 18 |
| Dogbane, Hemp | IL/MO | 2-20 | 21 |
| Johnsongrass | MW/SE/SW/CA | 20-60 | 45 |
| Muhly, Wirestem | PA | 2 | 15 |
| Nutsedge, Yellow | MW/SE/NE/NP/CA | 10-70 | 21 |
| Quackgrass | MW/NE/UT | 10-70 | 27 |
| Thistle, Canada | NE/MW/NP/CO | 5-25 | 26 |

Source: Gianessi et al., 2002.

[1]**Regions**
US: United States
MW:Midwest
NE: Northeast
NP: Northern Plains
NW:Northwest
SE: Southeast
SW: Southwest
SP: Southern Plains

**States**
CA: California
CO: Colorado
ID: Idaho
IA: Iowa
MD: Maryland
MO: Missouri
ND: North Dakota
OH: Ohio
PA: Pennsylvania
SD: South Dakota
TN: Tennessee
UT: Utah
WI: Wisconsin
WY: Wyoming

## IX.E. Corn Acreage, Land Use, and the Conservation Reserve Program

Total corn acreage in the U.S. has remained relatively steady from 1996 to 2006 with a yearly average of 78 million acres indicating that biotechnology-derived corn, which has been commercially available for over a decade, has had little to no impact on total corn acreage. In 2007, the total corn acreage increased by 15% from 78 million to 92.9 million acres. This increase was attributed to increased demand from ethanol producers (http://www.ncga.com/node/83) and strong exports sales. The increase in total corn acres resulted primarily from fewer acres of soybean planted in the Corn Belt and Great Plains (USDA-NASS, 2007).

A small percentage of the increase in corn acreage came from additional corn plantings on land that had previously been enrolled in the Conservation Reserve Program (CRP). The CRP is a voluntary program offering annual rental payments over a 10-year contract period, as well as cost-share assistance, to producers establishing specific types of plant cover on marginal farmland. Of the 36M acres enrolled in the CRP in 2006, 21.2M acres or 59% were from Great Plains states with 7.9M acres or 22% from Midwest "corn-belt" states (USDA-FSA-CRP, 2007).

Re-enrollment for 15.7M eligible acres with CRP contracts due to expire in 2007 dropped by 2.6 M to 13.1M acres compared with 2006 (USDA-FSA-CRP, 2007). This drop could be attributed to the growing biofuels industry and worldwide concern over food shortages that have caused commodity prices to increase, with the result that some growers are not renewing their enrollment in the CRP. This trend is already underway and may be accelerated by the introduction of a corn hybrid with enhanced drought tolerance characteristics, regardless of whether the hybrid has been developed through biotechnology or conventional breeding. In this scenario, introduction of MON 87460 is no more likely to impact changes in corn acreage and land use than a conventionally bred drought tolerant hybrid. A decrease in commodity prices would likely be accompanied by an increase in CRP enrollment, despite the availability of drought-tolerant corn.

## IX.F. Mitigation and Remediation Measures Before and After Deregulation of MON 87460 in the U.S.

Under the Coordinated Framework for Regulation of Biotechnology, the responsibility for regulatory oversight of non-pesticidal biotechnology-derived crops falls on two lead federal agencies: FDA and USDA (OSTP, 1986). Deregulation of MON 87460 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 87460 cannot be released and marketed until both FDA and USDA have completed their reviews and assessments under their respective jurisdictions.

Food and feed from biotechnology-derived crops are subject to regulatory review by FDA under the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 346 a(d)]. Since 1992, FDA has used a voluntary consultation process to work together with biotechnology-derived product developers to identify and resolve any issues regarding the safety and nutritional content of food and feed from biotechnology-derived crops. Using the current

process, Monsanto has initiated and will complete a consultation with FDA prior to commercial distribution of MON 87460 (Section I.F.1).

The Animal and Plant Health Inspection Services (APHIS) of the United States Department of Agriculture (USDA) has responsibility under the Plant Protection Act (7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. The APHIS regulation at 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated.

Monsanto has provided to both the FDA and the USDA a comprehensive data package including: (1) the intended technical effect of the modification of the corn plants; (2) a molecular characterization of the modification, including the identities, sources, and functions of the introduced genetic material; (3) information on the expressed CSPB and NPTII proteins encoded by the introduced genes; (4) information on assessment of potential allergenicity and toxicity of the introduced proteins; (5) information on the compositional and nutritional characteristics of the food and feed derived form the regulated article and, in the case of the USDA, (6) information on the plant pest potential of the introduction of MON 87460, and (7) an assessment of the potential impact on NTOs and threatened or endangered species. Based on these data, there is no reason to expect that food and feed derived from MON 87460 constitute a risk to human health. Because these data demonstrate that MON 87460 does not pose a plant pest risk, it is not anticipated that mitigation or remediation measures will be necessary for this product.

Monsanto employs a rigorous product stewardship program that demonstrates respect for our customers, their markets and the environment. Our market stewardship program considers many factors to support global integration and assure increased transparency. In keeping with past practice, we will not launch MON 87460 without first gaining regulatory approval from the key corn import countries to assure global compliance and support the flow of international trade. We commit to best industry practices on seed quality assurance and control to prevent adventitious presence of unapproved traits. Before commercializing MON 87460, a detection method will be made available to grain producers, processors, and buyers. Our stewardship policy is the shared responsibility of Monsanto, our licensees, and our customers to assure that our products are used properly. We are committed to our legal and ethical obligation to ensure that our products and technologies are safe and environmentally responsible, and do not pose undue risks to human health or the environment during any stage of their life cycle. As such, Monsanto has policies in place to meet these commitments as we research, develop, design, manufacture, market, and discontinue products through their product life cycle.

### IX.G. Conclusions

MON 87460 has been shown to be no different from conventional corn in its phenotypic, environmental, or compositional characteristics, with the exception of the drought tolerance trait (Sections VII and VIII). Thus, MON 87460 is similar to conventional corn in its agronomic characteristics and plant pest potential. Tillage, crop rotation, planting density, hybrid selection, and irrigation practices have historically been modified to

optimize corn yields in the Great Plains where precipitation use efficiency is a primary concern. As biotechnology-derived agricultural products have offered safe and effective alternatives to weed and insect control in the past, MON 87460 is another option for growers to help minimize production risk by providing increased yield stability under water-limited conditions.

Monsanto is not aware of any study results or observations associated with MON 87460 that would suggest that an increased plant pest risk would be anticipated to result from its introduction. MON 87460 reduces yield loss under water-limited conditions. The greatest benefit is expected to occur in regions that are suitable for corn production but prone to frequent drought stress, such as the Western Dryland region of the Great Plains. As demonstrated by field and laboratory study results, the only phenotypic difference between MON 87460 and conventional corn is reduced yield loss under water-limited conditions, due to expression of the CSPB protein.

The plant pest assessment of MON 87460 was based on multiple lines of evidence developed from a detailed characterization of MON 87460 compared to conventional corn, followed by a risk assessment on detected differences. The risk assessment considered various factors including: (1) the negligible risk for MON 87460 or its progeny to establish or persist in the environment without human assistance, (2) minimal availability of suitable hosts or habitats for MON 87460, and (3) the negligible risk for MON 87460 to cause damage to plants and plant products.

The assessment for potential impacts of MON 87460 on agronomic practices was based on an evaluation of the current production, land use, and agronomic practices for corn, with particular reference to the Western Dryland region of the Great Plains. Those assessments indicate that the introduction of MON 87460 is no more likely to impact agronomic and land use practices in the Great Plains than the use of conventionally bred drought tolerant corn. Finally, due to the inherently low plant pest potential of modern corn and the lack of (a) weediness and horizontal gene transfer potential, (b) any impact on disease or injury to plants or plant pests in the field, or (c) any observed effects on non-target or beneficial organisms in the agro-ecosystem, it is concluded that MON 87460 is unlikely to pose a plant pest risk.

Based on the data and information presented in this petition, it is concluded that MON 87460 is unlikely to be a plant pest. The adoption of MON 87460 may increase economic and environmental benefits primarily in the Western Dryland region of the Great Plains, due to the protection of corn yield under water-limited conditions, but is not expected to have a significant environmental impact. Therefore, Monsanto Company requests a determination from APHIS that MON 87460 and any progeny derived from crosses between MON 87460 and other commercial corn be granted non-regulated status under 7 CFR Part 340.

## IX.H. References


AOSCA. 2004. Operational Procedures and Crop Standards. Association of Official Seed Certifying Agencies.

Baker, H.G. 1965. Characteristics and modes of origin of weeds. Page 147-172 in The Genetics of Colonizing Species. Baker, H.G. and G.L. Stebbins (eds.). Academic Press, New York.

Bannert, M. 2006. Simulation of transgenic pollen dispersal by use of different grain colour maize. Doctor of Sciences dissertation. Swiss Federal Institute of Technology Zurich. Diss. ETH No. 16508.

Blumenthal, J.M., D.J. Lyon and W.W. Stroup. 2003. Optimal plant population and nitrogen fertility for dryland corn in western Nebraska. Agronomy Journal. 95:878-883.

Bosnic, A.C. and C.J. Swanton. 1997. Influence of barnyard grass (*Echinochloa crus-galli*) time of emergence and density of corn (*Zea mays*). Weed Science. 45:276-282.

Boyer, J. S. and M. E. Westgate. 2004. Grain yields with limited water. Journal of Experimental Botany. 55:2385-2394.

Bradford, K.J. 2006. Methods to maintain genetic purity of seed stocks. Division of Agricultural and Natural Resources Publication 81289. University of California-Oakland. http://www.plantsciences.ucdavis.edu/bradford/8l89.pdf

Campos, H., M. Cooper, G.O. Edmeades, C. Loffler, J.R. Schussler, and M. Ibanez. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. Maydica. 51:369-381.

Carey, J.B. and J.J. Kells. 1995. Timing of total post-emergence herbicide applications to maximize weed control and corn (*Zea mays*) yield. Weed Technology. 9:356-361.

Castiglioni, P., D. Warner, R.J. Bensen, D.C. Anstrom, J. Harrison, M. Stoecker, M. Abad, G. Kumar, S. Salvador, R. D'Ordine, S. Navarro, S. Back, M. Fernandes, J. Targolli, S. Dasgupta, C. Bonin, M.H. Luethy, and J.E. Heard. 2008. Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiology. 147:446-455.

CCIA. 2005. Seed Certification. California Crop Improvement Association. http://ccia.ucdavis.edu/seed_cert/seedcert_index.htm.

Claassen, M. M. and R. H. Shaw. 1970. Water deficit effects on corn. II. Grain components. Agronomy Journal. 62: 652-655.

Crockett, L. 1977. Wildly Successful Plants: North American Weeds. University of Hawaii Press, Honolulu, Hawaii.

Davis, J. and G. Peterson. 2002. Dryland Corn Newsletter, Cooperative Extension, Colorado State University, Vol. 22 (3) 1-2. http://www.extsoilcrop.colostate.edu/Newsletters/documents/2002_corn.pdf

Demanèche, S., H. Sanguin, and P. Simonet. 2008. Antibiotic-resistant soil bacteria in transgenic plant fields. Proceedings of the National Academy of Sciences 105: 3957-3962.

Dicke, F.F. and W.D. Guthrie. 1988. The most important corn insects. Pages 769-868 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

EFSA. 2007. Statement of the Scientific Panel on Genetically Modified Organisms on the safe use of the *npt*II antibiotic resistance marker gene in genetically modified plants. European Food Safety Authority. Brussels, Belgium http://www.efsa.europa.eu.

EFSA. 2009. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ). Use of antibiotic resistance genes as marker genes in genetically modified plants. EFSA Journal. 1034: 1-82.

Fausay, J.C., J.J. Kells, S.M. Swinton, and K.A. Renner. 1997. Giant foxtail interference in non-irrigated corn. Weed Science. 45:256-260.

FDA. 1994. U.S. Food and Drug Administration. Secondary direct food additives permitted in food for human consumption: food additives permitted in feed and drinking water of animals: aminoglycoside 3'-phosphotransferase II; final rule. Federal Register.

FDA. 1998. U.S. Food and Drug Administration. Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants (Draft Guidance released September 4, 1998).

Flavell, R.B., E. Dart, R.L. Fuchs, and R.T. Fraley. 1992. Selectable marker genes: safe for plants? BioTechnology. New York. 10:141-144.

Fuchs, R.L., R.A. Heeren, M.E. Gustafson, G.J. Rogan, D.E. Bartnicki, R.M. Leimgruber, R.F. Finn, A. Hershman, and S.A. Berberich. 1993a. Purification and characterization of microbially expressed neomycin phosphotransferase II (NPTII) protein and its equivalence to the plant expressed protein. BioTechnology. New York. 11:1537-1542.

Fuchs, R.L., J.E. Ream, B.G. Hammond, M.W. Naylor, R.M. Leimgruberand, and S.A. Berberich. 1993b. Safety assessment of the neomycin phosphotransferase II (NPTII). BioTechnology. New York. 11:1543-1547.

Galinat, W.C. 1988. The origin of corn. Pages 1-31 in Corn and Corn Improvement., G.F. and J.W. Dudley (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin.

Gianessi, L.P., C.S. Silvers, S. Sankula, and J.E. Carpenter. 2002. Plant biotechnology: current and potential impact for improving pest management in U.S. agriculture. an analysis of 40 case studies. National Center for Food & Agricultural Policy, Washington, D.C.

Graumann, P., T.M. Wendrich, M.H.W. Weber, K. Schroder, and M.A. Marahiel. 1997. A family of cold shock proteins in *Bacillus subtilis* is essential for cellular growth and for efficient protein synthesis at optimal and low temperatures. Molecular Microbiology. 25:741-756.

Holm, L., J.V. Pancho, J.P. Herberger, and D.L. Plucknett. 1979. Introduction. Pages i-vii in A Geographical Atlas of World Weeds. John Wiley and Sons, New York.

Hubbard, K. 1997. Great Plains Region: Climate System Characteristics. Presentation at Great Plains Climate Change Workshop 5/97.

Jemison, J.M. and M. Vayda. 2000. Pollen transport from genetically modified corn. Page 145 in 2000 Agronomy Abstracts. American Society of Agronomy (ASA), Madison, Wisconsin.

Kalaitzandonakes, N. and A. Magnier. 2004. Biotech labeling standards and compliance costs in seed production. Choices. 19:1-9.

Keeler, K. 1989. Can genetically engineered crops become weeds? BioTechnology. 7:1134-1139.

Kiely, T., Donaldson, D. and Grube, A. 2004. Pesticide Industry Sales and Usage: 2000 and 2001 Market Estimates. Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency.

Knake, E.L. and F.W. Slife. 1965. Giant foxtail seeded at various times in corn and soybeans. Weeds. 13:331-334.

Knake, E.L., J.D. Doll, J.F. Miller and O.E. Strand. 1990. Annual Grass Weeds in Corn. National Corn Handbook, Michigan State University.

Knezevic, S.Z. and K.G. Cassman. 2003. Use of herbicide-tolerant crops as a component of an integrated weed management program. Online. Crop Management doi:10.1094/CM-2003-0317-01-MG.

Luna, S.V., J.M. Figueroa, B.M. Baltazar, R.L. Gomez, R. Townsend, and J.B. Schoper. 2001. Maize pollen longevity and distance isolation requirement for effective pollen control. Crop Science. 41:1551-1557.

Mangelsdorf, P.C. 1974. Corn: its Origin, Evolution, and Improvement. Harvard University Press. Cambridge, Massachusetts.

Mapp, H.P. 1988 Irrigated agriculture on the High Plains: an uncertain future. Western Journal of Agricultural Economics. 13(2): 339-347.

McGuire, V.L., 2007, Changes in water level and storage in the High Plains aquifer, predevelopment to 2005: U.S. Geological Survey Fact Sheet. 2007-3029, 2 p

Muenscher, W.C. 1980. Weeds. 2nd ed. Cornell University Press, New York.

Nap, J.P., J. Bijvoet, and W.J. Stiekema. 1992. Biosafety of kanamycin-resistant transgenic plants: an overview. Transgenic Research. 1:239-249.

Nielson R. L., D.J. Wishart (eds.). 2004. Encyclopedia of the Great Plains, University of Nebraska Press, p. 39-40.

Norwood, C.A. 2001. Planting date, hybrid maturity and plant population effects on soil water depletion, water use and yield of dryland corn. Agronomy Journal. 93:1034-1042.

OECD. 1998. Report of the OECD Workshop on the Toxicological and Nutritional Testing of Novel Foods. Organization for Economic Co-operation and Development. Paris, France.

Office of Science and Technology Policy. 1986. Coordinated Framework for Regulation of Biotechnology. 51 FR 23302.

Olson, R.A. and D.H. Sander. 1988. Corn production. Pages 639-686 in Corn and Corn Improvement, 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Opie, J. 1996. Does its history doom the Plains to failure? Putting chaos theory to work. Pages 5-18 in Cathy Bruce and Lori Triplett. The Great Plains Symposium 1996: The Ogallala Aquifer: Sharing the Knowledge for the Future. The Great Plains Foundation.

Parton, W.J., D.S. Ojima and D.S. Schimel. 1994. Environmental changes in grasslands: assessment using models. Climate Change. 28:111-141.

Peterson, D. 2005. Land & Water, University of Illinois Extension, College of Agricultural, Consumer & Environmental Sciences, Number 6.

Riebsame, W.E. 1990. The United States Great Plains. Ch. 34, p. 561-575 in B.L. Turner II et. al., The Earth as Transformed by Human Action. Cambridge University Press, NY.

Russell, W.A. and A.R. Hallauer. 1980. Corn. Pages 299-312 in Hybridization of Crop Plants. Fehr, W.R. and H.H. Hadley (eds.). American Society of Agronomy, and Crop Science Society of America, Publishers, Madison, Wisconsin.

Schindler, T., P.L. Graumann, D. Perl, S.F. Ma, F.X. Schmid, and M.A. Marahiel. 1999. The family of cold shock proteins of *Bacillus subtilis* - stability and dynamics *in vitro* and *in vivo*. Journal of Biological Chemistry. 274:3407-3413.

Shurtleff, M.C. 1980. Compendium of Corn Diseases. 2nd ed. American Phytopathological Society, St. Paul, Minnesota.

Skold, M.D. 1997. Agricultural Systems and Economic Characteristics on the Great Plains. 4p. Briefing Document for Great Plains Climate Change Workshop 5/97.

Smika, D.E., and G.A. Wicks. 1968. Soil water storage during fallow in the central Great Plains as influenced by tillage and herbicide treatments. Soil Science Society of America Proceedings. 32:591-595.

Smith, D.R. and D.G. White. 1988. Diseases of corn. Pages 687-766 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Sundstrom, F.J., J. Williams, A. Van Deynze, and K.J. Bradford. 2002. Identity preservation of agricultural commodities. Division of Agricultural and Natural Resources Publication 8077. University of California-Oakland. http://anrcatelog.ucdavis.edu

Tanaka, D.L., and J.K. Aase. 1987. Fallow method influences on soil water and precipitation storage efficiency. Soil Tillage Research. 9:307-316.

Unger, P.W. and A.F. Wiese. 1979. Managing irrigated winter wheat residues for water storage and subsequent dryland grain sorghum production. Soil Science Society of America Journal. 42:582-588.

University of Missouri. 1998. Corn Insect Pests: A Diagnostic Guide. O'Day, M., A. Becker, A. Keaster, L. Kabrick, K. Steffey (eds.). University of Missouri-Columbia, Columbia, Missouri.

USDA-ARS Dryland Cropping Systems Management for the Central Great Plains. Nielsen, D., M. Virgil, J. Benjamin, F. Calderon and M. Mikha. 2007. 2007 Annual Report. Agricultural Research Service of the United States Department of Agriculture http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=410321&showpars=true&fy=2007 Accessed January 17, 2009.

USDA-ERS. 2002. Soil, nutrient, and water management systems used in U.S. Corn Production Agriculture Information Bulletin. No. 774. Electronic report from the Economic Research Service, United States Department of Agriculture. http://www.ers.usda.gov.

USDA-ERS. 2003. Agricultural Resources Environmental Indicators, Chapter 2.1: Irrigation Resources and Water Costs, Economic Research Service, United States Department of Agriculture http://www.ers.usda.gov.

USDA-FSA-CRP. 2007. News Release: USDA Announces Results of Intentions to Re-enroll and Extend CRP Contracts from United States Department of Agriculture, Farm Service Agency, Conservation Reserve Program (USDA-FSA-CRP).

USDA-NASS. 2006a. Agricultural chemical usage 2005 field crops summary. United States Department of Agriculture, National Agricultural Statistics Service. Ag Ch1 (06).

USDA-NASS. 2006b. USDA-NASS. 2006. National Agricultural Statistics Service, United States Department of Agriculture. Acreage Report, Cr Pr2-5 (6-06) a. Accessed June 30, 2006.

USDA-NASS. 2007 National Agricultural Statistics Service, United States Department of Agriculture. Acreage Report, Cr Pr 2-5 (6-07) Accessed June 30, 2008.

Welcker, C., B. Boussuge, C. Bencivenni, J-M. Ribaut and F. Tardieu. 2007. Are source and sink strengths genetically linked in maize plants subjected to water deficit? A QTL study of the responses of leaf growth and of anthesis-silking interval to water deficit. Journal of Experimental Botany. 58:339 - 349.

Westgate, M.E., M.E. Otegui, and F.H. Andrade. 2004. Physiology of the corn plant in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

Wilkes, H.G. 1967. Teosinte: the Closet Relative of Maize. Bussey Inst. Harvard Univ., Cambridge, Massachusetts, p. 69.

## X. Adverse Consequences of Introduction

Monsanto knows of no study results or observations associated with MON 87460 suggesting that adverse environmental consequences would be anticipated from its introduction. MON 87460 reduces yield loss under water-limited conditions. Under well-watered conditions, grain yield for MON 87460 is not different from conventional corn. Like conventional corn, MON 87460 is still subject to yield loss under water-limited conditions, particularly during flowering and grainfill periods when corn yield potential is most sensitive to stress by disrupting kernel development. Under severe water deficit, corn grain yield for MON 87460, as well as conventional corn, can be reduced to zero.

The data and information presented in this petition demonstrate that MON 87460 is unlikely to pose an increased plant pest potential compared to conventional corn. This conclusion is reached based on multiple lines of evidence developed from a detailed characterization of the product compared to conventional corn, followed by risk assessment on detected differences. Modern corn has inherently low plant pest potential because it is poorly suited to survive without human assistance and is not capable of surviving as a weed due to intense selection for domestication purposes during its evolution as a crop. The characterization studies for MON 87460 included molecular and protein analyses, which confirmed the insertion of a single functional copy of the *cspB* and *nptII* expression cassettes at a single locus within the corn genome and that the protein was expressed in tissues at levels that are efficacious for reduced yield loss under water-limited conditions. Allergenicity assessment concluded that the CSPB and NPTII proteins are unlikely to be allergens for humans. Toxicity tests, including acute oral gavage studies with mice for both CSPB and NPTII, showed no signs of adverse effects at high doses. Compositional analysis of key nutrients, anti-nutrients, and secondary metabolites showed that the forage and grain from MON 87460, and the foods and feeds derived from such, are compositionally equivalent to those derived from conventional corn. Extensive characterization of the plant phenotype also showed that MON 87460 is unchanged compared to conventional corn, with the exception of the intended modification. An endangered species risk assessment concluded that MON 87460 is unlikely to have adverse effects on these organisms. Finally, an assessment of agronomic practices confirmed that the introduction of MON 87460 is no more likely to impact land use, cultivation practices, or the management of weeds, diseases, and insects than the use of conventionally bred drought tolerant corn. Therefore, the risks for humans, animals, and other non-target organisms from MON 87460 are negligible under the conditions of use. The introduction of MON 87460 may increase economic and environmental benefits due to the protection of corn yields under water-limited conditions. Based on the results of these assessments, no adverse environmental consequences have been found to be associated with the introduction of MON 87460.

Therefore, on the basis of the benefits that this product offers for reduced yield loss under water-limited conditions, Monsanto requests that MON 87460 and any progeny derived from crosses between MON 87460 and other commercial corn be granted non-regulated status under 7 CFR Part 340.

## Appendix A. Materials and Methods Used for Molecular Analyses of MON 87460

### A.1. Materials

The DNA used in molecular analyses was isolated from MON 87460 seeds (seed lot number GLP-0604-17132-S). Additional DNA extracted from seeds of various generations of MON 87460 (seed lot numbers GLP-0704-18549-S; GLP-0604-17132-S ($F_1$ seed); GLP-0604-17132-S ($F_2$ grain); GLP-0704-18550-S; GLP-0609-17631-S; GLP-0609-17631-S; GLP-0703-18435-S) was used in generation stability analyses. The control DNA was isolated from the seed of a conventional corn with the same genetic background (seed lot number GLP-0604-17133-S). The reference substances included the PV-ZMAP595 plasmid, probe templates generated from this plasmid, and the size estimation molecular weight standards. As a positive control on Southern blots, PV-ZMAP595 plasmid DNA was digested with combination of enzymes to produce the banding patterns that were most relevant to the assessment of the test substance digested with appropriate enzyme(s). The plasmid DNA was digested first and then added to pre-digested conventional corn genomic DNA. The molecular weight standards include the 1 kb DNA Extension Ladder (Invitrogen) and λ DNA/*Hin*d III fragments (Invitrogen) for size estimations on Southern blots. The 100 bp and 500 bp DNA ladders (Invitrogen) were used for size estimations for PCR analyses**.**

### A.2. Characterization of the Materials

The quality of the source materials from MON 87460 and conventional corn were verified by PCR analysis to confirm the presence or absence of MON 87460 except the materials used in the generational stability analyses where the identity of the materials was confirmed by the generation stability Southern blots themselves. The stability of the genomic DNA was confirmed in each Southern analysis by observation of the digested DNA sample on an ethidium bromide-stained agarose gel.

### A.3. DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA samples from MON 87460 and conventional corn used in the insert and copy number, copy number of each element, backbone analysis, and PCR analyses were isolated from corn seeds that were processed into a fine powder using a Harbil® 5G high-speed paint shaker. DNA was extracted from the processed seeds using the method described by Rogers and Bendich (Rogers and Bendich 1985).

Genomic DNA was isolated using the following method. Place about 6 grams of processed seed tissue in a 50 mL centrifuge tube and add ~16 ml of cetyltriethylammonium bromide (CTAB) extraction buffer [1.5% CTAB, 75 mM tris(hydroxymethyl)aminomethane (Tris) pH 8.0, 100 mM ethylenediaminetetraacetic acid (EDTA), 1.05 M NaCl, 0.75% polyvinyl pyrrolidone (PVP) (40K)] and 8-10 µL of 10 mg/mL RNase. Incubate the samples at 65°C for 25-35 minutes and mix halfway through the incubation. Let the samples cool to room temperature, and add 16 mL of 24:1 chloroform: isoamyl alcohol, mix for 5 minutes and centrifuge for 5 minutes at 16,000 × *g* and 20-25°C to separate the aqueous and organic phases. Transfer the upper aqueous phase to a clean 50 mL centrifuge tube, add 1.6 mL of 10% (w/v) CTAB (10% CTAB, 0.71M NaCl) solution, mix by inversion, and add 16 mL of 24:1 chloroform: isoamyl alcohol. Mix the tubes for 5 minutes before centrifuge for 5 minutes at 16,000 × *g* and 20-

25°C to separate the aqueous and organic phases. Transfer the upper aqueous phase to a clean 50 mL centrifuge tube which contains 15 mL of CTAB precipitation buffer (1% CTAB, 50 mM Tris HCl, pH 8.0, 10 mM EDTA, pH 8.0). Mix the tubes gently by inversion, and let stand at RT for 50-70 minutes. Centrifuge for 9-11 minutes at 16,000 × *g* and 20-25°C to pellet the DNA. Discard the supernatant. Add 2 mL of high salt TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA, pH 8.0, 1M NaCl) and incubate at 35-60°C with gentle shaking until the pellet goes into solution. Precipitate the DNA by adding 1/10 the volume of 3 M sodium acetate, pH 5.2, and two times the volume of 100% ethanol. Mix by inversion. Remove the DNA using a pipette tip, inoculation loop, or closed Pasteur pipette. Place the DNA in a clean 1.5 mL microcentrifuge tube containing 0.5-1.0 mL of 70% (v/v) ethanol, microcentrifuge for 5 minutes at maximum speed to pellet the DNA, and discard the supernatant. Dry the DNA pellet by vacuum drying for ≤10 minutes or by air drying for ≤2 hours. Resuspend the DNA pellet in 500-1000 µL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA, pH 8.0). To facilitate resuspension of the DNA, additional TE buffer may be added and/or the solution may be heated up to 70°C for 1-4 hours. Store the DNA in a 4°C refrigerator or -20°C or -80°C freezer.

**A.4. Quantification of Genomic DNA**

Quantification of DNA samples was performed using a Hoefer DyNA Quant 200 Fluorometer with Roche molecular size marker IX as a DNA calibration standard.

**A.5. Restriction Enzyme Digestion of Genomic DNA**

Approximately 10 or 20 µg of genomic DNA extracted from the test and control substances were used for restriction enzyme digestions. When digesting genomic DNA with *Hin*d III (Roche) or *Eco*R V (Roche), 10X Buffer B (Roche) was used. When digesting genomic DNA with the enzyme combination *Eco*O109 I (New England BioLabs, Beverly, MA) and *Not* I (Roche), NEbuffer 4 (New England BioLabs) was used. Finally, 100× BSA (New England BioLabs) was added to the *Eco*O109 I/*Not* I digests to a final concentration of 1×. All digests were performed at 37°C in a total volume of approximately 500 µl using ~100 units of the appropriate restriction enzyme(s).

**A.6. DNA Probe Preparation for Southern Blot Analyses**

Probe template DNA containing sequences of plasmid PV-ZMAP595 was prepared by PCR amplification using a standard procedure based on Sambrook and Russell (Sambrook and Russell 2001). Approximately 25 ng of each probe template were radiolabeled with either $^{32}$P-deoxycytidine triphosphate (dCTP) or $^{32}$P-deoxyadenosine triphosphate (dATP) (6000 Ci/mmol) using the random priming method (RadPrime DNA Labeling System, Invitrogen) or PCR method. Probe locations relative to the genetic elements in plasmid PV-ZMAP595 are depicted in Figure IV-6 and Figure IV-7.

**A.7. Southern Blot Analyses of Genomic DNA**

Digested DNA was separated using 0.8% (w/v) agarose gel electrophoresis. Except for generational stability analyses, DNA samples were loaded on the gels for a long run and a short run in an effort to provide better resolution of larger DNA fragments while retaining smaller DNA fragments on the gel. After transferring the DNA to the membrane, Southern blots were hybridized at 55°C, 60°C, or 65°C. The table below lists the temperature and radiolabeling conditions of the probes used in this study. Multiple

exposures of each blot were then generated using Kodak Biomax MS film in conjunction with one Kodak Biomax MS intensifying screen in a -80°C freezer.

| Probe | DNA Probe | Labeling Method | Probe labeled with dNTP ($^{32}$P) | Hybridization Temperature (°C) |
|---|---|---|---|---|
| 1 | T-DNA Probe 1 | RadPrime | dCTP | 65 |
| 2 | T-DNA Probe 2 | RadPrime | dCTP | 65 |
| 3 | T-DNA Probe 3 | RadPrime | dCTP | 65 |
| 4 | Backbone Probe 1 | RadPrime | dCTP | 65 |
| 5 | Backbone Probe 2 | RadPrime | dCTP | 65 |
| 6 | Backbone Probe 3 | RadPrime | dCTP | 65 |
| 7 | P-*Ract1* Probe | RadPrime | dCTP | 65 |
| 8 | I-*Ract1* Probe | PCR | dCTP | 65 |
| 9 | CS-*cspB* Probe | PCR | dATP | 60 |
| 10 | T-*tr7* Probe | PCR | dATP | 55 |
| 11 | *loxP* + P-*35S* Probe | RadPrime | dCTP | 60 |
| 12 | CS-*nptII* Probe | RadPrime | dCTP | 65 |
| 13 | T-*nos* + *loxP* + Left Border Probe | RadPrime | dATP | 60 |

dNTP = deoxyribonucleotide triphosphate

**A.8. DNA Sequence Analyses of the MON 87460 Insert**

Overlapping PCR products were generated that span the insert in MON 87460. These products were sequenced to determine the nucleotide sequence of the insert in MON 87460 as well as the nucleotide sequence of the genomic DNA flanking the 5' and 3' ends of the insert.

The PCR analyses were conducted using approximately 75 ng of genomic DNA template or approximately 10 ng of plasmid DNA in a 50 µl reaction volume containing a final concentration of 2 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM each deoxyribonucleotide triphosphate (dNTP), and 1 unit of DNA polymerase mix. The DNA polymerase mix used to generate the products was Platinum *Taq* (Invitrogen) or Platinum High Fidelity *Taq* (Invitrogen).

Aliquots of each PCR reaction were separated on a 1.0 % (w/v) agarose gel and visualized by ethidium bromide staining to verify that the products were of the expected size prior to sequencing. The PCR product was sequenced with the multiple primers used for PCR amplification. In addition, primers internal to the PCR primers were used to sequence other regions of the amplified product. All sequencing was performed by the Monsanto Genomics Sequencing Center using dye-terminator chemistry.

**A.9. PCR and DNA Sequence Analyses of the Parental Corn Genome**

To demonstrate that the DNA sequences flanking the insert in MON 87460 are native to the corn genome, PCR analysis was performed on genomic DNA from both MON 87460 and conventional corn. The primers used in this analysis were designed from the DNA sequences flanking the insert in MON 87460. One primer designed from the genomic DNA sequence flanking the 5' end of the insert was paired with a second primer located in the genomic DNA sequence flanking the 3' end of the insert.

The PCR analyses were conducted using approximately 75 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgCl_2$, 0.2 µM of each primer, 0.1 mM each dNTP, and 1 unit of Platinum *Taq* DNA polymerase High Fidelty (Invitrogen).

Aliquots of each PCR reaction were separated on a 1.0% (w/v) agarose gel and visualized by ethidium bromide staining to verify that the product was of the expected size prior to sequencing. The PCR product was sequenced with the primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using dye-terminator chemistry.

**A.10. References:**

Rogers, S. O. and A. J. Bendich. 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissue. Plant Molecular Biology. 5:69-76.

Sambrook, J. and D. Russell. 2001. Chapter 5 Protocol 1: Agarose gel electrophoresis. Pages 5.4 to 5.13 in Molecular cloning: a laboratory manual. 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

## Appendix B. Materials, Methods, and Results for Characterization of CSPB Protein

The expression levels of CSPB protein in different tissues of MON 87460 are relatively low. Therefore, it was necessary to produce the protein in a high-expressing, recombinant microorganism in order to obtain sufficient quantities of the protein for safety studies. A recombinant CSPB protein was produced in *Escherichia coli*, the sequence of which was engineered to match that of CSPB protein produced in MON 87460. The equivalence of the physicochemical characteristics and functional activity between the MON 87460-produced and *E. coli*-produced CSPB protein was confirmed by a panel of analytical techniques, including sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis, matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), glycosylation analysis, and assay of biological activity. The details of the materials, methods, and results are described below.

### B.1. Materials and Methods

#### B.1.1. Protein Purification

The MON 87460-produced CSPB protein was purified from the grain of MON 87460. The CSPB protein was purified at ~4 °C from an extract of ground grain using a combination of ammonium sulfate fractionation, anion exchange chromatography, immunoaffinity chromatography, and size exclusion chromatography. Protein purification records are archived at Monsanto Company under Orion lot 10000842, and the purification methods are described below.

The ground grain (10 kg) was mixed with a Tris-borate extraction buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) for 17 h at approximately a 1:10 sample weight to buffer volume ratio. To remove lipids, diatomaceous earth (Advanced Minerals Corp, Goleta, CA) was added to a final concentration of 7.5% (w/v) and mixed for 3 hours. The final slurry was filtered using an Ertel Alsop filter press (Kingston, NY) with Die 42 micro media filter pads and a Cuno filter (45115-12-90S, Hagedorn & Gannon Co., Inc). The filtrate was concentrated by diafiltration utilizing a polysulfone hollow fiber cartridge with a 3 kDa Molecular Weight Cut Off (MWCO) (surface area: 3.25 $m^2$, GE Healthcare, Piscataway, NJ). An ammonium sulfate precipitate was prepared by the addition of ammonium sulfate salt to the clarified extract to a final saturation of 40% and was allowed to dissolve overnight at 4 °C. After centrifugation, the ammonium sulfate pellet was discarded and the supernatant collected and diafiltered against 20 mM Tris-HCl pH 7.0, resulting in a final volume of 14 L.

The sample was loaded onto a 4.4 L (14 cm x 20 cm) Q Sepharose Fast Flow anion exchange resin column (GE Healthcare, Piscataway, NJ ), which was equilibrated with AEC buffer A (20 mM Tris-HCl, pH 7.0). The bound CSPB was eluted with a linear salt gradient that increased from 0 M to 0.5 M sodium chloride (in AEC buffer A) over 44 L. Fractions containing MON 87460-produced CSPB protein were identified by western blot analysis and totaled 9.2 L. These fractions were pooled and concentrated using diafiltration to a final volume of ~0.6 L.

The concentrated sample containing MON 87460-produced CSPB protein was re-circulated over two AminoLink (Pierce, Rockford, IL) columns (2.4 ml: 1.6x1.2 cm; 4.4 ml: 1.6x2.2 cm) to which a monoclonal anti-CSPB antibody (Leinco Technologies Inc, St Louis, MO) had been conjugated. Bound CSPB protein was eluted using 100 mM triethylamine buffer and neutralized with 1/20$^{th}$ volume of 1 M sodium phosphate, pH 6.8. The process was repeated a total of 19 times to capture and elute most of the CSPB protein present in the concentrated AEC pool. After analysis of fractions by western blot, those containing CSPB protein were combined to a final volume of 205 ml. The pool was concentrated by diafiltration to approximately 27 ml and then divided into three 9 ml samples that were further purified by size exclusion chromatography on a 320 ml, 60 cm bed height, Sephacryl S-100 HR column (GE Healthcare, Piscataway, NJ) equilibrated in 20 mM Tris-HCL pH 7.0. The fractions containing CSPB protein were identified by western blot and a final pool of ~105 ml was concentrated by diafiltration with a mini cartridge to approximately 12 ml. Further concentration to 0.5 ml was accomplished by placing the solution into a slide-A-lyzer dialysis cassette (MWCO: 3.5 kDa, size: 0.5-3 ml, Pierce, Rockford, IL) and removing the excess of solvent (~11.5 ml) by exposure to a water absorbing polymer powder (Aquacide I, EMD, Gibbstown, NJ). The concentrated pool of MON 87460-produced protein was submitted to the Analytical Protein Standard (APS) program as 23 aliquots that were assigned APS lot 1000842.

### B.1.2. Protein Concentration

The concentration of the MON 87460-produced CSPB protein was estimated using quantitative densitometric analysis of silver stained SDS-PAGE. The *E. coli*-produced CSPB protein (amounts ranging from 10 to 60 ng) was used to create a standard curve. Aliquots of the MON 87460-produced CSPB protein and reference standard were diluted in 20 mM Tris-HCl pH 7.0 and 5× Laemmli buffer (5× LB), heated at 100.3 °C for 3 min, and applied to a pre-cast tricine 10 - 20% polyacrylamide gradient 18-well gel. A 50-fold diluted MON 87460-produced protein solution was mixed with 5× LB and three different amounts were loaded in duplicate on the gel. Electrophoresis was performed at a constant voltage of 200 V for 45 min. Pre-stained molecular weight markers (Invitrogen SeeBlue Plus2, Carlsbad, CA) were loaded in parallel.

The gel was stained using the Owl Silver Staining Kit Protocol (Owl Separation Systems, Portsmouth, NH). The following steps were performed during the gel staining procedure:

1. Fixing for 10 min in 150 ml of fixing solution (60 ml deionized water, 75 ml methanol, and 15 ml acetic acid);
2. 15 min incubation in 150 ml of a second fixing solution (82.5 ml deionized water, 45 ml methanol, 15 ml acetic acid and 7.5 ml Reagent Bottle 1);
3. 10 min incubation in 150 ml of a pretreatment solution (75 ml methanol, 7.5 ml Pretreatment Reagent, and 67.5 ml deionized water);
4. Washing for 5 min with 150 ml of deionized water;
5. 15 min staining in 150 ml of the silver staining solution (7.5 ml Staining Solution A, 7.5 ml Staining Solution B, and 135 ml deionized water);
6. Washing three times for 2 min each with 150 ml of deionized water;

7. Development of stained protein bands occurred in 150 ml of developer solution (7.5 ml Concentrated Developer and 142.5 ml deionized water) for 8 min, and was stopped by addition of 7.5 ml stopping solution for 10 min;
8. The gel was washed three times for 2 min each with 150 ml of deionized water and the gel was then changed to 50 ml of a 20% ethanol solution.

Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA) using the lane finding and contour tool. The raw data were exported to a Microsoft Excel (version 2002, SP3) file for the construction of the calibration curve and the final concentration determination of the MON 87460-produced CSPB concentration.

**B.1.3. Immunoblot Analysis**

Immunoblot analysis was performed to confirm the identity of the CSPB protein purified from MON 87460 and to compare the immunoreactivity of the MON 87460- and *E coli*-produced CSPB proteins.

The MON 87460- and *E. coli*-produced CSPB proteins were loaded onto the same gel at equal amounts of 3, 6, and 9 ng. Aliquots of each protein were diluted in 20 mM Tris-HCl pH 7.0 and mixed with 5× LB, heated at 100 °C for 3 min, and applied to a pre-cast tricine 10 - 20% polyacrylamide gradient 15-well gel. The three amounts of each protein were loaded in duplicate on the gel. Electrophoresis was performed at a constant voltage of 170 V for 70 min. Pre-stained molecular weight markers (SeeBlue Plus2 Prestained, Invitrogen, Carlsbad, CA) were loaded in parallel to verify electrotransfer of protein to the membrane and estimate the size of the immunoreactive bands observed. Electrotransfer to a 0.45 μm nitrocellulose membrane (Invitrogen, Carlsbad, CA) was performed for 90 min at a constant voltage of 35 V.

The membrane was blocked for 1 hour with 5% (w/v) non-fat dry milk (NFDM) in 1× phosphate-buffered saline containing Tween-20 (PBST). The membrane was probed with a 1:1000 dilution of goat anti-CSPB antibody (Orion lot 10000798, aliquot # 101) in 5% (w/v) NFDM in PBST for 14 hr. Excess antibody was removed using three 10 min washes with PBST. Finally, the membrane was probed with horseradish peroxidase-conjugated rabbit anti-goat IgG (Sigma, St. Louis, MO) at a dilution of 1:10000 in 5% (w/v) NFDM in PBST for 60 min. Excess HRP-conjugate was removed using three 10 min washes with PBST. All incubations were performed at room temperature, except for the primary antibody which was incubated at 4°C. Immunoreactive bands were visualized using the ECL detection system (Amersham Biosciences, Piscataway, NJ) and exposed (10, 30, and 60 s) to BioMax XAR film (Eastman Kodak, Rochester, NY). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

The immunoreactive bands of the MON 87460-produced CSPB protein in each lane migrating to the same level as the reference standard protein were quantitated and compared to the signals corresponding to the CSPB reference standard protein. Quantitation of the blot was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA) using the lane finding and contour tool. The raw data were exported to a Microsoft Excel (version 2002, SP3) file

for the pair wise comparison of all the loads. An average percent difference was calculated for each comparison to determine the immunoreactivity equivalence.

### B.1.4. N-Terminal Sequencing

N-terminal sequencing by Edman degradation was used to confirm the identity of the MON 87460-produced CSPB and to determine if the N-terminal methionine was present in the protein.

*Protein Blot for N-Terminal Analysis*

An aliquot of MON 87460-produced CSPB was removed from storage, diluted with 20 mM Tris-HCl pH 7.0 and mixed with 5× LB to a final concentration of 10 ng/µl, heated at 100.3 °C for 3 min, and loaded in duplicate at 250 ng per lane onto a tricine 10-20% gradient polyacrylamide 10-well gel. Pre-stained molecular weight markers (SeeBlue Plus2 Prestained, Invitrogen, Carlsbad, CA) were loaded in parallel to verify electrotransfer of protein to the membrane and estimate the size of the stained bands observed. Electrophoresis was performed at a constant voltage of 170 V for 70 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 90 min at a constant voltage of 25 V. The blot was stained with Ponceau S (Sigma, St. Louis, MO) to visualize the markers and the CSPB protein.

*N-Terminal Sequencing*

The bands corresponding to MON 87460-produced CSPB protein were excised from the blot and N-terminal sequence analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapiller and Hood, 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and 785 Programmable Absorbance Detector and Procise™ Control Software (version 1.1a) were used. Chromatographic data were collected using Atlas[99] software (version 3.59a, LabSystems, Altrincham, Cheshire, England). A phenylthiohydantoin (PTH)-amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to chromatographically calibrate the instrument for each analysis. This mixture served to verify system suitability criteria such as percent peak resolution and relative amino acid chromatographic retention times. A control protein (10 pmole β-lactoglobulin, Applied Biosystems) was analyzed before and after the analysis of the two CSPB protein bands that were analyzed as a single sample, to verify that the sequencer met performance criteria for repetitive yield and sequence identity.

### B.1.5. MALDI-TOF Mass Analysis

MALDI-TOF mass spectrometry was used to confirm the molecular weight of the MON 87460-produced CSPB. Since the protein was determined to be very pure (97%) prior to this analysis, it was not deemed necessary to separate the protein by SDS-PAGE.

An aliquot of the MON 87460-produced CSPB protein was diluted ten-fold to a final concentration of ~12 µg/ml. Mass spectral analyses were performed as follows. Mass calibration of the instrument was performed using an external peptide mixture from a Sequazyme Peptide Mass Standards kit (Applied Biosystems). Samples (0.3 µl) from each of the tryptic samples were co-crystallized with 0.75 µl sinapinic acid on the analysis plate. The sample was analyzed in the 1000 to 25000 Da range using 200 shots at a laser intensity setting of 2603 (a unit-less MALDI-TOF instrument specific value).

Protonated (MH+) peptide masses were observed monoisotopically in linear mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical mass of the expected CSPB (plant) protein sequence based upon the nucleotide sequence. Peaks were not assessed if the peak heights were less than approximately twice the baseline noise, or when a mass could not be assigned due to overlap with a stronger signal ± 2 Da from the mass analyzed.

*MALDI-TOF Tryptic Mass Map Analysis*

MALDI-TOF mass spectrometry was used to confirm the identity of the MON 87460-produced CSPB protein. Since the protein was determined to be very pure (97%) prior to this analysis, it was not deemed necessary to separate the protein by SDS-PAGE.

An aliquot of the MON 87460-produced CSPB protein was diluted ten-fold to a final concentration of ~12 µg/ml. A 30 µl sample was transferred to a micro vial tube and evaporated to dryness in a Speed-Vac concentrator. The sample was digested for 16 hr at 37 °C with 660 ng of trypsin (Promega, Madison, WI) in 20 µl of a 25 mM ammonium bicarbonate buffer.

Ten µl of the trypsin digested sample was transferred to a separate micro vial for guanidination of the peptides using the ProteoMass™ guanidination kit (Sigma, St. Louis, MO). To the tube, 10 µl of guanidination reagent (O-methylisourea hemisulfate) solution and 10 µl of base (2.85 M $NH_4OH$) were added and the tube was vortexed. The tube was incubated at 65 °C for 30 min, then 10 µl of stop solution (10% trifluoroacetic acid (TFA)) was added.

Mass spectral analyses were performed as follows. Mass calibration of the instrument was performed using an external peptide mixture from a Sequazyme Peptide Mass Standards kit (Applied Biosystems). Samples (0.3 µL) from each of the trypsin digested samples were co-crystallized with 0.75 µL α-cyano-4-hydroxycinnamic acid on the analysis plate. The sample was analyzed in the 500 to 5000 Da range using 100 shots at a laser intensity setting of 1783 and 2175 (a unit-less MALDI-TOF instrument specific value). Protonated (MH+) peptide masses were observed monoisotopically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical trypsin digest of the expected MON 87460-produced CSPB protein sequence based upon the nucleotide sequence. Masses were calculated for each theoretical peptide and compared to the raw mass data. Experimental masses (MH+) were assigned to peaks in the 500 to 1000 Da range if there were two or more isotopically resolved peaks, and in the 1000 to 5000 Da range if there were three or more isotopically resolved peaks in the spectra. Peaks were not assessed if the peak heights were less than approximately twice the baseline noise, or when a mass could not be assigned due to overlap with a stronger signal ± 2 Da from the mass analyzed. Known autocatalytic fragments from trypsin digestion were identified in the raw data. The tryptic mass map coverage was considered acceptable if ≥ 40 % of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments.

### B.1.6. Molecular Weight and Purity Estimation – SDS-PAGE

Aliquots of the *E. coli*-produced reference standard and MON 87460-produced CSPB proteins were diluted with 20 mM Tris-HCl, pH 7.0 and mixed with 5× LB to a final protein concentration of 10 ng/µl. The MON 87460-produced protein was analyzed in duplicate at 50, 100, and 150 ng of total protein per lane. The *E. coli*-produced CSPB protein reference standard was analyzed at 100 ng of purity corrected full-length protein. All samples were heated in a thermo-block at 100.3 °C for 3 min and applied to a pre-cast tricine 10-20% polyacrylamide gradient 10-well mini-gel (Invitrogen, Carlsbad, CA). Pre-stained molecular weight markers (Invitrogen SeeBlue Plus2, Carlsbad, CA) were loaded in parallel. Electrophoresis was performed at a constant voltage of 170 V for 70 min.

The gel was stained using the Owl Silver Staining Kit Protocol (Owl Separation Systems, Portsmouth, NH). The same procedure described previously in this appendix was followed, though the solutions were proportionately adjusted to a final volume of 50 ml. Also, the development of protein bands occurred during incubation of the gel in the developer solution.

Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA). The molecular weight markers were used to estimate the apparent molecular weight of the MON 87460-produced CSPB protein. For the purity evaluation, all visible bands within each lane were quantified. The purity and estimated molecular weight of the MON 87460-produced CSPB protein were reported as the average of the six values obtained by densitometric analysis.

### B.1.7. Glycosylation Analysis

Glycosylation analysis was used to determine whether the MON 87460-produced CSPB protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 87460-produced CSPB protein, the *E. coli*-produced CSPB reference standard protein, and the positive controls, transferrin (~ 76 – 81kDa, Sigma-Aldrich, St. Louis, MO), and horseradish peroxidase (~ 40kDa, Pierce, Rockford, IL) were each diluted with 20 mM Tris-HCl pH 7.0 and mixed with 5× LB. These samples were heated at 100 °C for 3 min, cooled, and loaded on a tricine 10-20% polyacrylamide gradient 10-well mini-gel. Each sample was loaded at 25 and 50 ng per lane. SeeBlue Plus2 pre-stained protein molecular weight markers (Invitrogen) were loaded to verify electrotransfer of the proteins to the membrane and the CandyCane™ Glycoprotein Molecular Weight Standards (Molecular Probes, Eugene, OR) were loaded as positive/negative controls and markers for molecular weight. Electrophoresis was performed at a constant voltage of 170 V for 60 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen) was performed for 90 min at a constant voltage of 35 V.

Carbohydrate detection was performed directly on the PVDF membrane using the Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit (Molecular Probes). The manufacturer's protocol was followed. All steps were performed at room temperature. The PVDF membrane was fixed in 25 ml of a solution containing 50% methanol and 5% glacial acetic acid for 45 min, the solution was then changed and the membrane was incubated overnight. Two, 10 min washes (50 ml each) with 3% (v/v) glacial acetic acid

(wash solution) were followed by a 20 min oxidation in 25 ml of an oxidizing solution containing periodic acid (Component C from kit). Membrane was washed three times, 10 min each, in 50 ml of wash solution. The blot was then incubated in 25 ml of Pro-Q Emerald Staining Solution that was prepared using the kit reagents. After 1 hr of staining in the dark, two 20 min, 50 ml wash cycles were followed by two 30 min, 50 ml wash cycles. The final wash cycles included two 50 ml, 1 min deionized water washes followed by three 5 min methanol washes(EMD, San Diego, CA). The blot was then scanned using the BioRad Molecular Imager FX using the Alexa 488 illumination setting (Quantity One software; version 4.6, build 036) in order to visualize the fluorescently-labeled glycosylated proteins.

After glycosylation analysis the blot was stained to visualize the proteins present on the membrane. Proteins were stained using the SYPRO® Ruby Protein Blot Stain (Molecular Probes). Sections 2.4 to 2.6 of the manufacturer's instructions were followed and all steps were performed at room temperature and incubations were done on a shaking table. The blot used for glycosylation was stained in 10 ml of the SYPRO staining solution for 15 min. The solution was discarded and the blot was washed twice for 5 min in 50 ml of deionized water. The blot was stored in 25 ml of deionized water. The blot was then scanned using the BioRad Molecular Imager FX using the SYPRO Ruby illumination setting (Quantity One software; version 4.6, build 036) in order to visualize the fluorescently-labeled proteins.

**B.1.8. Functional Activity Assay**

In order to assess the functional activity of the MON 87460-produced CSPB protein and to compare its activity to the *E. coli*-produced CSPB reference standard protein, aliquots of the MON 87460-produced CSPB protein and *E. coli*-produced CSPB reference standard protein were analyzed for their ability to unfold polynucleotide hairpin structures. Activity is expressed as the amount of DLP that is unfolded by CSPB. The probe consists of a custom synthesized 35-base oligonucleotide DNA fragment with a 6-FAM fluorescent label at the 5' end and a black hole quencher at the 3' end. The oligonucleotide probe forms a double strand stem of six base pairs due to complementary bases located at the 5' and 3' ends. The 23 nucleotides (dT) in the middle form a loop. and the binding of CSPB to the loop will separate the double strands of the probe, thus separating the fluorophore from the quencher, allowing fluorescence to be emitted and measured.

The assay was carried out on a micro titer plate. A calibration curve using the 6-FAM was constructed from serial dilutions of a 100 nM stock solution of the 6-FAM. The dilutions were done in Assay buffer (25 mM Tris-HCl, 100 mM NaCl, 2 mM EDTA, pH 7.5) and the final concentrations of the 6-FAM were 0.234, 0.468, 0.938, 1.875, 3.75, 7.50, and 15.00 pmoles/well. The sample wells were prepared by adding 175 µl of a reagent solution containing 0.34 µM DLP in the assay buffer. The plate was incubated at 30.1 °C for 30 min. Then, 25 µl of dilutions of each MON 87460- and *E.coli*-produced CSPB protein (3 µg total CSPB), in triplicate, were added to the test wells and the plate was incubated at 30 °C in a SpectraMax M2 plate reader (Molecular Devices, Sunnyvale, CA). The fluorescence was determined with an excitation wavelength of 485 nm and an emission wavelength of 520 nm using a template created within the SpectraMax Pro GxP software (version 5.0.1).

### B.1.9. Storage Stability

The short-term stability of the MON 87460-produced CSPB protein during storage in a freezer set to maintain -80°C was evaluated by comparing the purity and molecular weight values obtained on day 0 to the purity and molecular weight values obtained on day 14 of storage. Day 0 stability analysis corresponds to the purity and molecular weight determination. On day 14, an aliquot was removed from a -80 °C freezer, diluted with 20 mM Tris-HCl pH 7.0 and mixed with 5× LB to a final concentration of 10 ng/µl, heated at 100 °C for 3 min, and loaded in duplicate (50, 100, and 150 ng per lane) onto a tricine 10 - 20% gradient polyacrylamide 10-well gel. Staining and densitometric analysis were performed as described for Molecular Weight and Purity Estimation-SDS-PAGE. The protein samples were considered to have undergone degradation if a > 10% decrease in purity and/or molecular weight was observed relative to the value determined on Day 0.

## B.2. Results

### B.2.1. CSPB Protein N-terminal Sequence Analysis

Sequencing of the first 15 amino acids comprising the N-terminal of MON 87460-produced CSPB protein produced the expected result (Table B-1). The N-terminal methionine was not detected. This result is expected as removal of the N-terminal methionine, catalyzed by methionine aminopeptidase, is a common modification that occurs co-translationally before completion of the nascent protein chain and has no effect on protein structure or activity (Arfin and Bradshaw, 1988; Polevoda and Sherman, 2000). The N-terminal sequence information, therefore, confirms the identity of the CSPB protein isolated from MON 87460 and the intactness of its N-terminus.

### B.2.2. CSPB Protein MALDI-TOF Mass Spectrometry Analysis

The identity of the MON 87460-produced CSPB protein was further confirmed by tryptic peptide mass mapping analysis using MALDI-TOF MS. Protein identification made by peptide mapping is reliable if the measured coverage of the sequence is 15% or higher with a minimum of five matched peptides (Jensen et al., 1997). Observed tryptic peptides were considered a match to the expected tryptic mass when differences in molecular weight of less than one Dalton (Da) were found between the observed and predicted fragment masses. Such matches were made without consideration for potential natural amino acid modifications such as glycosylation. The protein sample was heat-denatured, chemically reduced, alkylated, digested with trypsin, guanidinated, and the masses of the tryptic peptides were measured.

CSPB is a small protein with a limited number of the trypsin-digested peptides that are amenable to identification by MALDI-TOF. There were four unique peptide fragments identified that matched expected masses of the CSPB trypsin-digested peptides. The identified masses were used to assemble a coverage map indicating the matched peptide sequences for the entire CSPB protein (Figure B-1), resulting in an 88% (58 out of 66 amino acids) coverage of the total protein. This analysis confirmed the identity of the MON 87460-produced CSPB protein. Table B-2 presents the tryptic masses of CSPB.

### B.2.3. CSPB Protein Immunoreactivity

A western blot analysis using goat anti-CSPB serum was conducted to determine the relative immunoreactivity of the MON 87460-produced CSP protein and the *E. coli*-produced CSPB reference standard. The results demonstrated that the anti-CSPB antibody recognized the MON 87460-produced CSPB that migrated identically to the *E. coli*-produced reference standard protein (Figure B-2). Furthermore, the immunoreactive signal increased with increasing levels of CSPB loading. Immunoreactivities between the MON 87460- and *E. coli*-produced proteins were similar based on densitometric analysis of the western blot. Based on the analysis, the MON 87460- and *E. coli*-produced CSPB proteins demonstrated equivalent immunoreactive properties, which confirmed the identity and equivalence of the two proteins.

**Table B-1. N-terminal Amino Acid Sequence Analysis of the CSPB Protein Purified from Grain Tissue of MON 87460**

| Amino acid[1] residue # from the N-terminus → | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Predicted CSPB Sequence[2] → | M | V | E | G | K | V | K | W | F | N | S | E | K | G | F | G |
| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| | \| |
| Observed Sequence→ | - | V | E | G | K | V | K | W | F | N | S | E | K | G | F | G |

1 The single letter amino acid code is: E, Glutamic acid; F, Phenylalanine; G, Glycine; K, Lysine; M, Methionine; N, Asparagine, S, Serine; V valine and W, Tryptophan.

2 The predicted amino acid sequence of the CSPB protein was deduced from the coding region of the full length *cspB* gene present in MON 87460.

**Table B-2. Summary of the Tryptic Masses Identified for the MON 87460-Produced CSPB Protein Using MALDI-TOF Mass Spectrometry**

Only experimental masses that matched expected masses are listed in the table.

| Observed Mass (Da) | | Expected Mass (Da) | Mass Difference (Da) | AA Position | Fragment Sequence |
|---|---|---|---|---|---|
| Crude Sample | Guanidinated Sample | | | | |
| 810.42 | - | 810.38 | 0.04 | 7-12 | WFNSEK |
| - | 852.51 | 852.38 | 0.13 | 7-12G | WFNSEK |
| 885.54 | - | 885.48 | 0.06 | 56-64 | GPQAANVTK |
| - | 927.64 | 927.48 | 0.16 | 56-64G | GPQAANVTK |
| 1878.11 | - | 1877.92 | 0.19 | 39-55 | TLEEGQAVSFEIVEGNR |
| - | 2903.32 | 2903.36 | 0.04 | 13-38G | GFGFIEVEGQDDVFVHFSAIQGEGFK |

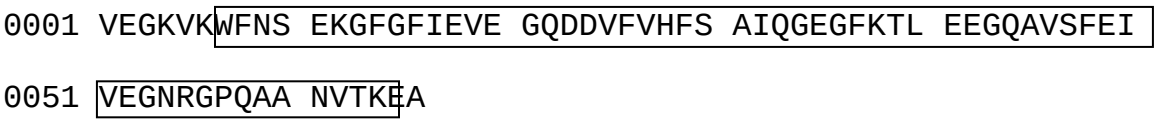



**Figure B-1. MALDI-TOF MS Coverage Map of the CSPB Protein Isolated from MON 87460**

The amino acid sequence of the plant-produced CSPB protein was deduced from the coding region of the full-length *cspB* gene present in MON 87460. Boxed regions correspond to tryptic peptide masses that were identified from the protein sample using MALDI-TOF MS. In total, 88% (58 of 66 total amino acids) of the expected protein sequence were identified.

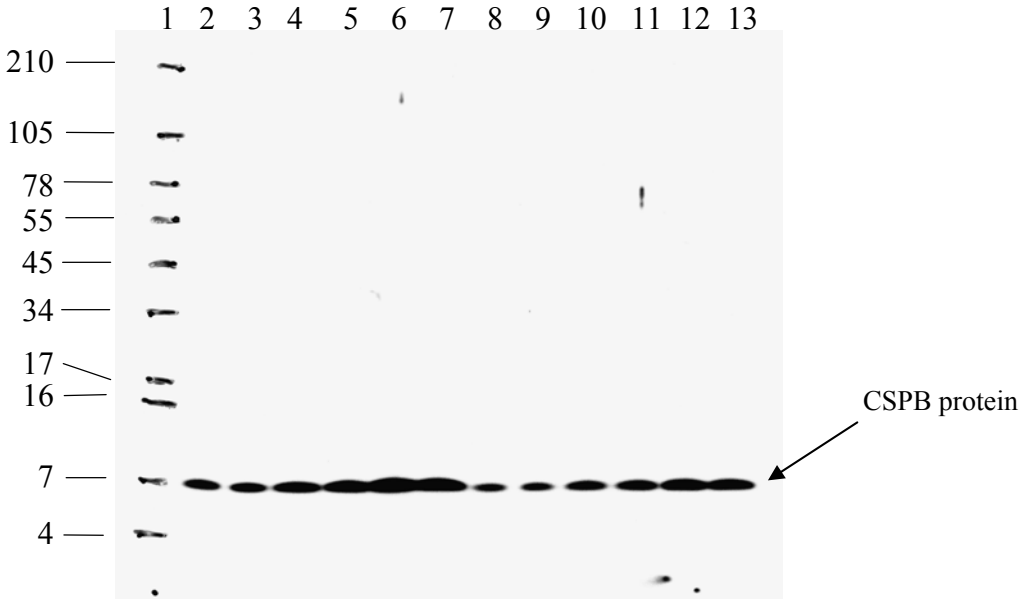



**Figure B-2. Western Blot Analysis of MON 87460- and *E. coli*-produced CSPB Proteins**

Aliquots of the purified, MON 87460- and *E. coli*-produced CSPB proteins were separated by SDS-PAGE, and electrotransferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was probed with goat anti-CSPB serum and developed using an enhanced chemiluminescense (ECL) system (GE Healthcare). Approximate molecular weights (kDa) of markers loaded in Lane 1 are shown on the left side of the blot.

| Lane | Sample | Amount Loaded (ng) |
|---|---|---|
| 1 | See Blue® Plus2 Pre-Stained molecular weight markers | — |
| 2 | *E. coli*-produced CSPB reference standard | 3 |
| 3 | *E. coli*-produced CSPB reference standard | 3 |
| 4 | *E. coli*-produced CSPB reference standard | 6 |
| 5 | *E. coli*-produced CSPB reference standard | 6 |
| 6 | *E. coli*-produced CSPB reference standard | 9 |
| 7 | *E. coli*-produced CSPB reference standard | 9 |
| 8 | MON 87460-produced CSPB protein | 3 |
| 9 | MON 87460-produced CSPB protein | 3 |
| 10 | MON 87460-produced CSPB protein | 6 |
| 11 | MON 87460-produced CSPB protein | 6 |
| 12 | MON 87460-produced CSPB protein | 9 |
| 13 | MON 87460-produced CSPB protein | 9 |

### B.2.4. CSPB Protein Molecular Weight Equivalence

The equivalence in apparent molecular weight of the purified MON 87460- and the *E. coli*-produced CSPB proteins was demonstrated using SDS-PAGE (Figure B-3). The MON 87460-produced CSPB protein migrated with a molecular weight indistinguishable to that of the *E. coli*-produced protein standard analyzed concurrently (Figure B-3). Based on comparable electrophoretic mobilities, the MON 87460- and *E. coli*-produced CSPB proteins were determined to have equivalent apparent molecular weights.

The predicted mass of the MON 87460-produced CSPB protein was also confirmed by MALDI-TOF MS. The average mass obtained for CSPB was 7220 Da. This experimentally obtained mass differs from the theoretical mass calculated for the CSPB reference standard protein by 131 Da. The difference between the expected and the observed mass for MON 87460-produced CSPB corresponds to the mass of methionine (131 Da). The absence of the N-terminal methionine was confirmed by N-terminal sequencing (Section B.2.1).

### B.2.5. CSPB Protein Glycosylation Equivalence

Some eukaryotic proteins are post-translationally modified by the addition of carbohydrate moieties (Rademacher et al., 1988). These carbohydrate moieties may be complex, branched polysaccharide structures, simple oligosaccharides or monosaccharides. In contrast, prokaryotic organisms such as non-virulent *E. coli* strains used for cloning and expression purposes, lack the necessary biochemical synthetic capacity required for protein glycosylation. An investigation of glycosylation status therefore is necessary to confirm that the MON 87460-produced CSPB protein is equivalent to the *E. coli*-produced CSPB protein. Results of this analysis confirm that the proteins are equivalent in this respect.

To assess whether potential post-translational glycosylation of the MON 87460-produced CSPB protein occurred, the purified protein sample was subjected to glycosylation analysis. The *E. coli*-produced CSPB reference standard represented a negative control. The positive controls were the transferrin and horseradish peroxidase (HRP) proteins which are known to have multiple covalently-linked carbohydrate modifications. The transferrin protein and HRP, as well as the purified CSPB protein isolated from MON 87460 and *E. coli* were separated on SDS-PAGE, transferred to a PVDF membrane, and glycosylation analysis was performed to detect carbohydrate moieties on the proteins. The results of this analysis are shown in Figure B-4. The positive controls, transferrin and HRP, were detected at the expected molecular weights of ~75 and ~50 kDa, respectively, in a concentration-dependent manner (Figure B-4, Panel A, Lanes 4-5 and 2-3). No detectable signal was observed for the MON 87460- and *E. coli*-produced CSPB proteins (Figure B-4, Panel A, Lanes 6-7 and 8-9). To confirm that sufficient MON 87460- and *E. coli*-produced CSPB proteins were present for carbohydrate detection and glycosylation analysis, the membrane was stained with SYPRO® Ruby stain to detect proteins (Figure B-4, Panel B). Both MON 87460- and *E. coli*-produced CSPB were clearly detected on the membrane (Figure B-4, Panel B, Lanes 6-9). These results demonstrate that the MON 87460-produced CSPB protein is not glycosylated and, thus is equivalent to the *E. coli*-produced CSPB reference standard.

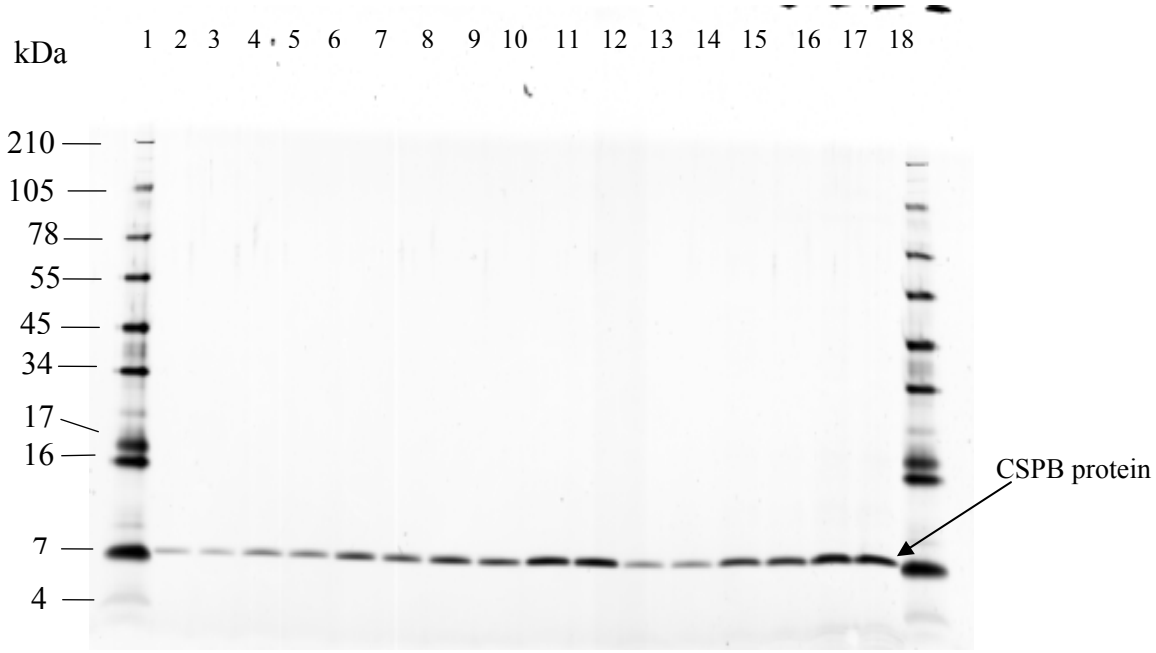



**Figure B-3. SDS-PAGE of *E. coli*- and MON 87460-produced CSPB Proteins**

Aliquots of the MON 87460-produced CSPB and the *E. coli*-produced CSPB reference standard were separated by a tricine 10-20% polyacrylamide gradient gel and stained with an Owl Silver Staining kit. Approximate molecular weights (kDa) of markers loaded in Lanes 1 and 18 are shown on the left side of the gel.

| Lane | Sample | Amount Loaded (ng) | Amount Loaded (µl) |
|---|---|---|---|
| 1 | See Blue® Plus2 Pre-Stained molecular weight markers | — | 15 |
| 2 | *E. coli*-produced CSPB standard | 10 | — |
| 3 | *E. coli*-produced CSPB standard | 10 | — |
| 4 | *E. coli*-produced CSPB standard | 20 | — |
| 5 | *E. coli*-produced CSPB standard | 20 | — |
| 6 | *E. coli*-produced CSPB standard | 30 | — |
| 7 | *E. coli*-produced CSPB standard | 30 | — |
| 8 | *E. coli*-produced CSPB standard | 40 | — |
| 9 | *E. coli*-produced CSPB standard | 40 | — |
| 10 | *E. coli*-produced CSPB standard | 60 | — |
| 11 | *E. coli*-produced CSPB standard | 60 | — |
| 12 | MON 87460-produced CSPB protein | — | 10 |
| 13 | MON 87460-produced CSPB protein | — | 10 |
| 14 | MON 87460-produced CSPB protein | — | 20 |
| 15 | MON 87460-produced CSPB protein | — | 20 |
| 16 | MON 87460-produced CSPB protein | — | 30 |
| 17 | MON 87460-produced CSPB protein | — | 30 |
| 18 | See Blue® Plus2 Pre-Stained molecular weight markers | — | 15 |

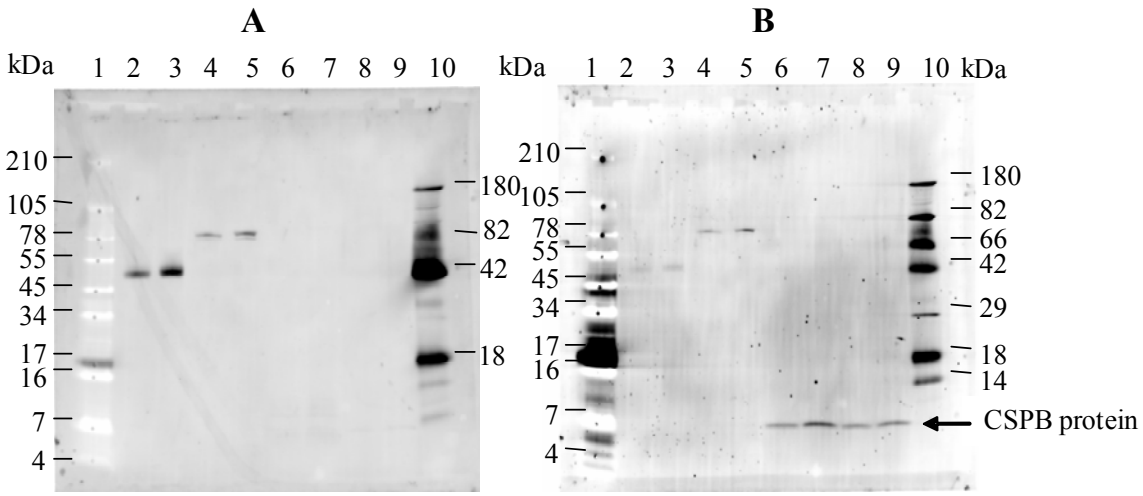



**Figure B.4. Glycosylation Analysis of the MON 87460-produced CSPB Protein**

Aliquots of the MON 87460-produced CSPB protein, *E. coli*-produced CSPB reference standard (negative control), horseradish peroxidase (positive control) and transferrin (positive control) were separated by SDS-PAGE (10-20% gradient) and electrotransferred to a PVDF membrane. (**A**) Where present, periodate-oxidized protein-bound carbohydrate moieties reacted with Pro-Q Emerald 488 glycoprotein stain and emitted a fluorescent signal at 488 nm (Lanes 1-5). (**B**) The same blot was stained with SYPRO Ruby. The signal was captured using a Bio-Rad Molecular Imager FX. Approximate molecular weights (kDa) correspond to the See Blue® Plus2 pre-stained dual color molecular weight marker loaded in Lane 1 and CandyCane glycosylated markers loaded in Lane 10.

| Lane | Sample | Amount (ng) |
|---|---|---|
| 1 | See Blue® Plus2 Pre-Stained molecular weight markers | — |
| 2 | Horseradish Peroxidase (positive control) | 25 |
| 3 | Horseradish Peroxidase (positive control) | 50 |
| 4 | Transferrin (positive control) | 25 |
| 5 | Transferrin (positive control) | 50 |
| 6 | MON 87460-produced CSPB | 25 |
| 7 | MON 87460-produced CSPB | 50 |
| 8 | *E. coli*-produced CSPB (negative control) | 25 |
| 9 | *E. coli*-produced CSPB (negative control) | 50 |
| 10 | CandyCane Glycoprotein molecular weight standards | — |

### B.2.6. CSPB Protein Functional Activity Equivalence

The functional activities of the *E. coli*- and MON 87460-produced CSPB proteins were measured using an assay where protein unfolds or "melts" a DNA-hairpin structure. Results confirm the two proteins exhibit similar functional activity. The DNA-hairpin structure is labeled with a fluorophore at the 5'- and quencher at the 3'-terminus. Due to the close proximity of the fluorescent tag and quencher in the hairpin conformation, the fluorescence is efficiently quenched. When a CSPB protein "melts" the hairpin conformation, the fluorescent tag and quencher are spatially separated which permits fluorescence. This assay has been broadly utilized to characterize the specificity of a variety of CSPs including CSD-containing proteins identified in bacteria and plants (Karlson et al., 2002; Kim et al., 2007; Phadtare et al., 2002).

In this assay protein specific activity is expressed as the amount (pmol) of open Dual Labeled Probe (DLP) that is induced by a microgram (µg) of CSPB. The *E. coli*- and MON 87460-produced CSPB proteins were considered functionally equivalent if the specific activity of one protein was within 25% of the other.

The DLP consists of a custom synthesized 35-base oligonucleotide DNA fragment with a fluorescein amidite derived from 6-carboxyfluorescein (6-FAM) label at the 5' end and a black hole quencher at the 3' end. The oligonucleotide probe forms a double strand stem of six base pairs due to the complementary bases located at the 5' and 3' ends. The 23 nucleotides (dT) in the middle form a loop. CSPB has been shown to have a high affinity for poly dT sequences and its binding to the loop will separate the double strands of the probe, which separates the fluorophore from the quencher, allowing fluorescence to be emitted and measured.

MON 87460-produced CSPB had a specific activity of 0.660 pmole open DLP/µg protein and the *E. coli*-produced reference standard had a specific activity of 0.757 pmole open DLP/µg protein. The difference in specific activities was 12.8% (Table B-3). These results clearly demonstrate that the CSPB proteins derived from MON 87460 and *E. coli* have equivalent functional activities.

**Table B-3. CSPB Functional Assay Results**

Assay activity is expressed as the amount (pmol) of open Dual Labeled Probe (DLP) that is induced by a microgram (µg) of CSPB. The probe consists of a custom synthesized 35-base oligonucleotide DNA fragment with a 6-FAM fluorescent label at the 5' end and a black hole quencher at the 3' end. Fluorescence was determined with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The amount of open probe was determined relative to the standard curve prepared from serial dilutions of 6-FAM. The opening of the DLP was measured for both proteins, using 3 µg of CSPB for each replicate. The activity represents the average of three replicates.

| **Specific Activity (pmoles opened DLP/µg CSPB ± S.E.)** | | **% Difference[1] (MON 87460 vs. *E. coli*) CSPB** |
|---|---|---|
| **MON 87460 – CSPB** | ***E. coli* – CSPB** | 12.8 |
| 0.660 ± 0.029 | 0.757 ± 0.032 | |

[1] Percent difference was calculated as follows:

$$\frac{\left|ActivityEcoli - ActivityPlant\right|}{ActivityEcoli} \times 100 = \%Difference$$

Note: S.E. = $SD/\sqrt{3}$

### B.2.7. Conclusions of the CSPB Protein Characterization

A comparison of the MON 87460-produced CSPB to the *E. coli*-produced CSPB reference protein standard confirmed the identity of the MON 87460-produced CSPB protein and established the equivalence of the plant produced protein to the *E. coli*-produced CSPB reference protein standard. The molecular weight of the MON 87460- and *E. coli*-produced CSPB proteins was estimated by SDS-PAGE. SDS-PAGE demonstrated that the proteins migrated at the same molecular weight indicating that the CSPB proteins from both sources are equivalent in their molecular weight. The electrophoretic mobility and immunoreactive properties of the MON 87460-produced CSPB protein were equivalent to those of the *E. coli*-produced CSPB reference standard. The N-terminus of the MON 87460-produced CSPB was consistent with the predicted amino acid sequence translated from the *cspB* coding sequence, and the MALDI-TOF mass spectrometry analysis yielded peptide masses consistent with the expected peptide masses from the translated *cspB* coding sequence. The MON 87460- and the *E. coli*-produced CSPB reference standard were also equivalent based on the functional activities and the lack of glycosylation. Taken together, these data provide a detailed characterization of the CSPB protein isolated from MON 87460 and establish its equivalence to the *E. coli*-produced CSPB reference protein standard.

**B.3. References:**


Aebersold, R. 1993. Mass spectrometry of proteins and peptides in biotechnology. Current Opinion in Biotechnology. 4:412-419.

Arfin, S.M. and R.A. Bradshaw. 1988. Cotranslational processing and protein turnover in eukaryotic cells. Biochemistry-US. 27:7979-7984.

Billeci, T.M. and J.T. Stults. 1993. Tryptic mapping of recombinant proteins by matrix-assisted laser desorption/ionization mass spectrometry. Analytical Chemistry. 85:1709-1716.

Hunkapiller, M.W. and L.E. Hood. 1983. Protein sequence analysis: automated microsequencing. Science. 219:650-659.

Jensen, O.L., A.V. Podtelejnikov and M. Mann. 1997. Identification of the components of simple protein mixtures by high-accuracy peptide mass mapping and database searching. Analytical Chemistry. 69:4741-4750.

Karlson, D., K. Nakaminami, T. Toyomasu and R. Imai. 2002. A cold-regulated nucleic acid-binding protein of winter wheat shares a domain with bacterial cold shock proteins. Journal of Biological Chemistry. 277:35248-35256.

Kim, J.S., S.J. Park, K.J. Kwak, Y.O. Kim, J.Y. Kim, J. Song, B. Jang, C.-H. Jung and H. Kang. 2007. Cold shock domain proteins and glycine-rich RNA-binding proteins from *Arabidopsis thaliana* can promote the cold adaptation process in *Escherichia coli*. Nucleic Acids Research. 35:506-516.

Phadtare, S., S. Tyagi, M. Inouye and K. Severinov. 2002. Three amino acids in *Escherichia coli* CspE surface-exposed aromatic patch are critical for nucleic acid melting activity leading to transcription antitermination and cold acclimation of cells. Journal of Biological Chemistry. 277:46706-46711.

Polevoda, B. and F. Sherman. 2000. N-terminal acetylation of eukaryotic proteins. Journal of Biological Chemistry. 275:36479-36482.

Rademacher, T.W., R.B. Parekh, and R.A. Dwek. 1988. Glycobiology. Annual Review of Biochemistry. 57:785-838.

## Appendix C. Materials, Methods, and Results for Characterization of NPTII Protein

The NPTII protein produced in MON 87460 was characterized and its equivalence to a previously characterized *E. coli*-produced NPTII reference substance was demonstrated. Demonstration of the equivalence between *E. coli*- and MON 87460-produced NPTII proteins allows utilization of previous safety assessment data performed on *E. coli* produced NPTII to confirm the safety of the NPTII protein in MON 87460. The analyses employed for the characterization of MON 87460-produced NPTII protein and establishment of the equivalence between MON 87460- and *E. coli*-produced proteins included western blot and SDS-PAGE analyses. The details of the materials, methods, and results are described below.

### C.1. Materials and Methods

#### C.1.1. Preparation of Protein Extracts from Leaf Samples

Frozen leaf samples from MON 87460 and conventional corn with a genetic background similar to that of the test material were extracted using phosphate saline buffer containing 0.1 % Triton X-100 (PBST) and Complete Mini (protease inhibitor) EDTA-free protein inhibitor (Roche, Indianapolis, IN) as the extraction buffer. Samples of leaf tissues (0.22 g of MON 87460 and 0.21 g of conventional control) were place in a polypropylene mesh bag from a Plant Protein Extraction kit (Pierce, Rockford, IL) and the extraction buffer was added to the bag at a tissue to buffer ratio of 1:5 (w/v). With the open end of the bag upright and held closed, the lower portion of the bag was placed on a hard flat surface, and pressed and rubbed with the backend of a marker pen 15 times. The extracts were transferred to 1.5 ml labeled tubes and centrifuged for 5 min at approximately 15,000 $\times$ *g* at room temperature. Each supernatant was transferred to a clean, labeled 1.5 ml tube, stored on ice and used within the day for the analysis.

To produce a spiked assay control, 5 µl of a 0.5 mg/ml NPTII protein reference standard solution was mixed with 35 µl leaf extract from conventional corn. The resulting final concentration of NPTII protein was 0.0625 mg/ml.

#### C.1.2. SDS-PAGE and Immunoblotting

Aliquots of 40 µl of each sample were mixed with 10 µl of 5$\times$ loading buffer (5$\times$LB) and heated for three min at 96.2 ºC. A pre-cast tris-glycine 4-20% gradient polyacrylamide SDS 12-well gel (Invitrogen, Carlsbad, CA) was loaded with the following samples:

5, 10, and 15 µl of leaf extract from MON 87460,

5, 10, and 15 µl of the spiked assay control,

10 µl of NPTII reference standard,

10 µl of leaf extract from conventional corn,

5 µl of Precision Plus Protein WesternC molecular weight markers in triplicate (Bio-Rad, Hercules, CA).

Electrophoresis was performed at a constant voltage of 125 V for 90 min. Proteins separated by SDS-PAGE were transferred to a nitrocellulose membrane (0.45 µm pore size, Invitrogen, Carlsbad, CA) at a constant voltage of 25 V for 90 min.

The membrane was blocked for 18 h at ~ 4 ºC with 5% (w/v) NFDM in PBST. From this point on, all incubations were performed at room temperature. The membrane was probed with a 1:2000 dilution of a rabbit anti-NPTII antibodies (Sigma. St. Louis, MO, Cat No N6412) in 1% (w/v) NFDM in PBST for 60 min. Excess antibodies were removed using three 5 min washes with PBST. The membrane was probed with HRP-conjugated goat anti-rabbit IgG (Vector lab, Burlingame, CA) secondary antibody at a dilution of 1:5000 in 1% (w/v) NFDM in PBST for 60 min. Precision Protein StrepTactin-HRP conjugates (Bio-Rad, Hercules, CA) were added to the secondary antibody incubation solution at a dilution of 1:50000 (a 10-fold dilution was made first with PBST, then a 1 to 5000 dilution was made in the incubation solution) to visualize the position of the WesternC protein molecular weight markers. Excess HRP-conjugates were removed using three 5 min washes with PBST. Immunoreactive bands were visualized using the ECL detection system (GE healthcare, Piscataway, NJ) and films were exposed for 5, 10 and 20 s to Hyperfilm ECL high performance chemiluminescence film (GE Healthcare, GE healthcare, Piscataway, NJ). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

### C.1.3. Immunoblot Analysis

The 10 s exposure film was scanned using a Bio-Rad GS-800 densitometer (Hercules, CA) and used for the image analysis. The image analysis was performed using Quantity One software (Version 4.6, Bio-Rad, Hercules, CA). The apparent molecular weights of the MON 87460-produced NPTII protein and the NPTII reference standard in the spiked assay control were determined relative to the known values of the Precision Plus Protein WesternC molecular weight markers loaded on the gel. The apparent molecular weight was calculated as the average value for all loads of each sample and the average values were compared.

### C.1.4. Equivalence criteria

The equivalence of the MON 87460- and *E. coli*-produced NPTII proteins was established by direct comparison of their apparent molecular weight and immunoreactivity with NPTII specific antibodies. The criteria for these tests were pre-established during developmental work taking into consideration the inherent variability of each analytical method. These criteria were as follows:

The immunoreactive band corresponding to the NPTII protein from the leaf extract of MON 87460 should migrate to the same position as the NPTII protein in the spiked assay control. No immunoreactive band with the same mobility as the NPTII protein reference standard should be observed in leaf extract from conventional corn.

The apparent molecular weight of the MON 87460-produced NPTII protein should be within ± 10% of the *E. coli*-produced NPTII protein in the spiked assay control.

## C.2. Results

### C.2.1. Identity and Function of the NPTII Protein

The NPTII protein functions as a selectable marker in the initial laboratory stages of plant cell selection following transformation (Horsch at al., 1984; DeBlock et al., 1984). NPTII uses adenosine triphosphate (ATP) to phosphorylate neomycin and related aminoglycoside antibiotics, thereby inactivating them. Cells that produce the NPTII enzyme selectively survive exposure to these aminoglycosides. The *nptII* coding sequence is derived from the prokaryotic *E. coli* transposon *Tn5* (Beck et al., 1982). The purpose of inserting the gene encoding the NPTII protein into corn cells along with CSPB was to have an effective method for selecting cells after transformation. In general, the frequency of plant cells that are transformed is often low, ranging from $1x10^{-5}$ to $lx10^{-4}$ of cells treated (Fraley et al., 1983). Therefore, the selectable marker, NPTII, facilitates the screening process.

### C.2.2. Characterization of the NPTII Protein

The NPTII protein produced in MON 87460 was characterized and its equivalence to a previously characterized *E. coli*-produced NPTII reference substance was demonstrated. Demonstration of the equivalence between *E. coli*- and MON 87460-produced NPTII proteins allows utilization of previous safety assessment data to confirm the safety of the NPTII protein in MON 87460. The analyses employed for the characterization of MON 87460-produced NPTII protein and establishment of the equivalence between MON 87460- and *E. coli*-produced proteins included:

1. immunoblot analysis to establish protein identity through immunoreactivity with NPTII–specific antibody and demonstrate immuno-equivalence between MON 87460 and *E. coli*-produced NPTII proteins.
2. SDS-PAGE to assess the apparent molecular weight of the protein and establish equivalence of the apparent molecular weight between MON 87460- and *E. coli*-produced proteins.

### C.2.3. NPTII Protein Immunoreactivity

Immunoblot analysis established that MON 87460-produced NPTII and *E. coli*-produced NPTII have equivalent immunoreactive properties. The expression levels of NPTII protein in MON 87460 leaf tissue allowed detection of the protein with an NPTII-specific antibody directly in leaf extracts without additional enrichment. An extract was also prepared from a leaf sample of conventional corn with a similar genetic background as MON 87460 to serve as a negative control for the presence of the NPTII protein. To ensure that the electromobility of the NPTII protein had not been altered as a result of matrix effects, the reference substance was spiked into the leaf extract from conventional corn and analyzed alongside the leaf extract from MON 87460. The leaf extract from MON 87460, *E. coli*-produced NPTII protein, and NPTII-spiked conventional corn leaf extract were subjected to a reducing and denaturing SDS-PAGE and then transferred to a nitrocellulose membrane for detection using an anti-NPTII antibody. A co-migrating immunoreactive band was observed in the leaf extract from MON 87460 (Figure C-1, Lanes 4-6), leaf NPTII-spiked conventional corn leaf extract (Figure C-1, Lanes 7-9), and

pure *E. coli*-produced NPTII protein (Figure C-1, Lane10). As expected, the immunoreactive signal increased with increased loading levels of the leaf extract from MON 87460 and increased amount of the leaf extract from conventional corn spiked with the *E. coli*-produced NPTII protein. No immunoreactive bands were observed in the leaf extract from conventional corn (Figure C-1, Lane 3). Based on this analysis, the MON 87460- and *E. coli*-produced NPTII proteins demonstrated equivalent immunoreactive properties, which confirmed both the identity and equivalence of the two proteins.

### C.2.4. NPTII Protein Molecular Weight Equivalence

The molecular weight of the MON 87460-produced NPTII protein and its equivalence to the molecular weight of the *E. coli*-produced NPTII reference standard was confirmed using densitometric analysis of the western blot (Figure C-1). The electromobility of the MON 87460-produced protein was indistinguishable from the electromobility of the *E. coli*-produced NPTII protein. The estimated molecular weight of the MON 87460-produced NPTII protein was 27.4 kDa, which was similar to the previously determined molecular weight of the *E. coli*-produced NPTII reference standard (27.1 kDa). Based on the identical electrophoretic mobility and apparent molecular masses, the MON 87460- and *E. coli*-produced NPTII proteins have equivalent molecular weights.

### C.2.5. Conclusions of the NPTII Protein Characterization

MON 87460-produced and *E. coli*-produced NPTII proteins have equivalent immunoreactivities and apparent molecular weights. The results of this analysis confirmed the identity of the MON 87460-produced NPTII protein and established the equivalence of the plant produced protein to the *E. coli*-produced NPTII reference protein standard. A western blot analysis was utilized to compare the immunoreactivity and apparent molecular weight of the MON 87460-produced NPTII protein to that of the previously characterized *E. coli*-produced NPTII reference protein standard. The MON 87460- and *E. coli*-produced NPTII proteins displayed similar immunoreactivity with NPTII-specific antibody and had identical electromobility on SDS-PAGE. Taken together, these data establish equivalence between the MON 87460-produced and *E. coli*-produced NPTII reference protein standard.

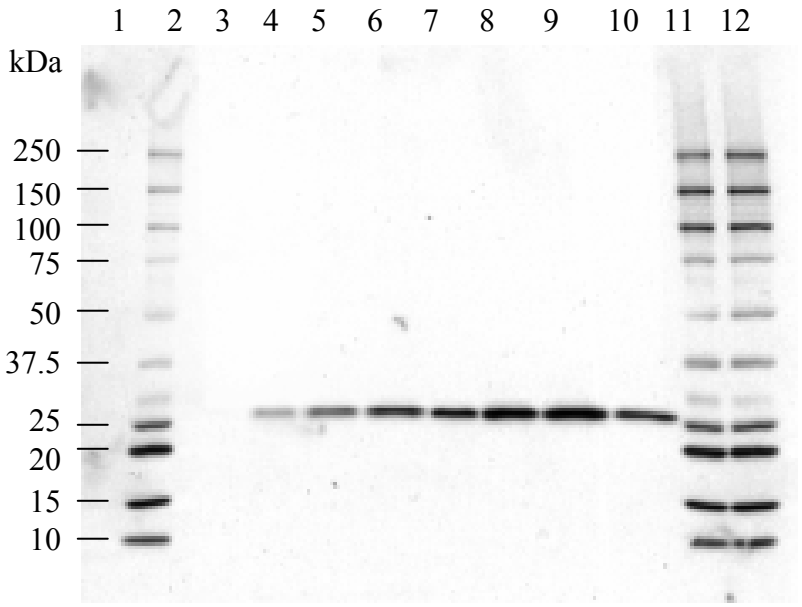



**Figure C-1. Western Blot Analysis of the MON 87460- and *E. coli*-produced NPTII Protein**

Corn leaf extracts from MON 87460 and conventional corn. *E. coli*-produced NPTII and *E. coli*-produced NPTII spiked into leaf extract of conventional corn were separated by SDS-PAGE and electrotransferred to a nitrocellulose membrane. The membrane was probed with rabbit anti-NPTII antibody and an HPR-conjugated secondary antibody and visualized using an ECL system. Approximate molecular weights (kDa) are shown on the left and correspond to the protein marker loaded in Lanes 2, 11 and 12. The 10 s exposure is shown and is representative of the bands observed in the other exposures.

| Lane | Sample | Amount Loaded (ng) | Amount Loaded (µl) |
|---|---|---|---|
| 1 | Empty | — | — |
| 2 | Precision Plus Protein WesternC markers | — | 5 |
| 3 | Leaf extract from conventional corn | — | 10 |
| 4 | Leaf extract from corn MON 87460 | — | 5 |
| 5 | Leaf extract from corn MON 87460 | — | 10 |
| 6 | Leaf Extract from corn MON 87460 | — | 15 |
| 7 | *E. coli*-produced NPTII spiked* | 0.25 | 5 |
| 8 | *E. coli*-produced NPTII spiked* | 0.5 | 10 |
| 9 | *E. coli*-produced NPTII spiked* | 0.75 | 15 |
| 10 | *E. coli*-produced NPTII | 0.5 | 10 |
| 11 | Precision Plus Protein WesternC markers | — | 5 |
| 12 | Precision Plus Protein WesternC markers | — | 5 |

* *E. coli*-produced NPTII spiked in leaf extract from conventional corn

**C.3. References:**

Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss and H. Schaller. 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon *Tn5*. Gene. 19:327-336.

DeBlock, M., L. Herrera-Estrella, M.V. Montagu, J. Schell and P. Zambryski. 1984. Expression of foreign genes in regenerated plants and in their progeny. EMBO Journal. 3:1681-1689.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L. Hoffmann and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proceedings of the National Academy of Sciences USA. 80:4803-4807.

Horsch, R.B., R.T. Fraley, S.G. Rogers, P.R. Sanders, A. Lloyd and N. Hoffmann. 1984. Inheritance of functional foreign genes in plants. Science. 223:496-498.

## Appendix D. Materials and Methods for Expression Levels of CSPB and NPTII Proteins

The levels of the CSPB and NPTII proteins in various tissues of MON 87460 were determined using enzyme-linked immunosorbent assays (ELISA). The materials and methods for the ELISA analysis, as well as a description of the tissue types, are provided below. To produce the tissues for analysis, MON 87460 and conventional corn were each planted in field trials conducted during two different growing seasons. The first season was conducted at six sites in the U.S. during 2006 under typical agronomic practices and water conditions. The second season was conducted at four sites in Chile during 2006/2007 using a strip-plot design to establish two water treatment levels (well-watered and water-limited) to assess for any changes in CSPB and NPTII protein levels under different soil moisture conditions. The sites were located in the major corn-growing regions of the U.S. and Chile. Forage, stover, silk, pollen, and grain samples were collected at appropriate times of plant development. Over-season leaf (OSL), over-season root (OSR), and over-season whole plant (OSWP) samples were collected four times (1-4) over the season corresponding to plant growth stages V2-V4, V6-V8, V10-V12, and pre-VT (pre-tasseling), respectively. The expression levels of CSPB and NPTII proteins in these tissues are shown in Tables VI-1 to VI-4. As discussed previously (Section I.B.5, and in more detail in Section VIII.C and Table VIII-3), the QUI site in the Chile 2006/2007 study was not established with the appropriate water stress treatments; therefore, data and analysis for the QUI site are presented separately in Section D.3.

### D.1. U.S. 2006 Study

#### D.1.1. Field Design

Tissue samples were collected from six field trials conducted in the U.S. during 2006. The trials were located in Iowa, Illinois, Indiana, Kansas, and Nebraska which represent the major corn-growing regions of the U.S. and provide a range of environmental conditions that would be encountered in the commercial production of corn. At each site, three replicated plots of MON 87460 and a conventional control hybrid were planted using a randomized complete block field design. Over-season leaf (OSL), over-season whole plant (OSWP), over-season root (OSR), pollen, silk, forage, forage root, grain, stover, and senescent root tissues were collected from each replicated plot at all field sites.

#### D.1.2. Description of Collected Tissues

**Leaf.** Leaves were randomly collected from plants in each plot at each site. Twenty leaves were combined to form the leaf sample for each plot. There were 18 leaf samples across all sites for OSL-1, OSL-3, and OSL-4 and 17 leaf samples for OSL-2. OSL samples were collected as follows:

| *Over-season leaf (OSL)* | *Corn development stage* | *Days after planting (DAP)* |
|---|---|---|
| OSL-1 | V2-V4 | 15-22 |
| OSL-2 | V6-V8 | 27-38 |
| OSL-3 | V10-V12 | 41-56 |
| OSL-4 | pre-VT (pre-tasseling) | 49-63 |

**Whole Plant**. The aerial portion of the plant was collected from four plants in each plot at each site at the V2-V4 stage and combined to form the whole plant sample. Two plants were collected and combined to form the whole plant samples for the later growth stages. OSWP samples were collected as follows:

| *Overseason whole plant (OSWP)* | *Corn development stage* | *DAP* |
|---|---|---|
| OSWP-1 | V2-V4 | 15-22 |
| OSWP-2 | V6-V8 | 27-38 |
| OSWP-3 | V10-V12 | 41-56 |
| OSWP-4 | pre-VT (pre-tasseling) | 49-63 |

**Root**. Roots remaining after collection of whole plants from each plot were combined to form the root sample. Therefore, roots from four plants at the V2-V4 stage and roots from two plants at later stages were combined. OSR samples were collected as follows.

| *Overseason root (OSR)* | *Corn development stage* | *DAP* |
|---|---|---|
| OSR-1 | V2-V4 | 15-22 |
| OSR-2 | V6-V8 | 27-38 |
| OSR-3 | V10-V12 | 41-56 |
| OSR-4 | pre-VT (pre-tasseling) | 49-63 |
| Forage root | early dent stage (R4-R6) | 90-103 |
| Senescent root | after harvest | 135-151 |

**Pollen and Silk**. Approximately 10 ml of pollen was collected from multiple tassels in each plot at each site at pollination, approximately 59-68 days after planting. Silks were collected approximately 58-74 days after planting from five plants, except for the Indiana and Iowa sites, where silks were collected from ten plants. Silks were only collected from ears of plants that were covered with shoot bags to preserve their genetic identity.

**Forage and Forage Root**. Two whole plants in each plot at each site were cut above the soil surface at an early dent stage, at approximately 96-109 days after planting, and then combined to form the forage sample. The roots of these plants were combined to form the forage root sample.

**Grain, Stover, and Senescent Root**. Grain was harvested at maturity from all plants in each plot at each site and dried to a moisture content of 11-17%. Following harvest, approximately 136-158 days after planting, two whole plants in each plot at each site were cut above the soil surface and combined to form the stover sample. The roots of these plants were also removed, washed and combined to form a senescent root sample.

All tissue samples, except grain, were stored and shipped on dry ice for processing and analysis. Grain was stored and shipped at room temperature. All tissue samples were stored in a –80ºC freezer upon receipt.

CSPB expression levels were determined in all 19 tissue types described above. Because of the extensive historical safety data for NPTII, the number of tissues evaluated for NPTII expression was fewer than those evaluated for CSPB protein. The NPTII levels were evaluated in four of the 19 tissue types including OSL-1 (V2-V4), OSR-1 (V2-V4), forage and grain. These four tissues were selected to span the life cycle of corn. Moisture content was measured for all tissue types. Protein levels for all tissue types are provided in µg/g fresh weight tissue (fwt) and µg/g dry weight tissue (dwt) basis.

### D.1.3. Tissue Processing and Protein Extraction

All samples produced at the field sites were shipped to Monsanto's processing facility in Creve Coeur, MO. During the processing step, dry ice was combined with the individual samples, and vertical cutters or mixers were used to thoroughly grind and mix the tissues. Processed samples were transferred into capped tubes and stored in a -80°C freezer until use.

CSPB and NPTII were extracted from all tissues by shaking tubes mounted in a Harbil mixer for a specified period of time. Each extraction tube contained eight ¼" diameter Chrome-steel beads, buffer and a tissue to buffer ratio as specified below.

**Table D-1. Protein Extraction Methods for U.S. 2006 Tissue Samples**

| *Protein* | *Tissue* | *Extraction Buffer* | *T:B Ratio*[1] | *Shake Time (minutes)* | *Sample Clarification Method* |
|---|---|---|---|---|---|
| **CSPB/NPTII** | Leaf [2] | PBST/BSA[3] | 1:100 | 7.0 | Serum filter |
| **CSPB/NPTII** | Root[4] | PBST/BSA | 1:20 | 7.0 | Serum filter |
| **CSPB/NPTII** | Forage[5] | TB[6] | 1:30 | 7.0 | Serum filter |
| **CSPB/NPTII** | Grain | TB | 1:25 | 10.5 | Serum filter |
| **CSPB** | Silk | PBST/BSA | 1:50 | 7.0 | Serum filter |
| **CSPB** | Pollen | TB | 1:50 | 10.5 | Serum filter |

[1]T:B Ratio – Tissue to buffer ratio
[2]Overseason leaf (OSL1, OSL2, OSL3, and OSL4)
[3]1x Phosphate Buffered Saline + 0.05% (w/v)Tween + 0.1%(w/v) Bovine Serum Albumin
[4]Overseason root (OSR1, OSR2, OSR3, and OSR4), forage root, and senescent root
[5]Forage, overseason whole plant (OSWP1, OSWP2, OSWP3, and OSWP4), and stover
[6]1x Tris borate buffer (0.1 M Tris, 0.1 M $Na_2B_4O_7 \cdot 10H_2O$, 0.01 M $MgCl_2$, 0.05% (v/v) Tween-20, pH 7.8).

Following shaking, insoluble material was removed from the extracts using a serum filter (Fisher Scientific, Pittsburgh, PA). The clarified extracts were aliquot, and stored frozen in a -80°C freezer until ELISA analysis.

### D.1.4. CSPB Antibodies

Goat polyclonal CSPB-specific IgG was purified by Protein G-agarose affinity chromatography followed by affinity chromatography on AminoLink immobilized CSPB protein (lot G-812159). The concentration of the CSPB protein affinity purified IgG was determined to be 0.8 mg/ml by spectrophotometric methods. The purified antibody was stored in 137 mM NaCl, 10 mM $Na_2HPO_4 \cdot 7\ H_2O$, 1 mM $KH_2PO_4$, and 2.7 mM KCl, pH 7.4 (1X phosphate buffered saline (PBS)). CSPB protein affinity purified IgG was used as the well coating antibody.

Protein G agarose affinity purified goat polyclonal anti-CSPB was coupled with biotin (Sigma, St. Louis, MO) according to the manufacturer's instructions and assigned lot G-806080-2. The detection reagent was NeutrAvidin (Pierce, Rockford, IL) conjugated to HRP.

### D.1.5. NPTII Antibodies

Rabbit polyclonal antibodies (lot G-805224) specific for the NPTII protein were purified using Protein-A agarose affinity chromatography by TechServ Associates (St. Louis, MO). The concentration of the purified IgG was determined to be 5.6 mg/ml by spectrophotometric methods. The purified antibody was stored in 20 mM potassium phosphate, 150 mM NaCl, pH 7.3 and preserved with 0.01% (w/v) sodium azide.

The purified NPTII antibodies were coupled with biotin (Sigma, St. Louis, MO) according to the manufacturer's instructions and assigned lot G-814147. The detection reagent was NeutrAvidin (Pierce, Rockford, IL) conjugated to HRP.

### D.1.6. CSPB ELISA Method

Affinity-purified goat anti-CSPB capture antibodies were diluted in coating buffer (15 mM Na2CO3, 35 mM NaHCO3, pH 9.6) and immobilized onto 96-well microtiter plates at 2.0 µg/ml followed by incubation in a 4°C refrigerator for >8 h. Prior to each step in the assay, plates were washed with 1X PBS containing 0.05% (w/v) Tween-20 (1X PBST). Plates were blocked with the addition of 150 µl per well of 1X PBST with 1% BSA for 60 to 70 min at 37°C. CSPB protein standard or sample extract was added at 100 µl per well and incubated for 1 h at 37°C. The captured CSPB protein was detected by the addition of 100 µl per well of biotinylated goat anti-CSPB antibodies and NeutrAvidin-HRP (Pierce). Plates were developed by adding 100 µl per well of HRP substrate, 3,3',5,5'-tetramethylbenzidine (TMB; Kirkegaard & Perry, Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 3 M H3PO4. Quantitation of the CSPB protein was accomplished by interpolation from a CSPB protein standard curve that ranged from 0.05 – 1.6 ng/ml.

### D.1.7. NPTII ELISA Method

Rabbit anti-NPTII capture antibodies were diluted in coating buffer (15 mM $Na_2CO_3$ and 35 mM $NaHCO_3$, pH 9.6) and immobilized onto 96-well microtiter plates at 5.0 µg/ml followed by incubation in a 4°C refrigerator for $\geq$8 h. Prior to each step in the assay, plates were washed with 1X PBST. NPTII protein standard or sample extract was added at 100 µl per well and incubated for 1 h at 37°C. The captured NPTII protein was detected by the addition of 100 µl per well of biotinylated rabbit anti-NPTII antibodies

and NeutrAvidin-HRP. Plates were developed by adding 100 µl per well of TMB. The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M $H_3PO_4$. Quantitation of the NPTII protein was accomplished by interpolation from a NPTII protein standard curve that ranged from 0.094 – 3.0 ng/ml.

### D.1.8. Moisture Analysis

All tissues were analyzed for moisture content using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). A homogeneous tissue-specific site pool (TSSP) was prepared using the test and control samples of a given tissue type grown at a given site. These pools were prepared for all tissues in this study. The average percent moisture for each TSSP was calculated from triplicate analyses. A TSSP Dry Weight Conversion Factor (DWCF) was calculated as follows:

$$DWCF = 1 - [Mean\ \%\ TSSP\ Moisture / 100]$$

The DWCF was used to convert protein levels assessed on a µg/g fresh weight (fwt) basis into levels reported on a µg/g dry weight (dwt) basis using the following calculation:

$$Protein\ Level\ in\ Dry\ Weight = \frac{(Protein\ Level\ Fresh\ Weight)}{(DWCF)}$$

The protein levels that were reported to be less than or equal to the limit of detection (LOD) or less than the limit of quantitation (LOQ) on a fresh weight basis were not reported on a dry weight basis.

### D.1.9. Data Analyses

All CSPB and NPTII ELISA plates were analyzed on a SPECTRAmax Plus (Molecular Devices, Sunnyvale, CA) or a SPECTRAFluor Plus (Tecan, Research Triangle Park, NC) microplate spectrophotometer, using a dual wavelength detection method. All protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of 620-650 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO version 4.7.1 or SOFTmax Pro GxP version 5.0.1. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve fit. Following the interpolation from the standard curve, the amount of protein (ng/ml) in the tissue was reported on a µg/g fwt basis. For all proteins, this conversion utilized a sample dilution factor and a tissue-to-buffer ratio. The protein values in µg/g fwt were also converted to µg/g dwt by applying the DWCF. Microsoft Excel 2002 (Version 10.6834.6830 SP3, Microsoft, Redmond, WA) was used to calculate the CSPB and NPTII protein levels in corn tissues.

## D.2. Chile 2006/2007 Study

### D.2.1. Field Design

The second season field trial was designed to assess protein levels in MON 87460 under a range of typical environmental conditions relevant to its commercial production. This trial was conducted in Chile during 2006/2007 using two water treatment levels (well-

watered and water-limited) to assess the impact of different soil moisture conditions on protein expression. The levels of the CSPB and NPTII proteins in various tissues of MON 87460 that are relevant to the risk assessment were assessed by a validated ELISA. OSL, OSWP, OSR, pollen, silk, forage, forage root, grain, stover, and senescent root tissues were collected from four field sites. The trial locations were Calera de Tango (CT), Colina (CL), Lumbreras (LUM) and Quillota (QUI), covering a range of environmental conditions representative of commercial corn production areas for MON 87460. At each site, three replicated plots of MON 87460, as well as the conventional control, were planted using a strip-plot design with three replicates per site. The whole-plot for each replicate was an irrigation treatment (well-watered or water-limited). The sub-plot for each irrigation treatment was substance type (test and control substances), which was randomized in strips across the irrigation treatments to assess for any changes in protein levels under different soil moisture conditions. Well-watered plots were irrigated to achieve optimal yield, whereas water-limited plots were managed to impose a drought stress by withholding irrigation during the late vegetative through early grain fill growth stages (i.e., approximately V10 through R2). For a site to be included in the combined-site analysis, commercial reference hybrids had to exhibit phenotypic responses indicative of a treatment effect. Specifically, reference hybrids in the water-limited plots had to exhibit a minimum 15% reduction in yield compared to the same reference hybrids planted in the well-watered plots. Moderate water deficits result in approximately a 15% yield loss annually for corn grown in both temperate and tropical regions (Barker et al., 2005). Assessments for plant height, ear height and days to 50% silking were also made as reduced height and a delay in silking are indicators of moisture deficit in corn (Campos et al., 2006). Reference hybrids at CT, CL and LUM exhibited the expected phenotypic response. Results from the Chile 2006/2007 represent combined-site data from three (CL, CT, LUM) of the four sites established. As discussed previously (Section VIII.C, Table VIII-3), the QUI site in the Chile 2006/2007 study was not established with the appropriate water stress treatments; therefore, data and analysis for the QUI site are presented below in Section D.3.

### D.2.2. Description of Collected Tissues

**Leaf.** The youngest leaves were randomly collected from plants in each plot at each site. Forty leaves were combined to form the leaf sample for each plot. Over-season leaf samples were collected as follows:

| *Over-season leaf (OSL)* | *Corn development stage* | *Days after planting (DAP)* |
|---|---|---|
| OSL-1 | V2-V4 | 13-20 |
| OSL-2 | V6-V8 | 32-38 |
| OSL-3 | V10-V12 | 48-52 |
| OSL-4 | ~VT | 62-65 |

**Whole Plant**. The aerial portion of the plant was collected from four plants in each plot at each site at the V2-V4 stage and combined to form the whole plant sample. Overseason whole plant samples were collected as follows:

| *Overseason whole plant (OSWP)* | *Corn development stage* | *DAP* |
|---|---|---|
| OSWP-1 | V2-V4 | 13-20 |
| OSWP-2 | V6-V8 | 32-38 |
| OSWP-3 | V10-V12 | 48-52 |
| OSWP-4 | ~VT | 62-65 |

**Root**. Roots remaining after collection of whole plants from each plot were combined to form the root sample. Overseason root samples were collected as follows:

| *Overseason root (OSR)* | *Corn development stage* | *DAP* |
|---|---|---|
| OSR-1 | V2-V4 | 13-20 |
| OSR-2 | V6-V8 | 32-38 |
| OSR-3 | V10-V12 | 48-52 |
| OSR-4 | ~VT | 62-65 |
| Forage root | early dent stage | 106-113 |
| Senescent root | after harvest | 151-153 |

**Pollen and Silk**. Quantities of pollen ranging from 0.5-40 ml and averaging approximately 10 ml were collected from multiple tassels in each plot at each site at pollination, approximately 67-74 days after planting. Silks were collected approximately 64-72 days after planting from five plants. Silks were only collected from ears of plants that were covered with shoot bags to preserve the genetic identity.

**Forage and Forage Root**. Four whole plants in each plot at each site were cut above the soil surface at an early dent stage, approximately 106-113 days after planting, and then combined to form the forage sample. The roots of these plants were also removed, washed and combined to form the forage root sample.

**Grain, Stover, and Senescent Root**. Grain was harvested at maturity from all plants in each plot at each site and dried to a moisture content of 11-17%. Following harvest, approximately 151-153 days after planting, four whole plants in each plot at each site were cut above the soil surface and combined to form the stover sample. The roots of these plants were also removed, washed and combined to form a senescent root sample.

All tissue samples, except grain, were stored and shipped on dry ice for processing and analysis. Grain was stored and shipped at room temperature. All tissue samples were stored in a –80 ºC freezer upon receipt. Tissue samples were extracted and analyzed by a validated ELISA according to applicable standard operating procedures (SOPs).

CSPB expression levels were determined in all 19 tissue types described above. Given the extensive historical safety data for NPTII, the number of tissues evaluated for NPTII expression was fewer than those evaluated for CSPB protein. The NPTII levels were

evaluated in four of the 19 tissue types including OSL1 (V2-V4), OSR1 (V2-V4), forage, and grain. These four tissues were selected to span the life cycle of corn.

Moisture content was measured for all tissue types. Protein levels for all tissue types are provided in µg/g fwt and µg/g dwt basis.

### D.2.3. Tissue Processing and Protein Extraction

Same as described for U.S. 2006 study.

### D.2.4. CSPB Antibodies

Same as described for U.S. 2006 study.

### D.2.5. NPTII Antibodies

Same as described for U.S. 2006 study.

### D.2.6. CSBP ELISA Method

Same as described for U.S. 2006 study.

### D.2.7. NPTII ELISA Method

Same as described for U.S. 2006 study.

### D.2.8. Moisture Analysis

Same as described for U.S. 2006 study.

### D.2.9. Data Analysis

Same as described for U.S. 2006 study.

### D.2.10. References

Barker, T., H. Campos, M. Cooper, D. Dolan, G. Edmeades, J. Habben, J. Schlusser, D. Wright and C. Zinselmeier. 2005. Improving drought tolerance in maize. Pages 173-253 in Plant Breeding Reviews. Vol 25. J. Janick, (ed.). John Wiley and Sons, Inc., Hoboken, NJ.

Campos, H., M. Cooper, G.O. Edmeades, C. Loffler, J.R. Schussler, and M. Ibanez. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. Maydica. 51:369-381.

## D.3. Supplementary Protein Level Data for QUI Site in Chile 2006/2007 Study

This section presents protein expression data for MON 87460 grown at an individual site (QUI) of the Chile 2006/2007 study. As discussed previously (Section VIII.C, Table VIII-3), the QUI site in the Chile 2006/2007 study was not established with the appropriate water stress treatments; therefore, data and analysis for the QUI site are presented in this appendix. Table D-2 presents results for CSPB and Table D-3 presents results for NPTII. Evaluation of the overall data set confirmed expression levels were as expected. No unexpected values for either CSPB or NPTII were observed. The QUI site results reported below do not impact the conclusion of protein expression studies of MON 87460 presented in Section VI.C.

**Table D-2. CSPB Protein Levels in Tissues Collected from MON 87460 Produced at the QUI site in Chile during 2006/2007 under Well-Watered and Water-Limited Conditions**

| Tissue Type | Well-Watered | | Water-Limited | | LOQ / LOD (µg/g fwt) |
|---|---|---|---|---|---|
| | Mean (SD)[1] Range[2] (µg/g fwt.)[3] | Mean (SD) Range (µg/g dwt.)[4] | Mean (SD) Range (µg/g fwt.) | Mean (SD) Range (µg/g dwt.) | |
| **OSL-1** | 0.46 (0.10)<br>0.36 - 0.56 | 3.1 (0.68)<br>2.4 - 3.7 | 0.47 (0.076)<br>0.39 - 0.53 | 2.5 (0.40)<br>2.0 - 2.8 | 0.015 / 0.0069 |
| **OSL-2** | 0.28 (0.037)<br>0.24 - 0.31 | 1.5 (0.19)<br>1.3 - 1.6 | 0.31 (0.015)<br>0.29 - 0.32 | 1.6 (0.077)<br>1.5 - 1.6 | 0.015 / 0.0069 |
| **OSL-3** | 0.11 (0.036)<br>0.084 - 0.15 | 0.50 (0.16)<br>0.37 - 0.67 | 0.11 (0.049)<br>0.061 - 0.16 | 0.46 (0.21)<br>0.26 - 0.68 | 0.015 / 0.0069 |
| **OSL-4** | 0.093 (0.0015)<br>0.092 - 0.095 | 0.44 (0.0073)<br>0.44 - 0.45 | 0.10 (0.031)<br>0.059 - 0.12 | 0.48 (0.16)<br>0.30 - 0.58 | 0.015 / 0.0069 |
| **OSR-1** | 0.12 (0.023)<br>0.10 - 0.14 | 1.2 (0.23)<br>0.95 - 1.4 | 0.10 (0.0063)<br>0.10 - 0.11 | 1.0 (0.063)<br>0.99 - 1.1 | 0.0020 / 0.0018 |
| **OSR-2** | 0.13 (0.044)<br>0.10 - 0.18 | 1.3 (0.44)<br>1.0 - 1.8 | 0.10 (0.022)<br>0.081 - 0.12 | 0.92 (0.20)<br>0.74 - 1.1 | 0.0020 / 0.0018 |
| **OSR-3** | 0.046 (0.010)<br>0.036 - 0.056 | 0.46 (0.10)<br>0.36 - 0.56 | 0.052 (0.023)<br>0.033 - 0.077 | 0.52 (0.23)<br>0.33 - 0.77 | 0.0020 / 0.0018 |
| **OSR-4** | 0.048 (0.014)<br>0.035 - 0.062 | 0.40 (0.11)<br>0.29 - 0.52 | 0.059 (0.0029)<br>0.056 - 0.062 | 0.45 (0.022)<br>0.43 - 0.47 | 0.0020 / 0.0018 |
| **OSWP-1** | 0.34 (0.0032)<br>0.34 - 0.35 | 3.1 (0.029)<br>3.1 - 3.1 | 0.31 (0.039)<br>0.29 - 0.36 | 2.8 (0.35)<br>2.6 - 3.2 | 0.0045 / 0.0043 |
| **OSWP-2** | 0.20 (0.023)<br>0.18 - 0.23 | 2.2 (0.26)<br>2.0 - 2.5 | 0.18 (0.019)<br>0.16 - 0.20 | 2.5 (0.28)<br>2.3 - 2.8 | 0.0045 / 0.0043 |
| **OSWP-3** | 0.082 (0.0079)<br>0.073 - 0.087 | 0.68 (0.066)<br>0.61 - 0.72 | 0.077 (0.0054)<br>0.071 - 0.081 | 0.70 (0.049)<br>0.64 - 0.74 | 0.0045 / 0.0043 |
| **OSWP-4** | 0.11 (0.017)<br>0.092 - 0.12 | 0.74 (0.11)<br>0.61 - 0.81 | 0.12 (0.0058)<br>0.11 - 0.13 | 0.79 (0.039)<br>0.76 - 0.84 | 0.0045 / 0.0043 |
| **Forage Root** | 0.0090 (0.0013)<br>0.0080 - 0.010 | 0.056 (0.0082)<br>0.050 - 0.066 | 0.0062 (0.0027)<br>0.0038 - 0.0092 | 0.041 (0.018)<br>0.025 - 0.061 | 0.0020 / 0.0018 |
| **Senescent Root** | 0.0051 (0.0041)<br>0.0021 - 0.010 | 0.032 (0.025)<br>0.013 - 0.061 | 0.0028 (N/A[5])<br>N/A | 0.018 (N/A)<br>N/A | 0.0020 / 0.0018 |
| **Forage** | 0.023 (0.0030)<br>0.021 - 0.027 | 0.10 (0.013)<br>0.091 - 0.12 | 0.021 (0.0040)<br>0.017 - 0.025 | 0.091 (0.017)<br>0.073 - 0.11 | 0.0045 / 0.0043 |
| **Stover** | 0.014 (0.00059)<br>0.013 - 0.015 | 0.048 (0.0020)<br>0.046 - 0.050 | 0.012 (0.0041)<br>0.0082 - 0.016 | 0.039 (0.013)<br>0.026 - 0.053 | 0.0045 / 0.0043 |
| **Silk** | 0.053 (0.014)<br>0.036 - 0.066 | 0.66 (0.17)<br>0.45 - 0.82 | 0.040 (0.012)<br>0.032 - 0.054 | 0.40 (0.12)<br>0.32 - 0.54 | 0.0075 / 0.0047 |
| **Pollen** | 12 (1.1)<br>12 - 14 | 18 (1.7)<br>17 - 20 | 11 (0.84)<br>10 - 12 | 17 (1.2)<br>16 - 18 | 0.050 / 0.045 |
| **Grain** | 0.043 (0.010)<br>0.031 - 0.051 | 0.050 (0.012)<br>0.036 - 0.059 | 0.036 (0.0091)<br>0.029 - 0.046 | 0.042 (0.011)<br>0.034 - 0.054 | 0.0038 / 0.0017 |

[1]The mean and standard deviation were calculated across sites (n=3 for well-watered and n=3 for water-limited, except silk where n=4 for well-watered and senescent root where n=1 under water-limited).
[2]Minimum and maximum values were determined for each tissue type across sites.
[3]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis.
[4]Protein levels are expressed as µg/g on a dry weight basis. The dry weight values were calculated by dividing the fresh weight by the dry weight conversion factors obtained from moisture analysis data.
[5]N/A – not applicable

**Table D-3. NPTII Protein Levels in Tissues Collected from MON 87460 Produced at the QUI site in Chile during 2006/2007 under Well-Watered and Water-Limited Conditions**

| Tissue Type | Well-Watered | | Water-Limited | | LOQ / LOD (µg/g fwt) |
|---|---|---|---|---|---|
| | Mean (SD)[1] Range[2] (µg/g fwt.)[3] | Mean (SD) Range (µg/g dwt.)[4] | Mean (SD) Range (µg/g fwt.) | Mean (SD) Range (µg/g dwt.) | |
| **OSL-1** | 0.42 (0.039)<br>0.38 - 0.45 | 2.8 (0.26)<br>2.5 - 3.0 | 0.50 (0.0093)<br>0.49 - 0.50 | 2.6 (0.049)<br>2.6 - 2.7 | 0.047/ 0.0090 |
| **OSR-1** | 0.044 (0.016)<br>0.025 - 0.055 | 0.44 (0.16)<br>0.25 - 0.55 | 0.036 (0.0094)<br>0.028 - 0.046 | 0.36 (0.094)<br>0.28 - 0.46 | 0.0075/ 0.0043 |
| **Forage** | 0.034 (0.0020)<br>0.032 - 0.036 | 0.15 (0.0088)<br>0.14 - 0.15 | 0.031 (0.0023)<br>0.028 - 0.033 | 0.13 (0.010)<br>0.12 - 0.14 | 0.0056/ 0.0024 |
| **Grain** | <LOQ (N/A[5])<br>N/A | N/A (N/A)<br>N/A | <LOQ (N/A)<br><LOD-<LOQ | N/A (N/A)<br>N/A | 0.0047 / 0.0024 |

[1]The mean and standard deviation were calculated across sites (n=3 for well-watered and n=3 for water-limited, except silk where n=4 for well-watered and senescent root where n=1 under water-limited).
[2]Minimum and maximum values were determined for each tissue type across sites.
[3]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis.
[4]Protein levels are expressed as µg/g on a dry weight basis. The dry weight values were calculated by dividing the fresh weight by the dry weight conversion factors obtained from moisture analysis data.
[5]N/A – not applicable

## Appendix E. Materials and Methods for Forage and Grain Compositional Analysis

Compositional comparisons between MON 87460 and a conventional control were performed using the principles and analytes outlined in the OECD consensus documents for corn composition (OECD, 2002; 2006). These principles are accepted globally and have been employed previously in assessments of corn products derived through biotechnology. The materials and methods for component analysis, as well as a description of the tissue types, are provided below.

The compositional assessment was conducted on forage and grain samples harvested from two different growing seasons. The first season was conducted in the U.S. during 2006 under typical agronomic practices and water conditions. The second season was conducted in Chile during 2006/2007 using a strip-plot design to establish two water treatment levels (well-watered and water-limited) to assess for any changes in compositional equivalence under different soil moisture conditions. Samples from the Chile 2006/2007 study were also analyzed for 11 additional secondary metabolites that are potentially associated with drought stress. Square brackets in the tables presented in this section denote ranges for the test and control or 99% tolerance intervals for the reference materials. As discussed previously (Section VIII.C, Table VIII-3), the QUI site in the Chile 2006/2007 study was not established with the appropriate water stress treatments; therefore, data and analysis for the QUI site are also presented below in Section E.8.

### E.1. Test, Control and Reference Substances

#### E.1.1. Test Substance

The test substance was MON 87460. Forage and grain tissues of corn MON 87460 were evaluated in this study.

#### E.1.2. Control Substance

The control substance was conventional corn hybrid with genetic background similar to MON 87460. The forage and grain tissues of the control substance were evaluated in this study.

#### E.1.3. Reference Substances

The reference substances were 15 conventional commercial corn hybrids. A single replicate of the forage and grain tissues from each reference substance was evaluated in this study. The following conventional corn hybrids were analyzed:

**Table E-1. Reference Substances for U.S. 2006 Composition Study**

| Material Name | Seed Lot No. | Field Code |
|---|---|---|
| DKC 61-42 | GLP-0603-16998-S | IAE |
| DKC 60-15 | GLP-0604-17072-S | IAE |
| DKC 63-78 | GLP-0604-17073-S | IAE |
| H8991 | GLP-0603-16996-S | IAW |
| DKC 61-50 | GLP-0603-16999-S | IAW |
| 33N29 | GLP-0604-17088-S | IAW |
| 33K39 | GLP-0604-17076-S | IL |
| M-3744 | GLP-0604-17077-S | IL |
| M-3765 | GLP-0604-17078-S | IL |
| BT-6512 | GLP-0604-17079-S | IN |
| B-625 | GLP-0604-17083-S | IN |
| B-645 | GLP-0604-17084-S | IN |
| S-2721 | GLP-0604-17146-S | KS |
| 32B33 | GLP-0604-17147-S | KS |
| 33H25 | GLP-0604-17071-S | KS |
| G-8424 | GLP-0604-17089-S | NE |
| NC+4822 | GLP-0604-17090-S | NE |
| 34N43 | GLP-0604-17091-S | NE |

**Table E-2. Reference Substances for Chile 2006/2007 Composition Study**

| Material Name | Seed Lot No. | Field Code |
|---|---|---|
| 33D11 | GLP-0604-17075-S | CL |
| BT 6011 | GLP-0610-17684-S | CL |
| Garst 8424 | GLP-0610-17687-S | CL |
| DKC62-30 | GLP-0609-17618-S | CL |
| 33N09 | GLP-0610-17691-S | CT |
| 33K39 | GLP-0604-17076-S | CT |
| BT 6613 | GLP-0610-17683-S | CT |
| DKC63-78 | GLP-0609-17613-S | CT |
| 33N29 | GLP-0604-17088-S | LUM |
| Garst 8445 | GLP-0610-17688-S | LUM |
| DKC61-50 | GLP-0609-17612-S | LUM |
| RX 715 | GLP-0609-17615-S | LUM |
| 34N43[1] | GLP-0604-17091-S | QUI[1] |
| BT 6610[1] | GLP-0610-17685-S | QUI[1] |
| Garst 8545[1] | GLP-0609-17689-S | QUI[1] |
| DKC60-15[1] | GLP-0609-17610-S | QUI[1] |

## E.2. Test, Control and Reference Substance Characterization

The identities of the forage and grain samples from each test, control, and reference substance were verified by the Study Director by confirming the chain-of-custody documentation supplied with the forage and grain collected from the plots. The grain of the test, control, and reference substances were also characterized, by event-specific PCR analysis, for the presence of the *cspB* coding region.

## E.3. Field Trial Description

*U.S. 2006*

Seed was planted in the spring of 2006 at six sites (IAE, IAW, IL, IN, KS, and NE) in the United States. Locations of the field sites are as follows: IAE, Benton County, Iowa; IAW, Greene County, Iowa; IL, Stark County, Illinois; IN, Parke County, Indiana; KS, Pawnee County, Kansas, and NE, York County, Nebraska. At each field site, the T/C/R seed starting substances were planted in a randomized complete block design with three replicates per block. Each block (replicate) consisted of five plots with one plot for each test, control, and reference substance. Production was managed according to normal agronomic field practices. Grain and forage samples were harvested from all plots at ambient temperature and forwarded to Monsanto Company (St. Louis, MO). A sub-sample for compositional analysis was obtained from each tissue sample harvested. These sub-samples were then ground and stored in a freezer set to maintain a temperature of -20$_o$C until their shipment on dry ice to Covance Laboratories Inc. (Madison, WI) for analysis.

*Chile 2006/2007*

Seed was planted in the winter of 2006 at four replicated field sites (CL, CT, LUM, and QUI) in Chile. Locations of the field sites are as follows: CL, Colina, Region Metropolitana; CT, Calera de Tango, Region Metropolitana; LUM, Lumbreras, Region Metropolitana, and QUI, Quillota, "V." The test and control substances were grown at all field sites. Four different conventional reference substances were also grown at each of the field sites. The field design incorporated a strip-plot design. The whole plot factor was irrigation treatment. Well-watered was irrigation management for optimal yield. Water-limited was irrigation management to target replacement of 55-65% of water evapotranspiration starting at plant growth stage ~V10 and continuing through ~R2. The design for the whole plot factor was a randomized complete block design. The strip-plot factor consisted of the test, control, and reference substances.

Grain and forage samples were harvested from all plots at ambient temperature and forwarded to Monsanto Company (St. Louis, MO). A sub-sample for compositional analysis was obtained from each tissue sample collected. These sub-samples were then ground and stored in a freezer set to maintain a temperature of -20$^o$C until their shipment on dry ice to Covance Laboratories, Inc. (Madison, WI) for analysis.

## E.4. Analytical Methods

Components assessed in forage samples included proximates (protein, fat, ash, and moisture), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent

fiber (NDF), calcium, and phosphorus. Components assessed in grain samples included proximates (protein, fat, ash, and moisture), carbohydrates by calculation, ADF, NDF, total detergent fiber (TDF), total amino acid composition, fatty acid composition, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc), vitamins (vitamin B1 [thiamine], vitamin B2 [riboflavin], vitamin B6 [pyridoxine], vitamin E, niacin, folic acid), furfural, raffinose, phytic acid, *p*-coumaric acid, and ferulic acid.

All compositional analyses were performed at Covance Laboratories, Inc. (Madison, Wisconsin). Methods for analysis were based on internationally-recognized procedures and literature publications. Brief descriptions of the methods utilized for the analyses are described below.

### E.4.1 Moisture

Sample was dried in a vacuum oven at approximately 100°C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The limit of quantitation was 0.100%.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Methods 926.08 and 925.09, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

### E.4.2 Ash

Sample was placed in an electric furnace at 550°C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The limit of quantitation was 0.100%.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Method 923.03, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

### E.4.3 Protein

Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a mercury catalyst mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25. The limit of quantitation was 0.100%.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Methods 955.04 and 979.09, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

Bradstreet, R. B., *The Kjeldahl Method for Organic Nitrogen*, Academic Press: New York, New York, (1965).

### E.4.4 Fat by Acid Hydrolysis (Forage Analysis)

Forage sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted with ether and hexane. The extract was evaporated on a steam bath, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract

was then evaporated again on a steam bath under nitrogen, dried, and weighed. The limit of quantitation was 0.100%.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Methods 922.06 and 954.02, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

**E.4.5 Fat by Soxhlet Extraction (Grain Analysis)**
The sample was weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed. The limit of quantitation was 0.100%.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Method 960.39 and 948.22, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005)

**E.4.6 Carbohydrate** (CHO)
The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

% carbohydrates = 100 % - (% protein + % fat + % moisture + % ash)

The limit of quantitation was 0.100%.

United States Department of Agriculture, "Energy Value of Foods," *Agriculture Handbook No. 74,* pp. 2-11, (1973).

**E.4.7 Acid Detergent Fiber**
Sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected on the frit and determined gravimetrically. The limit of quantitation was 0.100%.

*Forage and Fiber Analyses*, Agriculture Handbook No.379, United States Department of Agriculture, Washington, D.C. (1970).

**E.4.8 Neutral Detergent Fiber**
Sample was placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected on the frit and determined gravimetrically. The limit of quantitation was 0.100%.

*Approved Methods of the American Association of Cereal Chemists*, 9th Ed.,

Method 32.20, (1998).

*Forage and Fiber Analyses*, Agriculture Handbook No. 379, United States Department of Agriculture, (1970).

### E.4.9 Total Dietary Fiber

Duplicate samples were gelatinized with α-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The samples were filtered, and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for the other. The total dietary fiber in the sample was calculated using the protein and ash values. The limit of quantitation was 1.0%.

*Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 985.29, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

### E.4.10 Mineral Analysis by ICP Emission Spectrometry

The sample was dried, precharred, and ashed overnight in a muffle set to maintain 500°C. The ashed sample was re-ashed with nitric acid, treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured on the inductively coupled plasma spectrometer, with the emission of reference standards.

**Table E-3. Reference Calibration Ranges and Limits of Quantitation**

| Mineral | Reference Calibration Range (µg/ml) | Limit of Quantitation (ppm) |
|---|---|---|
| Calcium | 200, 1000 | 20.0 |
| Copper | 2, 10 | 0.50 |
| Iron | 10, 50 | 2.00 |
| Magnesium | 50, 250 | 20.0 |
| Manganese | 2, 10 | 0.30 |
| Phosphorus | 200, 1000 | 20.0 |
| Potassium | 200, 1000 | 100 |
| Sodium | 200, 1000 | 100 |
| Zinc | 10, 50 | 0.40 |

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Methods 984.27 and 985.01, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

### E.4.11 Amino Acid Composition

Samples were assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using an automated amino acid analyzer. The limit of quantitation was 0.100 mg/g.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Method 982.30, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

### E.4.12 Fatty Acid Composition

The lipid was extracted and saponified with 0.5N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride in methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The limit of quantitation was 0.00400%.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Method 996.06, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

*Official Methods and Recommended Practices of the AOCS*, 5th Ed., Method Ce 1-62, American Oil Chemists' Society: Champaign, Illinois, (1997).

### E.4.13 Folic Acid

Sample was hydrolyzed in a potassium phosphate buffer with the addition of ascorbic acid to protect the folic acid during autoclaving. Following hydrolysis by autoclaving, the sample was treated with a chicken-pancreas enzyme and incubated approximately 18 hours to liberate the bound folic acid. The amount of folic acid was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus casei*, with the growth response of a folic acid standard. This response was measured turbidimetrically. The limit of quantitation was 0.060 µg/g.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Methods 960.46 and 992.05, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

*Methods of Analysis for Infant Formulas*, Infant Formula Council, Atlanta, Georgia, Section C-2, (1985).

### E.4.14 Niacin

Sample was hydrolyzed with sulfuric acid and the pH was adjusted to remove interferences. The amount of niacin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus plantarum,* with the growth response of a niacin standard. This response was measured turbidimetrically. The limit of quantitation was 0.300 µg/g.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Method 944.13, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

### E.4.15 Thiamine Hydrochloride

Sample was autoclaved under weak acid conditions to extract the thiamine. The resulting solution was incubated with a buffered enzyme solution to release any bound thiamine. The solution was purified on a cation-exchange column. An aliquot was reacted with potassium ferricyanide to convert thiamine to thiochrome. The thiochrome was extracted into isobutyl alcohol, measured on a fluorometer, and quantitated by comparison to a known standard. The limit of quantitation was 0.01 mg/100g.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Methods 942.23, 953.17, and 957.17, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

**E.4.16 Vitamin $B_2$ (Riboflavin)**

Sample was hydrolyzed with dilute hydrochloric acid and the pH was adjusted to remove interferences. The amount of riboflavin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus casei*, with the growth response of multipoint riboflavin standards. The growth response was measured turbidimetrically. The limit of quantitation was 0.200 µg/g.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Methods 940.33 and 960.46, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

*The United States Pharmacopeia,* Twenty-Ninth Revision, p. 1913, United States Pharmacopeial Convention, Inc.: Rockville, Maryland, (2005).

**E.4.17 Pyridoxine Hydrochloride**

The sample was hydrolyzed with dilute sulfuric acid in the autoclave and the pH was adjusted to remove interferences. The amount of pyridoxine was determined by comparing the growth response of the sample, using the yeast *Saccharomyces carlsbergensis,* with the growth response of a pyridoxine standard. The response was measured turbidimetrically. The limit of quantitation was 0.070 µg/g.

*Official Methods of Analysis of AOAC INTERNATIONAL,* 18th Ed., Method 961.15, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

Atkins, L., Schultz, A. S., Williams, W. L., and Frey, C. N., "Yeast Microbiological Methods for Determination of Vitamins," *Industrial and Engineering Chemistry, Analytical Edition,* 15:141-144, (1943).

**E.4.18 Vitamin E**

The product was saponified to break down any fat and release vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated by high-performance liquid chromatography using a silica column. The limit of quantitation for this study was approximately 0.500 mg/100g.

Cort, W. M., Vincente, T. S., Waysek, E.H., and Williams, B. D., "Vitamin E Content of Feedstuffs Determined by High-Performance Liquid Chromatographic Fluorescence," *Journal of Agricultural Food Chemistry, 31*:1330-1333, (1983).

Speek, A. J., Schrijver, J., and Schreurs, W. H. P., "Vitamin E Composition of Some Seed Oils as Determined by High-Performance Liquid Chromatography with Fluorometric Quantitation," *Journal of Food Science, 50*(1):121-124, (1985).

McMurray, C. H., Blanchflower, W. J., and Rice, D. A., "Influence of Extraction Techniques on Determination of α-Tocopherol in Animal Feedstuffs," *Journal of the Association of Official Analytical Chemists,* 63(6):1258-1261, (1980).

### E.4.19 p-Coumaric Acid and Ferulic Acid

Sample was extracted with methanol using ultrasonication, hydrolyzed using 4N sodium hydroxide, buffered using acetic acid/sodium hydroxide, acidified with 3N hydrochloric acid, and filtered. The levels of p-coumaric and ferulic acids in the extract were determined by reverse phase high-performance liquid chromatography with ultraviolet detection. The limit of quantitation was approximately 50.0 ppm.

Hagerman, A. E. and Nicholson, R. L., "High-Performance Liquid Chromatographic Determination of Hydroxycinnamic Acids in Maize Mesocotyl," *Journal of Agricultural and Food Chemistry*, 30 (No. 6):1098-1102, (1982).

### E.4.20 Phytic Acid

Sample was extracted using 0.5M HCl with ultrasonication. Purification and concentration were accomplished on a silica-based anion-exchange column. The sample was analyzed on a polymer high-performance liquid chromatography column PRP-1, 5μm (150 x 4.1mm) with a refractive index detector. The limit of quantitation was approximately 0.100%.

Lehrfeld, Jacob, "HPLC Separation and Quantitation of Phytic Acid and Some Inositol Phosphates in Foods: Problem and Solutions," *Journal of Agricultural and Food Chemistry*, 42:2726-2731, (1994).

Lehrfeld, Jacob, "High-Performance Liquid Chromatography Analysis of Phytic Acid on a pH-Stable, Macroporous Polymer Column," *Cereal Chemistry*, 66(6):510-515, (1989).

### E.4.21 Raffinose

Sample was extracted with deionized water and the extract treated with a hydroxylamine hydrochloride solution in pyridine, containing phenyl-β-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography using a flame ionization detector. The limit of quantitation was 0.0500%.

Brobst, K. M., "Gas-Liquid Chromatography of Trimethylsilyl Derivatives," *Methods in Carbohydrate Chemistry*, Volume 6, Academic Press: New York, New York, (1972).

Mason, B. S., and Slover, H. T., "A Gas Chromatographic Method for the Determination of Sugars in Foods," *Journal of Agricultural and Food Chemistry*, 19(3):551-554, (1971).

### E.4.22 2-Furaldehyde (Furfural)

Ground sample was extracted with 4% trichloroacetic acid and injected directly on a high-performance liquid chromatography system for quantitation of free furfurals by ultraviolet detection. The limit of quantitation was 0.500 ppm.

Albala-Hurtado S., Veciana-Nogues, M. T., Izquierdo-Pulido, M., and Vidal-Carou, M. C., "Determination of Free and Total Furfural Compounds In Infant Milk Formulas

By High-Performance Liquid Chromatography," *Journal of Agricultural and Food Chemistry*, 45:2128-2133, (1997).

**E.4.23 Sugar and Sugar Alcohols** (SGAL)

Sugars and sugar alcohols were extracted from the sample with water. Aliquots were dried under inert gas and reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl- β -D-glucoside as the internal standard. The resulting oximes were converted to silyl derivatives with hexamethyldisilazane (HMDS) and trifluoracetic acid (TFA) treatment and analyzed by gas chromatography using a flame ionization detector. The limit of quantitation for this study was 0.0500%.

Mason, B. S. and Slover, H. T., "A Gas Chromatographic Method for the Determination of Sugars in Foods," Journal of Agricultural and Food Chemistry, 1971.

Brosbt, K., "Gas Liquid Chromatography of Trimethylsilyl Derivations," Methods in Carbohydrate Chemistry, 6:3-8, Academic Press, New York, NY. 1972.

**E.4.24 Free Proline**

The sample was extracted in acid. Determination was by high-performance liquid chromatography (HPLC) with fluorescence or diode array detection. Primary amino acids were derivatized with o-phthalaldehyde and the secondary amino acids were derivatized with fluorenylmethyl chloroformate before injection. The limit of quantitation for this study was 0.0100 mg/g.

R. Schuster, "Determination of Amino Acids in Biological, Pharmaceutical, Plant and Food Samples by Automated Precolumn Derivatization and HPLC," Journal of Chromatography, 1988, 431, 271-284

Henderson, J. W., Ricker, R. D., Bidlingmeyer, B. A., Woodward, C., "Rapid Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids, Amino Acid Analysis Using Zorbax Eclipse-AAA columns and the Agilent 1100 HPLC," Agilent Publication, 2000.

**E.4.25 Glycerol**

Glycerol was extracted from the sample with water. A portion of the extract was passed through glass microfiber filter paper and an appropriate dilution was made. The sample was injected onto a high performance anion exchange chromatograph (HPAEC) equipped with a Pulsed Amperometric Detector (PAD). The amount of glycerol present was quantitated relative to an external standard curve using regression analysis. The limit of quantitation for this study was 20 ppm.

Hanko, V. P. and Rohrer, J. S., "Determination of Carbohydrates, Sugar Alcohols, and Glycols in Cell Cultures and Fermentation Broths Using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection", Analytical Biochemistry, 283:192-199, (2000).

### E.4.26 Glycine Betaine, Choline, Salicylic acid and Abscisic Acid

Internal standard and extraction solvent (0.1% formic acid in 50:50 methanol:water) were added to the sample. After centrifugation and filtration samples were analyzed by Liquid chromatography using MS/MS for detection. Specific precursor-fragment transitions were monitored for each analyte using the multiple reaction monitoring (MRM) technique. The analytes were identified by comparison to reference standards using the retention time of the specific precursor-fragment response.

## E.5. Control of Bias

The test, control, and reference substances from each respective plot within the field sites were produced under similar agronomic conditions. To control and/or minimize bias, the samples were analyzed in the order specified by a computer-generated randomized sample list. The Study Director generated the randomized sample list and forwarded it to Covance Laboratories, Inc. prior to analysis.

## E.6. Statistical Analysis

### E.6.1. Data Processing

After compositional analyses were performed at Covance Laboratories, Inc., data spreadsheets were sent to Monsanto Company. The data were reviewed, formatted, and sent to Certus International, Inc. for statistical analysis. A statistical sub-report was generated by Certus and sent to Monsanto Company. The following formulas were used for re-expression of the data for statistical analysis:

**Table E-4. Unit Conversions**

| Component | From (X) | To | Formula |
|---|---|---|---|
| Proximates (excluding moisture), Fiber, Anti-nutrients | % FW | % DW | X/d |
| Calcium, Phosphorus, Magnesium, Potassium, Sodium | ppm FW | % DW | $(X/d) \times 10^{-4}$ |
| Copper, Iron, Manganese, Zinc | ppm FW | mg/kg DW | X/d |
| Secondary Metabolites | ppm FW | µg/g DW | X/d |
| Thiamine HCl | mg/100g FW | mg/kg DW | 10 (X/d) |
| Vitamin E | mg/g FW | mg/kg DW | $10^3$ (X/d) |
| Folic Acid, Niacin, Riboflavin, Pyridoxine HCl/Vitamin B6 | µg/g FW | mg/kg DW | X/d |
| Amino Acids (AA) | mg/g FW | % DW | X/(10*d) |
| Fatty Acids (FA) | % FW | % Total FA | $(100)X_j/\Sigma X$, for each $FA_j$ where $\Sigma X$ is over all the FA |
| 'X' is the individual sample value; 'd' is the fraction of the sample that is dry matter. | | | |

#### E.6.1.1. U.S. 2006 Data Processing

In order to complete a statistical analysis for a compositional constituent in this study, at least 50% of the values for an analyte had to be greater than the assay LOQ. Analytes with greater than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following 15 analytes with greater than 50% of observations

below the assay LOQ were excluded from statistical analysis: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid, sodium, and furfural. These components naturally occur at very low levels in corn.

For individual measurements below the assay's LOQ, where fewer than 50% of the total values were below the LOQ, results were assigned a value equal to half the quantitation limit. The following analytes were assigned values:

| | | Obs. Below LOQ | | | | |
|---|---|---|---|---|---|---|
| **Component** | **Units** | **N** | **(%)** | **Total N** | **LOQ** | **Value Assigned** |
| **Grain Fatty Acid** | | | | | | |
| 16:1 Palmitoleic | % FW | 13 | 24.1 | 54 | 0.0040 | 0.0020 |
| 22:0 Behenic | % FW | 1 | 1.9 | 54 | 0.0040 | 0.0020 |

The data were assessed for potential outliers using a studentized PRESS residuals calculation. A predicted residual sums of squares (PRESS) residual is the difference between any value and its predicted value from a statistical model that excludes the datum point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Extreme data points that are also outside of the ± 6 studentized PRESS residual range are considered for exclusion, as outliers, from the final analyses. For this study, no results had a PRESS residual value outside of the ± 6 studentized PRESS residual range.

### E.6.1.2. Chile 2006/2007 Data Processing

In order to complete a statistical analysis for a compositional constituent in this study, at least 50% of the values for an analyte had to be greater than the assay LOQ. Analytes with more than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following 16 analytes with more than 50% of observations below the assay LOQ were excluded from statistical analysis: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, sodium, and furfural.

Otherwise, results below the LOQ were assigned a value equal to half the quantitation limit. The following analytes were assigned values:

| | | Obs. Below LOQ | | | | |
|---|---|---|---|---|---|---|
| **Component** | **Units** | **N** | **(%)** | **Total N** | **LOQ** | **Value Assigned** |
| **Forage Proximate** | | | | | | |
| Total Fat | % FW | 9 | 7.9 | 114 | 0.10 | 0.050 |
| **Grain Fatty Acid** | | | | | | |
| 22:0 Behenic | % FW | 30 | 26.5 | 113 | 0.0040 | 0.0020 |
| **Grain Vitamin** | | | | | | |
| Vitamin E | mg/g FW | 5 | 4.4 | 113 | 0.0050 | 0.0025 |
| **Grain Anti-nutrients** | | | | | | |
| Raffinose | % FW | 2 | 1.8 | 113 | 0.050 | 0.025 |

Individual samples assigned a value are represented in Listing 2 of the Statistical Sub-report.

PRESS residuals were used to identify outliers. A PRESS residual is the difference between any value and its value predicted from a statistical model that excludes the datum point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Extreme datum points that are also outside of the ± 6 studentized PRESS residual range are considered for exclusion, as outliers, from the final analyses. The following result had a PRESS residual value outside of ± 6 range:

| **Site** | **Rep** | **Description** | **Analyte** | **ID** | **Sent Value** | **Value** | **PRESS Std Residual** |
|---|---|---|---|---|---|---|---|
| **Grain Mineral** | | | | | | | |
| CL | 1 | DM1718 | Copper | 0645B302-00804 | 12 | 13.5287 | 17.1470 |

The copper value was considered an outlier and was removed from further analysis. The outlier test procedure was reapplied to all remaining copper data to detect potential outliers that were masked in the first analysis. No further PRESS residuals were outside of ± 6 range.

### E.6.2. Statistical Methodology for U.S. 2006

At the field sites, the test, control, and reference substances were grown in single plots randomly assigned within each of three replication blocks. The compositional components for the test and control substances were statistically analyzed using a mixed model analysis of variance. The data from the six replicated sites were analyzed separately and as a combined data set.

Individual replicated site analyses used the model:

$$Y_{ij} = U + T_i + B_j + e_{ij} ,$$

where $Y_{ij}$ = unique individual observation, U = overall mean, $T_i$ = hybrid effect, $B_j$ = random block effect, and $e_{ij}$ = residual error.

Combined site analyses used the model:

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk},$$

where $Y_{ijk}$ = unique individual observation, U = overall mean, $T_i$ = hybrid effect, $L_j$ = random location effect, $B(L)_{jk}$ = random block within location effect, $LT_{ij}$ = random location by hybrid interaction effect, and $e_{ijk}$ = residual error. For each compositional component, the forage and grain from the test substance was compared to the conventional control.

A range of observed values from the reference substances was determined for each analytical component. Additionally, the reference substances data were used to develop population tolerance intervals. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial references (George et al., 2004; Ridley et al., 2002). Each tolerance interval estimate was based upon one observation per unique reference substance. Individual substances with multiple observations were summarized within sites to obtain a single estimate for inclusion in tolerance interval calculations. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero. SAS® software was used to generate all summary statistics and perform all analyses (SAS® Software Release 9.1, 2002-2003). Report tables present p-values from SAS® as either <0.001 or the actual value truncated to three decimal places.

### E.6.3. Statistical Methodology for Chile 2006/2007

All T/C/R substances were grown in single plots randomly assigned within each of three replication blocks. All corn compositional analysis components were statistically analyzed using a mixed model analysis of variance. The three replicated sites were analyzed both separately and combined. Individual replicated site analyses used model (1).

*(1)* $$Y_{ijk} = U + B_i + T_j + BT_{ij} + S_k + BS_{ik} + TS_{jk} + e_{ijk},$$

where $Y_{ijk}$ = unique individual observation, U = overall mean, $B_i$ = random block effect, $T_j$ = irrigation treatment effect, $BT_{ij}$ = random block by treatment interaction effect, $S_k$ = substance effect, $BS_{ik}$ = random block by substance effect, $TS_{jk}$ = treatment by substance interaction effect and $e_{ijk}$ = residual error.

Combined site analyses used model (2).

*(2)* $Y_{ijkl} = U + L_i + B(L)_{ij} + T_k + LT_{ik} + TB(L)_{ijk} + S_l + SB(L)_{ijl} + TS_{kl} + LS_{il} + LTS_{ikl} + e_{ijkl}$,

where $Y_{ijkl}$ = unique individual observation, U = overall mean, $L_i$ = random location effect, $B(L)_{ij}$ = random block within location effect, $T_k$ = irrigation treatment effect, $LT_{ik}$ = random location by treatment interaction effect,
$TB(L)_{ijk}$ = random treatment by block within location interaction effect,
$S_l$ = substance effect, $SB(L)_{ijl}$ = random substance by block within location interaction effect, $TS_{kl}$ = treatment by substance interaction effect,
$LS_{il}$ = random location by substance interaction effect, $LTS_{ikl}$ = random location by treatment by substance interaction effect and $e_{ijkl}$ = residual error.

For each component analysis, mean comparison tests of each test substance versus the conventional control substance within each irrigation treatment were conducted.

A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component within each irrigation treatment, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial conventional substances. Each tolerance interval estimate was based upon one observation per unique reference substance within each treatment. For each treatment, data were first summarized by substance within site and then by substance across sites. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

SAS® programming was used to generate all summary statistics and perform all analyses (Version 9.1.3, SAS Institute, Inc. 2002-2003). Report tables present p-values from SAS as either <0.001 or the actual value truncated to three decimal places.

## E.7. References

AACC. Method 32.20. 1998. Approved Methods of the American Association of Cereal Chemists, 9th Ed.

Albala-Hurtado S., M.T. Veciana-Nogues, M. Izquierdo-Pulido and M.C. Vidal-Carou. 1997. Determination of free and total furfural compounds in infant milk formulas by high-performance liquid chromatography. Journal of Agricultural and Food Chemistry. 45:2128-2133.

AOAC. International Methods 922.06 and 954.02. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 923.03. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

® SAS is a registered trademark of SAS Institute Inc.

AOAC. International Methods 926.08 and 925.09. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 940.33 and 960.46. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 942.23, 953.17 and 957.17. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 944.13. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC International Methods 955.04 and 979.09. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 960.39 and 948.22. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 960.46 and 992.05. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 961.15. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 982.30. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 984.27 and 985.01. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 985.29. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOAC. International Methods 996.06. 2005. Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOCS. Method Ce 1-62. 1997. Official Methods and Recommended Practices of the American Oil Chemists Society, 5th Ed. American Oil Chemists Society, Champaign, IL.

Atkins, L., A.S. Schultz, W.L. Williams, and C.N. Frey. 1943. "Yeast Microbiological Methods for Determination of Vitamins," Industrial and Engineering Chemistry, Analytical Edition. 15:141-144.

Bradstreet, R. B. 1965. Pages 97-100 in The Kjeldahl Method for Organic Nitrogen. Academic Press, New York, NY.

Brobst, K. M. 1972. Gas-liquid chromatography of trimethylsilyl derivatives. Analysis of corn syrup. Pages 3-8 in Methods in Carbohydrate Chemistry. Vol. 6. Academic Press, New York, NY.

Cort, W.M., T.S. Vincente, E.H. Waysek and B.D. Williams. 1983. Vitamin E content of feedstuffs determined by high-performance liquid chromatographic fluorescence. Journal of Agricultural and Food Chemistry. 31:1330-1333.

George, C., W.P. Ridley, J.C. Obert, M.A. Nemeth, M.L. Breeze, and J.D. Astwood. 2004. Composition of grain and forage from corn rootworm-protected corn event MON 863 is equivalent to that of conventional corn (*Zea mays* L.). Journal of Agricultural and Food Chemistry. 52:4149-4158.

Hagerman, A.E. and R. L. Nicholson. 1982. High-performance liquid chromatographic determination of hydroxycinnamic acids in the maize mesocotyl. Journal of Agricultural and Food Chemistry. 30:1098-1102.

Hanko, V.P. and J.A. Rohrer. 2000. Determination of carbohydrates, sugar alcohols, and glycols in cell cultures and fermentation broths using high-performance anion-exchange chromatography with pulsed amperometric detection. Analytical Biochemistry. 283:192-199.

Henderson, J.W., R.D. Ricker, B.A., Bidlingmeyer, C. Woodward. 2000. Rapid Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids, Amino Acid Analysis Using Zorbax Eclipse-AAA columns and the Agilent 1100 HPLC. Agilent Publication.

Lehrfeld, J. 1989. High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. Cereal Chemistry. 66:510-515.

Lehrfeld, J. 1994. HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: problem and solutions Journal of Agricultural and Food Chemistry. 42:2726-2731.

Mason, B.S. and H.T. Slover. 1971. A gas chromatographic method for the determination of sugars in foods. Journal of Agricultural and Food Chemistry. 19:551-554.

McMurray, C.H., W.J. Blanchflower and D.A. Rice. 1980. Influence of extraction techniques on determination of α-tocopherol in animal feedstuffs. Journal-Association of Official Analytical Chemists. 63:1258-1261.

Methods of Analysis for Infant Formulas. Section C-2. 1985. Infant Formula Council, Atlanta, GA.

OECD. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, antinutrients and secondary plant metabolites. Organisation for Economic Co-operation and Development. Paris, France.

OECD, 2006. An Introduction to the Food/Feed Safety Consensus Documents of the Task Force. Organisation for Economic Co-operation and Development. Paris, France.

Ridley, W.P., R.S. Sidhu, P.D. Pyla, M.A. Nemeth, M.L. Breeze, and J.D. Astwood. 2002. Comparison of the nutritional profile of glyphosate-tolerant corn event NK603 with that of conventional corn (*Zea mays* L.). Journal of Agricultural and Food Chemistry. 50:7235-7243.

Schuster, R. 1988. Determination of amino acids in biological, pharmaceutical, plant and food samples by automated precolumn derivatization and HPLC. Journal of Chromatography. 431:271-284.

Speek, A.J., J. Schrijver, and W.H.P. Schreurs. 1985. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric detection. Journal of Food Science. 50:121-124.

The United States Pharmacopeia. 2005. Twenty-Ninth Revision, p. 1913, United States Pharmacopeial Convention, Inc.: Rockville, Maryland.

USDA. 1970. Forage and Fiber Analysis. Agriculture Handbook No. 379. United States Department of Agriculture, Washington, D.C.

USDA. 1973. Energy value of foods. Pages 2-11 in Agriculture Handbook No. 74. United States Department of Agriculture, Washington, D.C.

**Table E-5. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 24.10 (0.96) [17.78 - 34.43] | 24.64 (0.96) [19.11 - 29.21] | -0.54 (0.92) [-6.50 - 8.60] | -2.49, 1.41 | 0.567 | (19.44 - 30.49) [13.04, 35.77] |
| Neutral Detergent Fiber (% DW) | 38.69 (1.99) [31.10 - 49.44] | 38.75 (1.99) [27.73 - 48.35] | -0.056 (1.08) [-11.60 - 6.51] | -2.33, 2.22 | 0.959 | (32.12 - 49.62) [24.23, 56.48] |
| **Mineral** | | | | | | |
| Calcium (% DW) | 0.21 (0.018) [0.14 - 0.30] | 0.22 (0.018) [0.13 - 0.33] | -0.018 (0.012) [-0.075 - 0.089] | -0.048, 0.013 | 0.189 | (0.12 - 0.25) [0.044, 0.35] |
| Phosphorus (% DW) | 0.18 (0.0089) [0.14 - 0.22] | 0.19 (0.0089) [0.14 - 0.23] | -0.0095 (0.0055) [-0.069 - 0.022] | -0.021, 0.0021 | 0.101 | (0.090 - 0.26) [0.074, 0.32] |
| **Proximate** | | | | | | |
| Ash (% DW) | 3.76 (0.35) [2.17 - 5.34] | 4.21 (0.35) [2.94 - 8.01] | -0.44 (0.37) [-3.73 - 1.22] | -1.39, 0.50 | 0.281 | (2.67 - 4.43) [1.52, 5.75] |
| Carbohydrates (% DW) | 86.45 (0.54) [83.78 - 88.75] | 85.77 (0.54) [81.88 - 89.26] | 0.68 (0.49) [-2.08 - 2.89] | -0.57, 1.93 | 0.220 | (84.97 - 88.89) [82.09, 90.80] |
| Moisture (% FW) | 70.94 (1.25) [64.70 - 77.90] | 71.46 (1.25) [66.50 - 75.70] | -0.52 (0.37) [-2.50 - 2.40] | -1.30, 0.25 | 0.174 | (64.20 - 75.50) [59.32, 81.14] |
| Protein (% DW) | 7.56 (0.25) [6.65 - 8.57] | 7.85 (0.25) [6.45 - 10.24] | -0.30 (0.20) [-2.63 - 1.14] | -0.71, 0.12 | 0.146 | (5.80 - 8.63) [4.92, 10.30] |
| Total Fat (% DW) | 2.23 (0.20) [1.07 - 3.24] | 2.17 (0.20) [1.28 - 2.88] | 0.059 (0.13) [-0.88 - 0.90] | -0.22, 0.34 | 0.659 | (1.60 - 3.62) [0, 4.67] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-6. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| Analytical Component[1] | Test Mean ± S.E. [Range] | Control Mean ± S.E. [1] [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Proximate** | | | | | | |
| Ash (% DW) | 1.54 (0.039) [1.33 - 1.83] | 1.46 (0.039) [1.32 - 1.79] | 0.082 (0.038) [-0.22 - 0.38] | 0.0033, 0.16 | 0.041 | (1.17 - 2.01) [0.55, 2.30] |
| Carbohydrates (% DW) | 84.22 (0.56) [81.40 - 87.04] | 84.10 (0.56) [81.31 - 86.05] | 0.13 (0.20) [-1.57 - 1.98] | -0.30, 0.56 | 0.539 | (82.11 - 87.06) [80.32, 89.92] |
| Moisture (% FW) | 9.94 (0.18) [9.12 - 11.00] | 10.09 (0.18) [9.17 - 11.20] | -0.15 (0.15) [-1.36 - 0.83] | -0.54, 0.25 | 0.377 | (8.74 - 11.30) [7.58, 12.13] |
| Protein (% DW) | 10.50 (0.54) [8.19 - 13.21] | 10.74 (0.54) [8.77 - 13.33] | -0.24 (0.18) [-2.05 - 1.35] | -0.61, 0.13 | 0.195 | (8.27 - 11.50) [6.26, 13.45] |
| Total Fat (% DW) | 3.74 (0.051) [3.44 - 4.06] | 3.71 (0.051) [3.57 - 3.96] | 0.029 (0.067) [-0.52 - 0.32] | -0.14, 0.20 | 0.678 | (2.95 - 4.40) [2.08, 5.12] |
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 3.03 (0.25) [1.57 - 4.94] | 3.02 (0.25) [1.94 - 4.08] | 0.0095 (0.36) [-2.51 - 3.00] | -0.79, 0.81 | 0.979 | (1.82 - 4.48) [0.62, 5.72] |
| Neutral Detergent Fiber (% DW) | 8.97 (0.32) [6.45 - 11.63] | 8.95 (0.32) [7.82 - 12.22] | 0.019 (0.46) [-4.07 - 3.32] | -1.00, 1.03 | 0.967 | (6.51 - 12.28) [3.45, 15.08] |
| Total Dietary Fiber (% DW) | 12.59 (0.34) [10.42 - 14.57] | 12.15 (0.34) [10.76 - 14.87] | 0.44 (0.35) [-3.32 - 3.67] | -0.28, 1.16 | 0.216 | (10.65 - 16.26) [8.11, 17.95] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-7. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
| Calcium (% DW) | 0.0054 (0.00019) [0.0047 - 0.0061] | 0.0054 (0.00019) [0.0048 - 0.0063] | -0.00006 (0.00007) [-0.00059 - 0.00056] | -0.00020, 0.00009 | 0.431 | (0.0036 - 0.0068) [0.0019, 0.0076] |
| Copper (mg/kg DW) | 1.89 (0.14) [1.47 - 4.61] | 1.86 (0.14) [1.54 - 3.43] | 0.022 (0.16) [-1.31 - 2.11] | -0.32, 0.37 | 0.892 | (1.14 - 2.56) [0.39, 3.21] |
| Iron (mg/kg DW) | 18.24 (0.62) [15.02 - 24.88] | 18.30 (0.62) [14.17 - 20.58] | -0.067 (0.50) [-2.34 - 7.02] | -1.34, 1.21 | 0.898 | (16.89 - 23.40) [13.28, 26.47] |
| Magnesium (% DW) | 0.11 (0.0042) [0.095 - 0.13] | 0.12 (0.0042) [0.095 - 0.13] | -0.0013 (0.0016) [-0.010 - 0.013] | -0.0047, 0.0020 | 0.418 | (0.091 - 0.14) [0.059, 0.16] |
| Manganese (mg/kg DW) | 6.79 (0.43) [5.02 - 8.64] | 6.89 (0.43) [5.50 - 8.34] | -0.097 (0.12) [-0.97 - 0.68] | -0.41, 0.22 | 0.462 | (4.83 - 8.05) [2.27, 9.92] |
| Phosphorus (% DW) | 0.31 (0.011) [0.27 - 0.35] | 0.32 (0.011) [0.27 - 0.37] | -0.0085 (0.0047) [-0.030 - 0.034] | -0.018, 0.0014 | 0.089 | (0.24 - 0.36) [0.20, 0.40] |
| Potassium (% DW) | 0.38 (0.0030) [0.36 - 0.39] | 0.38 (0.0030) [0.35 - 0.39] | 0.0019 (0.0037) [-0.025 - 0.038] | -0.0060, 0.0097 | 0.624 | (0.29 - 0.37) [0.26, 0.42] |
| Zinc (mg/kg DW) | 20.86 (0.95) [18.24 - 24.75] | 21.24 (0.95) [17.41 - 25.20] | -0.38 (0.32) [-3.02 - 1.85] | -1.03, 0.27 | 0.238 | (16.78 - 28.17) [11.61, 32.63] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-8. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| **Analytical Component[1]** | **Test Mean ± S.E. [1] [Range]** | **Control Mean ± S.E. [Range]** | **Mean ± S.E. [Range]** | **95% CI[1] (Lower,Upper)** | **p-Value** | **Commercial (Range) [99% Tolerance Int.[2]]** |
| Alanine (% DW) | 0.80 (0.047)<br>[0.60 - 1.04] | 0.82 (0.047)<br>[0.64 - 1.04] | -0.011 (0.013)<br>[-0.10 - 0.10] | -0.037, 0.016 | 0.410 | (0.60 - 0.91)<br>[0.43, 1.08] |
| Arginine (% DW) | 0.45 (0.019)<br>[0.33 - 0.54] | 0.44 (0.019)<br>[0.38 - 0.52] | 0.0067 (0.011)<br>[-0.071 - 0.087] | -0.022, 0.035 | 0.577 | (0.34 - 0.51)<br>[0.24, 0.60] |
| Aspartic acid (% DW) | 0.65 (0.028)<br>[0.52 - 0.79] | 0.66 (0.028)<br>[0.54 - 0.78] | -0.0062 (0.0075)<br>[-0.065 - 0.060] | -0.022, 0.0096 | 0.419 | (0.52 - 0.72)<br>[0.39, 0.84] |
| Cystine (% DW) | 0.23 (0.0085)<br>[0.19 - 0.27] | 0.23 (0.0085)<br>[0.20 - 0.26] | -0.0040 (0.0018)<br>[-0.016 - 0.012] | -0.0087, 0.00069 | 0.079 | (0.19 - 0.24)<br>[0.15, 0.27] |
| Glutamic acid (% DW) | 2.07 (0.12)<br>[1.52 - 2.66] | 2.09 (0.12)<br>[1.64 - 2.67] | -0.025 (0.034)<br>[-0.26 - 0.28] | -0.097, 0.046 | 0.462 | (1.54 - 2.32)<br>[1.06, 2.76] |
| Glycine (% DW) | 0.39 (0.013)<br>[0.33 - 0.45] | 0.39 (0.013)<br>[0.34 - 0.43] | 0.0019 (0.0041)<br>[-0.024 - 0.035] | -0.0085, 0.012 | 0.656 | (0.33 - 0.42)<br>[0.26, 0.47] |
| Histidine (% DW) | 0.32 (0.012)<br>[0.25 - 0.38] | 0.32 (0.012)<br>[0.27 - 0.37] | -0.00085 (0.0049)<br>[-0.029 - 0.040] | -0.013, 0.012 | 0.868 | (0.25 - 0.33)<br>[0.20, 0.36] |
| Isoleucine (% DW) | 0.38 (0.021)<br>[0.28 - 0.50] | 0.38 (0.021)<br>[0.31 - 0.48] | -0.0022 (0.0088)<br>[-0.042 - 0.070] | -0.025, 0.020 | 0.810 | (0.30 - 0.41)<br>[0.22, 0.49] |

**Table E-8 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Leucine (% DW) | 1.41 (0.088) [1.01 - 1.85] | 1.43 (0.088) [1.11 - 1.87] | -0.020 (0.026) [-0.20 - 0.22] | -0.075, 0.035 | 0.453 | (1.02 - 1.55) [0.68, 1.90] |
| Lysine (% DW) | 0.30 (0.0076) [0.26 - 0.34] | 0.30 (0.0076) [0.26 - 0.33] | 0.0024 (0.0040) [-0.023 - 0.027] | -0.0080, 0.013 | 0.579 | (0.27 - 0.32) [0.22, 0.36] |
| Methionine (% DW) | 0.22 (0.013) [0.16 - 0.28] | 0.22 (0.013) [0.17 - 0.26] | -0.00089 (0.0030) [-0.019 - 0.019] | -0.0086, 0.0068 | 0.777 | (0.17 - 0.24) [0.14, 0.28] |
| Phenylalanine (% DW) | 0.56 (0.031) [0.41 - 0.72] | 0.56 (0.031) [0.45 - 0.72] | -0.0059 (0.0090) [-0.067 - 0.074] | -0.025, 0.013 | 0.518 | (0.43 - 0.61) [0.30, 0.74] |
| Proline (% DW) | 1.01 (0.047) [0.78 - 1.23] | 1.02 (0.047) [0.83 - 1.21] | -0.0048 (0.017) [-0.082 - 0.17] | -0.050, 0.040 | 0.793 | (0.74 - 1.01) [0.56, 1.19] |
| Serine (% DW) | 0.53 (0.028) [0.40 - 0.64] | 0.53 (0.028) [0.43 - 0.67] | -0.0070 (0.0086) [-0.089 - 0.046] | -0.025, 0.011 | 0.430 | (0.39 - 0.60) [0.27, 0.70] |
| Threonine (% DW) | 0.37 (0.016) [0.30 - 0.45] | 0.37 (0.016) [0.31 - 0.45] | -0.00059 (0.0050) [-0.036 - 0.037] | -0.011, 0.010 | 0.908 | (0.29 - 0.40) [0.22, 0.46] |
| Tryptophan (% DW) | 0.066 (0.0027) [0.054 - 0.088] | 0.068 (0.0027) [0.055 - 0.085] | -0.0015 (0.0017) [-0.014 - 0.016] | -0.0050, 0.0021 | 0.394 | (0.047 - 0.070) [0.037, 0.081] |

**Table E-8 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Tyrosine (% DW) | 0.32 (0.024) [0.16 - 0.43] | 0.30 (0.024) [0.15 - 0.43] | 0.014 (0.022) [-0.12 - 0.21] | -0.044, 0.071 | 0.565 | (0.13 - 0.37) [0.0046, 0.54] |
| Valine (% DW) | 0.52 (0.024) [0.40 - 0.64] | 0.52 (0.024) [0.43 - 0.62] | -0.0019 (0.011) [-0.053 - 0.079] | -0.029, 0.026 | 0.866 | (0.42 - 0.54) [0.33, 0.62] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-9. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
| 16:0 Palmitic (% Total FA) | 12.12 (0.20) [11.60 - 15.21] | 11.94 (0.20) [11.45 - 12.38] | 0.18 (0.19) [-0.28 - 2.84] | -0.31, 0.66 | 0.394 | (8.80 - 13.33) [6.35, 16.03] |
| 16:1 Palmitoleic (% Total FA) | 0.17 (0.0073) [0.15 - 0.20] | 0.17 (0.0073) [0.15 - 0.23] | -0.0020 (0.0036) [-0.042 - 0.015] | -0.0095, 0.0055 | 0.576 | (0.059 - 0.15) [0, 0.21] |
| 18:0 Stearic (% Total FA) | 2.05 (0.033) [1.88 - 2.34] | 1.98 (0.033) [1.80 - 2.10] | 0.069 (0.022) [-0.041 - 0.33] | 0.013, 0.13 | 0.024 | (1.36 - 2.14) [1.00, 2.51] |
| 18:1 Oleic (% Total FA) | 20.26 (0.18) [19.32 - 21.08] | 20.49 (0.18) [19.50 - 21.77] | -0.23 (0.12) [-1.13 - 0.85] | -0.48, 0.015 | 0.064 | (21.17 - 33.71) [11.92, 39.78] |
| 18:2 Linoleic (% Total FA) | 63.34 (0.35) [59.90 - 65.07] | 63.34 (0.35) [61.88 - 64.70] | -0.0079 (0.26) [-3.07 - 1.05] | -0.67, 0.65 | 0.976 | (49.31 - 62.94) [45.91, 72.47] |
| 18:3 Linolenic (% Total FA) | 1.28 (0.012) [1.17 - 1.46] | 1.27 (0.012) [1.22 - 1.33] | 0.0066 (0.015) [-0.046 - 0.20] | -0.029, 0.042 | 0.673 | (0.89 - 1.56) [0.39, 1.85] |
| 20:0 Arachidic (% Total FA) | 0.41 (0.0078) [0.39 - 0.44] | 0.41 (0.0078) [0.37 - 0.45] | 0.0037 (0.0032) [-0.017 - 0.024] | -0.0031, 0.010 | 0.263 | (0.30 - 0.49) [0.23, 0.56] |
| 20:1 Eicosenoic (% Total FA) | 0.18 (0.0024) [0.17 - 0.19] | 0.19 (0.0024) [0.17 - 0.22] | -0.0078 (0.0027) [-0.035 - 0.0086] | -0.013, -0.0022 | 0.007 | (0.20 - 0.29) [0.15, 0.33] |
| 22:0 Behenic (% Total FA) | 0.20 (0.012) [0.14 - 0.27] | 0.20 (0.012) [0.14 - 0.27] | -0.0044 (0.013) [-0.099 - 0.078] | -0.037, 0.028 | 0.742 | (0.069 - 0.28) [0, 0.37] |

[1]FA = fatty acid S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-10. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| **Analytical Component[1]** | **Test Mean ± S.E. [1] [Range]** | **Control Mean ± S.E. [Range]** | **Mean ± S.E. [Range]** | **95% CI[1] (Lower,Upper)** | **p-Value** | **Commercial (Range) [99% Tolerance Int.[2]]** |
| Folic Acid (mg/kg DW) | 0.30 (0.012) | 0.30 (0.012) | 0.0058 (0.0059) | -0.0094, 0.021 | 0.371 | (0.19 - 0.31) |
| | [0.25 - 0.36] | [0.24 - 0.35] | [-0.033 - 0.041] | | | [0.13, 0.38] |
| Niacin (mg/kg DW) | 18.59 (0.77) | 18.52 (0.77) | 0.069 (0.53) | -1.29, 1.42 | 0.901 | (15.07 - 32.38) |
| | [15.53 - 22.23] | [15.26 - 21.85] | [-3.73 - 4.43] | | | [4.67, 36.68] |
| Thiamine HCl Vitamin B1 (mg/kg DW) | 3.31 (0.16) | 3.21 (0.16) | 0.094 (0.066) | -0.077, 0.26 | 0.216 | (2.43 - 4.17) |
| | [2.67 - 3.89] | [2.33 - 3.89] | [-0.44 - 0.54] | | | [1.84, 4.94] |
| Riboflavin/Vitamin B2 (mg/kg DW) | 1.54 (0.084) | 1.44 (0.084) | 0.10 (0.097) | -0.15, 0.36 | 0.331 | (0.95 - 2.42) |
| | [0.95 - 2.04] | [0.94 - 1.94] | [-0.88 - 0.60] | | | [0.047, 2.91] |
| Pyridoxine HCl/ Vitamin B6 (mg/kg DW) | 6.10 (0.25) | 6.24 (0.25) | -0.13 (0.17) | -0.48, 0.21 | 0.436 | (4.93 - 7.53) |
| | [5.03 - 7.49] | [5.21 - 7.41] | [-1.55 - 1.66] | | | [3.12, 8.09] |
| Vitamin E (mg/kg DW) | 14.73 (0.80) | 14.69 (0.80) | 0.045 (0.53) | -1.31, 1.40 | 0.935 | (5.96 - 17.70) |
| | [11.09 - 20.02] | [9.47 - 18.44] | [-3.95 - 4.77] | | | [0, 26.07] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-11. Comparison of the Anti-nutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control for Combined Sites in the U.S. during 2006 under Typical Agronomic Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Anti-nutrient** | | | | | | |
| Phytic Acid (% DW) | 0.83 (0.038) [0.60 - 1.00] | 0.84 (0.038) [0.69 - 1.09] | -0.0087 (0.028) [-0.15 - 0.19] | -0.080, 0.063 | 0.767 | (0.69 - 0.98) [0.50, 1.11] |
| Raffinose (% DW) | 0.19 (0.0081) [0.15 - 0.22] | 0.18 (0.0081) [0.15 - 0.22] | 0.014 (0.0082) [-0.036 - 0.050] | -0.0074, 0.035 | 0.155 | (0.079 - 0.19) [0.039, 0.26] |
| **Secondary Metabolite** | | | | | | |
| Ferulic Acid (µg/g DW) | 1772.22 (47.57) [1561.63 - 1966.67] | 1693.18 (47.57) [1245.83 - 1997.77] | 79.04 (62.05) [-210.94 - 533.93] | -80.47, 238.55 | 0.258 | (1205.75 - 2873.05) [395.96, 3485.38] |
| p-Coumaric Acid (µg/g DW) | 115.95 (4.15) [99.45 - 136.67] | 126.55 (4.15) [94.77 - 156.25] | -10.61 (4.60) [-38.32 - 27.59] | -22.44, 1.22 | 0.069 | (128.21 - 327.39) [7.61, 408.53] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero**.**

**Table E-12. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 28.37 (1.45) [17.95 - 34.70] | 30.43 (1.45) [24.98 - 35.12] | -2.07 (1.79) [-10.93 - 5.34] | -5.85, 1.72 | 0.264 | (25.07 - 37.22) [16.01, 45.98] |
| Neutral Detergent Fiber (% DW) | 42.02 (1.52) [36.08 - 50.00] | 44.51 (1.52) [39.08 - 47.24] | -2.49 (1.81) [-8.10 - 8.70] | -6.10, 1.11 | 0.171 | (37.84 - 49.16) [27.28, 58.88] |
| **Mineral** | | | | | | |
| Calcium (% DW) | 0.26 (0.020) [0.24 - 0.28] | 0.27 (0.020) [0.22 - 0.39] | -0.0091 (0.019) [-0.11 - 0.051] | -0.048, 0.029 | 0.628 | (0.17 - 0.36) [0.043, 0.46] |
| Phosphorus (% DW) | 0.16 (0.0077) [0.12 - 0.19] | 0.16 (0.0077) [0.13 - 0.20] | 0.0011 (0.0061) [-0.030 - 0.033] | -0.011, 0.013 | 0.852 | (0.13 - 0.18) [0.086, 0.22] |
| **Proximate** | | | | | | |
| Ash (% DW) | 4.71 (0.22) [4.25 - 5.35] | 4.89 (0.22) [3.88 - 6.05] | -0.18 (0.20) [-1.33 - 0.73] | -0.59, 0.22 | 0.366 | (4.12 - 6.12) [2.42, 8.00] |
| Carbohydrates (% DW) | 87.61 (0.42) [86.51 - 89.58] | 87.11 (0.42) [85.87 - 88.50] | 0.50 (0.40) [-0.52 - 2.34] | -0.29, 1.29 | 0.208 | (85.54 - 89.52) [82.51, 92.09] |
| Moisture (% FW) | 74.02 (0.73) [70.90 - 75.90] | 75.19 (0.73) [74.20 - 78.00] | -1.17 (0.70) [-4.40 - 1.70] | -2.65, 0.31 | 0.113 | (71.40 - 76.80) [69.22, 81.25] |
| Protein (% DW) | 6.53 (0.40) [5.29 - 7.10] | 6.71 (0.40) [6.01 - 7.44] | -0.18 (0.22) [-1.20 - 1.09] | -0.62, 0.25 | 0.407 | (5.56 - 7.39) [4.12, 8.77] |
| Total Fat (% DW) | 1.16 (0.16) [0.57 - 1.96] | 1.30 (0.16) [0.51 - 2.33] | -0.14 (0.23) [-0.71 - 0.68] | -0.60, 0.33 | 0.557 | (0.20 - 2.26) [0, 3.59] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-13. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Proximate** | | | | | | |
| Ash (% DW) | 1.44 (0.038) [1.35 - 1.53] | 1.42 (0.038) [1.26 - 1.60] | 0.015 (0.048) [-0.22 - 0.20] | -0.083, 0.11 | 0.751 | (1.14 - 1.47) [0.90, 1.76] |
| Carbohydrates (% DW) | 85.17 (0.27) [82.98 - 87.63] | 85.53 (0.27) [84.91 - 86.31] | -0.37 (0.36) [-2.41 - 2.56] | -1.08, 0.35 | 0.310 | (83.60 - 86.65) [81.08, 89.71] |
| Moisture (% FW) | 12.09 (0.15) [11.80 - 12.50] | 12.09 (0.15) [11.30 - 12.80] | 0 (0.21) [-0.70 - 0.70] | -0.44, 0.44 | 1.000 | (11.00 - 12.20) [10.10, 13.35] |
| Protein (% DW) | 9.50 (0.23) [7.57 - 11.32] | 9.32 (0.23) [8.55 - 9.77] | 0.18 (0.29) [-1.93 - 1.61] | -0.40, 0.76 | 0.533 | (8.69 - 11.33) [5.83, 13.57] |
| Total Fat (% DW) | 3.89 (0.082) [3.45 - 4.23] | 3.72 (0.082) [3.60 - 3.90] | 0.17 (0.071) [-0.42 - 0.61] | 0.018, 0.32 | 0.029 | (3.16 - 4.07) [2.47, 4.68] |
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 2.57 (0.21) [2.08 - 3.18] | 2.47 (0.21) [1.41 - 4.41] | 0.11 (0.27) [-1.43 - 0.96] | -0.44, 0.65 | 0.696 | (1.95 - 3.76) [0.29, 5.01] |
| Neutral Detergent Fiber (% DW) | 8.66 (0.34) [8.19 - 9.45] | 8.60 (0.34) [7.74 - 9.70] | 0.063 (0.44) [-1.41 - 1.62] | -0.86, 0.99 | 0.887 | (7.15 - 9.41) [5.23, 10.90] |
| Total Dietary Fiber (% DW) | 12.70 (0.44) [11.59 - 16.00] | 12.53 (0.44) [11.20 - 13.98] | 0.17 (0.50) [-1.15 - 2.97] | -0.90, 1.24 | 0.737 | (10.24 - 13.51) [6.72, 16.07] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-14. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Calcium (% DW) | 0.0047 (0.00045) [0.0035 - 0.0058] | 0.0045 (0.00045) [0.0036 - 0.0059] | 0.00014 (0.00029) [-0.00060 - 0.0012] | -0.00044, 0.00072 | 0.630 | (0.0032 - 0.0057) [0.00076, 0.0080] |
| Copper (mg/kg DW) | 1.86 (0.24) [1.58 - 2.17] | 1.87 (0.24) [1.47 - 2.81] | -0.011 (0.27) [-0.89 - 0.37] | -0.60, 0.58 | 0.966 | (1.29 - 4.16) [0, 5.74] |
| Iron (mg/kg DW) | 16.60 (0.68) [14.53 - 20.30] | 16.95 (0.68) [15.22 - 19.95] | -0.36 (0.61) [-2.07 - 1.58] | -1.58, 0.86 | 0.561 | (14.37 - 19.48) [10.40, 25.42] |
| Magnesium (% DW) | 0.12 (0.0034) [0.10 - 0.14] | 0.11 (0.0034) [0.10 - 0.11] | 0.0094 (0.0037) [-0.0076 - 0.028] | 0.0021, 0.017 | 0.012 | (0.095 - 0.13) [0.064, 0.16] |
| Manganese (mg/kg DW) | 6.27 (0.40) [5.25 - 7.08] | 6.03 (0.40) [4.64 - 7.58] | 0.24 (0.29) [-1.51 - 1.61] | -0.59, 0.87 | 0.434 | (4.55 - 9.02) [0.69, 10.70] |
| Phosphorus (% DW) | 0.32 (0.0099) [0.29 - 0.34] | 0.30 (0.0099) [0.26 - 0.33] | 0.019 (0.0098) [-0.034 - 0.086] | -0.00060, 0.038 | 0.057 | (0.27 - 0.36) [0.21, 0.40] |
| Potassium (% DW) | 0.40 (0.011) [0.37 - 0.41] | 0.40 (0.011) [0.36 - 0.45] | -0.0039 (0.0084) [-0.078 - 0.047] | -0.021, 0.013 | 0.643 | (0.32 - 0.42) [0.25, 0.47] |
| Zinc (mg/kg DW) | 22.00 (1.00) [19.20 - 25.09] | 21.02 (1.00) [18.36 - 25.34] | 0.99 (0.80) [-3.91 - 5.47] | -0.60, 2.57 | 0.219 | (18.12 - 29.69) [7.39, 38.63] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-15. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Alanine (% DW) | 0.71 (0.018) [0.63 - 0.86] | 0.70 (0.018) [0.63 - 0.76] | 0.017 (0.025) [-0.077 - 0.14] | -0.032, 0.066 | 0.500 | (0.66 - 0.89) [0.44, 1.06] |
| Arginine (% DW) | 0.39 (0.012) [0.32 - 0.47] | 0.38 (0.012) [0.32 - 0.43] | 0.0079 (0.017) [-0.030 - 0.14] | -0.026, 0.042 | 0.639 | (0.34 - 0.46) [0.23, 0.55] |
| Aspartic Acid (% DW) | 0.61 (0.013) [0.54 - 0.70] | 0.60 (0.013) [0.56 - 0.63] | 0.0088 (0.017) [-0.057 - 0.073] | -0.024, 0.042 | 0.598 | (0.58 - 0.77) [0.39, 0.88] |
| Cystine (% DW) | 0.22 (0.0043) [0.20 - 0.23] | 0.21 (0.0043) [0.20 - 0.22] | 0.0030 (0.0047) [-0.0082 - 0.017] | -0.0064, 0.012 | 0.527 | (0.20 - 0.24) [0.16, 0.27] |
| Glutamic Acid (% DW) | 1.84 (0.047) [1.63 - 2.21] | 1.80 (0.047) [1.62 - 1.97] | 0.043 (0.064) [-0.22 - 0.35] | -0.085, 0.17 | 0.503 | (1.64 - 2.26) [1.09, 2.72] |
| Glycine (% DW) | 0.35 (0.0063) [0.32 - 0.39] | 0.34 (0.0063) [0.31 - 0.35] | 0.0063 (0.0077) [-0.029 - 0.041] | -0.0090, 0.022 | 0.414 | (0.31 - 0.38) [0.26, 0.42] |
| Histidine (% DW) | 0.29 (0.0056) [0.26 - 0.34] | 0.29 (0.0056) [0.26 - 0.30] | 0.0034 (0.0074) [-0.031 - 0.037] | -0.011, 0.018 | 0.645 | (0.24 - 0.30) [0.20, 0.34] |
| Isoleucine (% DW) | 0.33 (0.0092) [0.29 - 0.42] | 0.33 (0.0092) [0.30 - 0.36] | 0.0014 (0.013) [-0.044 - 0.074] | -0.024, 0.026 | 0.908 | (0.30 - 0.41) [0.19, 0.49] |

**Table E-15 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Leucine (% DW) | 1.23 (0.034) [1.08 - 1.52] | 1.19 (0.034) [1.08 - 1.30] | 0.036 (0.046) [-0.13 - 0.28] | -0.057, 0.13 | 0.441 | (1.06 - 1.53) [0.66, 1.87] |
| Lysine (% DW) | 0.28 (0.0045) [0.26 - 0.31] | 0.28 (0.0045) [0.26 - 0.29] | 0.00075 (0.0055) [-0.027 - 0.018] | -0.010, 0.012 | 0.892 | (0.25 - 0.31) [0.19, 0.35] |
| Methionine (% DW) | 0.18 (0.0073) [0.16 - 0.21] | 0.18 (0.0073) [0.16 - 0.19] | 0.0032 (0.0065) [-0.021 - 0.027] | -0.0098, 0.016 | 0.625 | (0.18 - 0.23) [0.14, 0.26] |
| Phenylalanine (% DW) | 0.49 (0.012) [0.43 - 0.60] | 0.48 (0.012) [0.43 - 0.52] | 0.011 (0.017) [-0.056 - 0.10] | -0.023, 0.044 | 0.535 | (0.44 - 0.60) [0.28, 0.72] |
| Proline (% DW) | 0.88 (0.027) [0.77 - 1.07] | 0.87 (0.027) [0.82 - 0.95] | 0.012 (0.030) [-0.092 - 0.16] | -0.049, 0.072 | 0.705 | (0.72 - 0.99) [0.48, 1.18] |
| Serine (% DW) | 0.47 (0.012) [0.43 - 0.54] | 0.45 (0.012) [0.40 - 0.50] | 0.019 (0.016) [-0.041 - 0.071] | -0.013, 0.051 | 0.224 | (0.43 - 0.55) [0.32, 0.65] |
| Threonine (% DW) | 0.32 (0.0069) [0.28 - 0.37] | 0.32 (0.0069) [0.29 - 0.33] | 0.0043 (0.0090) [-0.033 - 0.040] | -0.014, 0.022 | 0.634 | (0.30 - 0.37) [0.23, 0.42] |
| Tryptophan (% DW) | 0.051 (0.0020) [0.039 - 0.063] | 0.051 (0.0020) [0.046 - 0.054] | 0.00095 (0.0025) [-0.013 - 0.012] | -0.0039, 0.0058 | 0.701 | (0.040 - 0.059) [0.022, 0.078] |

**Table E-15 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Tyrosine (% DW) | 0.23 (0.023)<br>[0.12 - 0.35] | 0.25 (0.023)<br>[0.13 - 0.32] | -0.022 (0.032)<br>[-0.11 - 0.079] | -0.089, 0.044 | 0.496 | (0.14 - 0.32)<br>[0, 0.53] |
| Valine (% DW) | 0.46 (0.011)<br>[0.42 - 0.56] | 0.46 (0.011)<br>[0.41 - 0.49] | 0.0017 (0.014)<br>[-0.054 - 0.077] | -0.027, 0.031 | 0.909 | (0.41 - 0.54)<br>[0.29, 0.62] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-16. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
| 16:0 Palmitic (% Total FA) | 10.93 (0.13) [10.63 - 11.60] | 11.15 (0.13) [10.96 - 11.32] | -0.22 (0.16) [-0.69 - 0.33] | -0.54, 0.10 | 0.173 | (9.53 - 12.33) [7.43, 14.09] |
| 18:0 Stearic (% Total FA) | 1.80 (0.045) [1.74 - 1.97] | 1.78 (0.045) [1.66 - 1.91] | 0.020 (0.047) [-0.11 - 0.22] | -0.078, 0.12 | 0.675 | (1.28 - 2.13) [0.60, 2.58] |
| 18:1 Oleic (% Total FA) | 20.93 (0.37) [20.29 - 21.28] | 21.01 (0.37) [19.78 - 21.93] | -0.079 (0.29) [-1.20 - 0.51] | -0.69, 0.53 | 0.786 | (22.13 - 31.09) [12.40, 36.28] |
| 18:2 Linoleic (% Total FA) | 64.51 (0.45) [63.92 - 65.27] | 64.21 (0.45) [63.22 - 65.48] | 0.30 (0.42) [-0.81 - 1.59] | -0.59, 1.19 | 0.485 | (55.17 - 64.97) [49.61, 73.18] |
| 18:3 Linolenic (% Total FA) | 1.18 (0.016) [1.15 - 1.23] | 1.21 (0.016) [1.19 - 1.25] | -0.027 (0.014) [-0.049 - -0.013] | -0.056, 0.00085 | 0.057 | (1.00 - 1.32) [0.72, 1.66] |
| 20:0 Arachidic (% Total FA) | 0.31 (0.011) [0.29 - 0.34] | 0.32 (0.011) [0.29 - 0.34] | -0.0054 (0.0058) [-0.026 - 0.0090] | -0.018, 0.0067 | 0.367 | (0.29 - 0.42) [0.19, 0.52] |
| 20:1 Eicosenoic (% Total FA) | 0.18 (0.0039) [0.17 - 0.20] | 0.19 (0.0039) [0.17 - 0.20] | -0.0041 (0.0039) [-0.016 - 0.0089] | -0.012, 0.0038 | 0.301 | (0.20 - 0.31) [0.10, 0.36] |
| 22:0 Behenic (% Total FA) | 0.15 (0.020) [0.062 - 0.26] | 0.13 (0.020) [0.063 - 0.17] | 0.015 (0.026) [-0.075 - 0.097] | -0.039, 0.070 | 0.559 | (0.061 - 0.33) [0, 0.48] |

[1]DW = dry weight; FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-17. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| **Analytical Component[1]** | **Test Mean ± S.E.[1] [Range]** | **Control Mean ± S.E. [Range]** | **Mean ± S.E. [Range]** | **95% CI[1] (Lower,Upper)** | **p-Value** | **Commercial (Range) [99% Tolerance Int.[2]]** |
| Folic Acid (mg/kg DW) | 0.26 (0.018) [0.23 - 0.29] | 0.27 (0.018) [0.22 - 0.33] | -0.0094 (0.016) [-0.085 - 0.029] | -0.044, 0.025 | 0.569 | (0.26 - 0.41) [0.11, 0.55] |
| Niacin (mg/kg DW) | 19.17 (1.77) [16.42 - 21.50] | 19.23 (1.77) [17.42 - 21.17] | -0.054 (1.81) [-1.62 - 2.69] | -3.66, 3.56 | 0.976 | (14.92 - 26.80) [5.96, 38.50] |
| Thiamine HCl/Vitamin B1 (mg/kg DW) | 2.86 (0.084) [2.61 - 3.19] | 2.86 (0.084) [2.74 - 3.06] | 0.00065 (0.088) [-0.22 - 0.44] | -0.19, 0.19 | 0.994 | (2.94 - 4.78) [1.01, 6.00] |
| Riboflavin/Vitamin B2 (mg/kg DW) | 2.01 (0.14) [1.61 - 2.54] | 1.97 (0.14) [1.46 - 2.63] | 0.040 (0.14) [-1.02 - 0.61] | -0.24, 0.32 | 0.781 | (1.62 - 2.62) [0.87, 3.38] |
| Pyridoxine HCl/Vitamin B6 (mg/kg DW) | 6.32 (0.27) [5.49 - 7.39] | 6.83 (0.27) [6.17 - 7.37] | -0.51 (0.33) [-1.32 - 0.21] | -1.21, 0.19 | 0.143 | (4.01 - 6.70) [1.86, 8.29] |
| Vitamin E (mg/kg DW) | 11.90 (0.40) [10.64 - 13.57] | 10.99 (0.40) [9.30 - 12.78] | 0.91 (0.54) [-0.81 - 2.32] | -0.23, 2.05 | 0.110 | (2.83 - 11.69) [0, 19.32] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-18. Comparison of the Anti-nutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Anti-nutrient** | | | | | | |
| Phytic Acid (% DW) | 0.76 (0.035) [0.58 - 0.93] | 0.76 (0.035) [0.63 - 0.90] | 0.0069 (0.043) [-0.21 - 0.31] | -0.083, 0.096 | 0.873 | (0.58 - 0.97) [0.28, 1.15] |
| Raffinose (% DW) | 0.11 (0.013) [0.075 - 0.12] | 0.11 (0.013) [0.077 - 0.14] | -0.0015 (0.0050) [-0.022 - 0.013] | -0.012, 0.0089 | 0.767 | (0.028 - 0.15) [0, 0.21] |
| **Secondary Metabolite** | | | | | | |
| Ferulic Acid (µg/g DW) | 1849.20 (114.60) [1265.68 - 2240.00] | 1753.19 (114.60) [820.14 - 2128.15] | 96.01 (160.59) [-660.93 - 1232.32] | -224.84, 416.86 | 0.552 | (1504.52 - 2224.72) [1019.70, 2703.40] |
| *p*-Coumaric Acid (µg/g DW) | 137.39 (15.51) [68.64 - 188.64] | 149.37 (15.51) [64.03 - 204.06] | -11.98 (18.53) [-88.07 - 87.50] | -50.95, 26.99 | 0.526 | (84.79 - 239.33) [0, 378.84] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-19. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 27.15 (1.45) [23.03 - 32.00] | 26.73 (1.45) [23.10 - 30.79] | 0.42 (1.79) [-6.30 - 8.90] | -3.36, 4.21 | 0.816 | (20.73 - 33.39) [11.54, 42.87] |
| Neutral Detergent Fiber (% DW) | 39.06 (1.52) [33.29 - 44.10] | 40.10 (1.52) [31.81 - 50.61] | -1.04 (1.81) [-9.65 - 8.94] | -4.65, 2.56 | 0.565 | (36.08 - 49.33) [25.58, 58.01] |
| **Mineral** | | | | | | |
| Calcium (% DW) | 0.32 (0.020) [0.20 - 0.44] | 0.34 (0.020) [0.26 - 0.41] | -0.023 (0.019) [-0.14 - 0.11] | 0.002, 0.015 | 0.219 | (0.21 - 0.37) [0.085, 0.50] |
| Phosphorus (% DW) | 0.16 (0.0077) [0.14 - 0.18] | 0.17 (0.0077) [0.15 - 0.21] | -0.0078 (0.0061) [-0.033 - 0.014] | -0.020, 0.0044 | 0.204 | (0.13 - 0.19) [0.077, 0.23] |
| **Proximate** | | | | | | |
| Ash (% DW) | 5.29 (0.22) [4.51 - 6.29] | 5.49 (0.22) [4.59 - 6.90] | -0.20 (0.20) [-1.02 - 0.94] | -0.60, 0.21 | 0.332 | (4.80 - 6.62) [3.59, 7.93] |
| Carbohydrates (% DW) | 85.92 (0.42) [84.14 - 88.81] | 86.02 (0.42) [84.51 - 87.52] | -0.10 (0.40) [-1.84 - 1.29] | -0.89, 0.69 | 0.798 | (84.11 - 87.54) [81.74, 90.41] |
| Moisture (% FW) | 74.98 (0.73) [72.00 - 77.40] | 75.42 (0.75) [73.00 - 77.60] | -0.44 (0.70) [-3.20 - 4.10] | -1.92, 1.04 | 0.552 | (73.40 - 77.50) [70.85, 80.94] |
| Protein (% DW) | 7.47 (0.40) [5.49 - 8.76] | 7.67 (0.40) [6.52 - 9.14] | -0.20 (0.22) [-1.02 - 0.36] | -0.63, 0.24 | 0.373 | (5.56 - 8.59) [2.94, 11.20] |
| Total Fat (% DW) | 1.32 (0.16) [0.50 - 1.92] | 0.84 (0.16) [0.20 - 1.66] | 0.47 (0.23) [-0.42 - 0.92] | 0.010, 0.94 | 0.045 | (0.20 - 1.76) [0, 3.25] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-20. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Proximates** | | | | | | |
| Ash (% DW) | 1.47 (0.038) [1.24 - 1.75] | 1.50 (0.038) [1.39 - 1.63] | -0.032 (0.048) [-0.31 - 0.35] | -0.13, 0.066 | 0.505 | (1.27 - 1.63) [1.06, 1.93] |
| Carbohydrates (% DW) | 84.21 (0.27) [82.64 - 85.64] | 84.10 (0.27) [82.95 - 85.98] | 0.11 (0.36) [-2.06 - 1.84] | -0.60, 0.82 | 0.754 | (82.10 - 85.17) [80.40, 87.76] |
| Moisture (% FW) | 12.10 (0.15) [11.60 - 12.50] | 11.98 (0.15) [11.30 - 12.50] | 0.12 (0.21) [-0.70 - 1.20] | -0.31, 0.56 | 0.563 | (11.70 - 13.20) [10.50, 14.11] |
| Protein (% DW) | 10.30 (0.23) [9.41 - 11.45] | 10.44 (0.23) [9.17 - 11.50] | -0.13 (0.29) [-1.61 - 1.41] | -0.71, 0.45 | 0.645 | (9.99 - 12.19) [8.12, 13.56] |
| Total Fat (% DW) | 4.02 (0.082) [3.71 - 4.28] | 3.96 (0.082) [3.47 - 4.23] | 0.054 (0.071) [-0.19 - 0.54] | -0.096, 0.20 | 0.459 | (3.18 - 4.22) [2.07, 5.10] |
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 2.59 (0.21) [1.85 - 3.58] | 2.33 (0.21) [1.83 - 3.05] | 0.26 (0.27) [-0.53 - 0.99] | -0.28, 0.80 | 0.342 | (1.83 - 3.39) [0.88, 4.63] |
| Neutral Detergent Fiber (% DW) | 8.87 (0.34) [7.33 - 11.31] | 8.22 (0.34) [7.91 - 8.66] | 0.64 (0.44) [-1.25 - 3.07] | -0.28, 1.57 | 0.161 | (6.08 - 10.36) [2.87, 13.22] |
| Total Dietary Fiber (% DW) | 12.48 (0.44) [10.78 - 14.43] | 12.15 (0.44) [11.06 - 13.70] | 0.33 (0.50) [-1.99 - 2.19] | -0.73, 1.40 | 0.515 | (10.57 - 14.56) [6.50, 17.54] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-21. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
| Calcium (% DW) | 0.0052 (0.00045) [0.0046 - 0.0065] | 0.0050 (0.00045) [0.0041 - 0.0063] | 0.00017 (0.00029) [-0.0014 - 0.0015] | -0.00041, 0.00075 | 0.563 | (0.0035 - 0.0070) [0, 0.010] |
| Copper (mg/kg DW) | 2.19 (0.24) [1.88 - 2.49] | 2.16 (0.24) [1.87 - 2.30] | 0.031 (0.27) [-0.40 - 0.62] | -0.56, 0.62 | 0.910 | (1.39 - 2.76) [0.22, 3.82] |
| Iron (mg/kg DW) | 17.67 (0.68) [16.38 - 19.27] | 18.60 (0.68) [16.12 - 22.21] | -0.93 (0.61) [-3.53 - 1.02] | -2.14, 0.29 | 0.131 | (15.90 - 24.66) [7.05, 30.38] |
| Magnesium (% DW) | 0.13 (0.0034) [0.11 - 0.14] | 0.13 (0.0034) [0.10 - 0.14] | 0.00044 (0.0037) [-0.022 - 0.024] | -0.0069, 0.0077 | 0.905 | (0.11 - 0.14) [0.083, 0.16] |
| Manganese (mg/kg DW) | 6.71 (0.40) [5.28 - 8.66] | 6.54 (0.40) [5.25 - 7.77] | 0.18 (0.30) [-1.13 - 2.28] | -0.45, 0.80 | 0.565 | (4.78 - 9.35) [0.72, 11.82] |
| Phosphorus (% DW) | 0.32 (0.0099) [0.25 - 0.36] | 0.33 (0.0099) [0.27 - 0.38] | -0.0095 (0.0098) [-0.074 - 0.075] | -0.029, 0.010 | 0.334 | (0.30 - 0.38) [0.25, 0.42] |
| Potassium (% DW) | 0.40 (0.011) [0.37 - 0.43] | 0.40 (0.011) [0.37 - 0.43] | -0.0024 (0.0082) [-0.038 - 0.038] | -0.019, 0.015 | 0.777 | (0.36 - 0.43) [0.29, 0.49] |
| Zinc (mg/kg DW) | 23.30 (1.00) [18.36 - 26.77] | 24.37 (1.00) [21.29 - 27.79] | -1.07 (0.80) [-3.62 - 2.49] | -2.66, 0.52 | 0.183 | (18.25 - 30.44) [6.01, 42.60] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-22. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Alanine (% DW) | 0.78 (0.018)<br>[0.67 - 0.85] | 0.79 (0.018)<br>[0.68 - 0.89] | -0.010 (0.025)<br>[-0.12 - 0.12] | -0.059, 0.039 | 0.682 | (0.77 - 0.96)<br>[0.59, 1.09] |
| Arginine (% DW) | 0.43 (0.012)<br>[0.41 - 0.44] | 0.42 (0.012)<br>[0.34 - 0.47] | 0.013 (0.017)<br>[-0.058 - 0.10] | -0.022, 0.047 | 0.457 | (0.41 - 0.50)<br>[0.32, 0.56] |
| Aspartic Acid (% DW) | 0.65 (0.013)<br>[0.59 - 0.71] | 0.65 (0.013)<br>[0.59 - 0.73] | -0.0072 (0.017)<br>[-0.090 - 0.098] | -0.040, 0.026 | 0.665 | (0.63 - 0.76)<br>[0.52, 0.88] |
| Cystine (% DW) | 0.23 (0.0043)<br>[0.22 - 0.25] | 0.23 (0.0043)<br>[0.20 - 0.24] | -0.00090 (0.0047)<br>[-0.021 - 0.027] | -0.010, 0.0085 | 0.848 | (0.20 - 0.26)<br>[0.15, 0.30] |
| Glutamic Acid (% DW) | 2.01 (0.047)<br>[1.74 - 2.21] | 2.03 (0.047)<br>[1.71 - 2.29] | -0.019 (0.064)<br>[-0.31 - 0.32] | -0.15, 0.11 | 0.772 | (1.94 - 2.44)<br>[1.51, 2.80] |
| Glycine (% DW) | 0.36 (0.0063)<br>[0.34 - 0.39] | 0.36 (0.0063)<br>[0.33 - 0.39] | 0.0018 (0.0077)<br>[-0.033 - 0.037] | -0.014, 0.017 | 0.818 | (0.35 - 0.42)<br>[0.30, 0.45] |
| Histidine (% DW) | 0.31 (0.0056)<br>[0.28 - 0.32] | 0.31 (0.0056)<br>[0.27 - 0.34] | -0.0022 (0.0074)<br>[-0.031 - 0.034] | -0.017, 0.013 | 0.768 | (0.27 - 0.33)<br>[0.23, 0.36] |
| Isoleucine (% DW) | 0.37 (0.0092)<br>[0.32 - 0.38] | 0.37 (0.0092)<br>[0.32 - 0.41] | -0.0065 (0.013)<br>[-0.052 - 0.048] | -0.031, 0.018 | 0.605 | (0.34 - 0.44)<br>[0.27, 0.50] |

**Table E-22 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Leucine (% DW) | 1.36 (0.034)<br>[1.16 - 1.47] | 1.37 (0.034)<br>[1.13 - 1.56] | -0.011 (0.046)<br>[-0.22 - 0.24] | -0.10, 0.081 | 0.806 | (1.29 - 1.65)<br>[0.98, 1.91] |
| Lysine (% DW) | 0.29 (0.0045)<br>[0.27 - 0.31] | 0.29 (0.0045)<br>[0.28 - 0.31] | -0.0044 (0.0055)<br>[-0.023 - 0.029] | -0.016, 0.0067 | 0.428 | (0.28 - 0.31)<br>[0.25, 0.34] |
| Methionine (% DW) | 0.20 (0.0073)<br>[0.18 - 0.22] | 0.20 (0.0073)<br>[0.16 - 0.22] | 0.0010 (0.0065)<br>[-0.023 - 0.038] | -0.012, 0.014 | 0.873 | (0.19 - 0.30)<br>[0.095, 0.35] |
| Phenylalanine (% DW) | 0.53 (0.012)<br>[0.46 - 0.58] | 0.54 (0.012)<br>[0.45 - 0.61] | -0.0044 (0.017)<br>[-0.079 - 0.086] | -0.038, 0.029 | 0.797 | (0.51 - 0.63)<br>[0.41, 0.72] |
| Proline (% DW) | 0.96 (0.027)<br>[0.85 - 1.04] | 0.97 (0.027)<br>[0.84 - 1.11] | -0.011 (0.030)<br>[-0.15 - 0.16] | -0.072, 0.050 | 0.722 | (0.78 - 1.03)<br>[0.64, 1.23] |
| Serine (% DW) | 0.51 (0.012)<br>[0.45 - 0.58] | 0.51 (0.012)<br>[0.43 - 0.59] | -0.0023 (0.016)<br>[-0.087 - 0.11] | -0.034, 0.030 | 0.886 | (0.48 - 0.60)<br>[0.36, 0.71] |
| Threonine (% DW) | 0.35 (0.0069)<br>[0.32 - 0.39] | 0.35 (0.0069)<br>[0.31 - 0.39] | 0.0015 (0.0090)<br>[-0.042 - 0.067] | -0.016, 0.019 | 0.868 | (0.33 - 0.39)<br>[0.28, 0.44] |
| Tryptophan (% DW) | 0.053 (0.0020)<br>[0.046 - 0.059] | 0.052 (0.0020)<br>[0.042 - 0.063] | 0.0012 (0.0025)<br>[-0.0044 - 0.0095] | -0.0037, 0.0060 | 0.639 | (0.043 - 0.063)<br>[0.031, 0.082] |

**Table E-22 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Tyrosine (% DW) | 0.29 (0.023) [0.18 - 0.33] | 0.24 (0.023) [0.12 - 0.35] | 0.050 (0.032) [-0.11 - 0.16] | -0.017, 0.12 | 0.133 | (0.25 - 0.41) [0.12, 0.52] |
| Valine (% DW) | 0.50 (0.011) [0.44 - 0.51] | 0.51 (0.011) [0.45 - 0.55] | -0.0078 (0.014) [-0.068 - 0.050] | -0.037, 0.021 | 0.590 | (0.47 - 0.58) [0.39, 0.64] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-23. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| 16:0 Palmitic (% Total FA) | 11.06 (0.13) [10.54 - 11.33] | 11.18 (0.13) [10.75 - 11.45] | -0.12 (0.16) [-0.39 - 0.32] | -0.45, 0.20 | 0.447 | (9.84 - 12.33) [7.71, 14.14] |
| 18:0 Stearic (% Total FA) | 1.86 (0.045) [1.73 - 1.95] | 1.86 (0.045) [1.68 - 2.08] | -0.0062 (0.047) [-0.15 - 0.25] | -0.10, 0.092 | 0.896 | (1.30 - 2.10) [0.71, 2.57] |
| 18:1 Oleic (% Total FA) | 20.99 (0.37) [20.20 - 21.60] | 20.83 (0.37) [19.59 - 21.98] | 0.16 (0.29) [-1.03 - 1.33] | -0.45, 0.77 | 0.589 | (20.78 - 29.13) [12.15, 35.55] |
| 18:2 Linoleic (% Total FA) | 64.29 (0.45) [63.27 - 65.10] | 64.30 (0.45) [62.75 - 65.65] | -0.0089 (0.42) [-1.32 - 0.94] | -0.90, 0.88 | 0.983 | (56.51 - 64.46) [50.63, 72.71] |
| 18:3 Linolenic (% Total FA) | 1.19 (0.016) [1.13 - 1.25] | 1.21 (0.016) [1.12 - 1.26] | -0.013 (0.014) [-0.079 - 0.049] | -0.041, 0.015 | 0.354 | (1.03 - 1.38) [0.67, 1.76] |
| 20:0 Arachidic (% Total FA) | 0.31 (0.011) [0.30 - 0.34] | 0.32 (0.011) [0.30 - 0.33] | -0.0034 (0.0058) [-0.025 - 0.014] | -0.016, 0.0087 | 0.561 | (0.30 - 0.41) [0.18, 0.52] |
| 20:1 Eicosenoic (% Total FA) | 0.1763 (0.0039) [0.16 - 0.19] | 0.1845 (0.0039) [0.17 - 0.20] | -0.0082 (0.0039) [-0.025 - 0.014] | -0.016, -0.00030 | 0.042 | (0.18 - 0.27) [0.11, 0.34] |
| 22.0 Behenic (% Total FA) | 0.12 (0.020) [0.058 - 0.20] | 0.12 (0.020) [0.059 - 0.15] | 0.0022 (0.026) [-0.092 - 0.13] | -0.052, 0.056 | 0.933 | (0.062 - 0.18) [0, 0.32] |

[1]FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-24. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| **Analytical Component[1]** | **Test Mean ± S.E. [1] [Range]** | **Control Mean ± S.E. [Range]** | **Mean ± S.E. [Range]** | **95% CI[1] (Lower,Upper)** | **p-Value** | **Commercial (Range) [99% Tolerance Int.[2]]** |
| Folic Acid (mg/kg DW) | 0.29 (0.018) [0.25 - 0.37] | 0.28 (0.018) [0.23 - 0.35] | 0.011 (0.016) [-0.062 - 0.13] | -0.023, 0.046 | 0.497 | (0.26 - 0.42) [0.098, 0.58] |
| Niacin (mg/kg DW) | 18.54 (1.77) [16.23 - 25.00] | 21.73 (1.77) [16.36 - 42.06] | -3.18 (1.81) [-24.26 - 2.78] | -6.79, 0.43 | 0.083 | (13.64 - 27.42) [2.23, 41.53] |
| Thiamine HCl/Vitamin B1 (mg/kg DW) | 3.10 (0.084) [2.84 - 3.42] | 2.98 (0.084) [2.71 - 3.19] | 0.12 (0.088) [-0.11 - 0.45] | -0.070, 0.30 | 0.203 | (2.87 - 4.33) [1.55, 5.85] |
| Riboflavin/Vitamin B2 (mg/kg DW) | 2.12 (0.14) [1.43 - 2.89] | 2.29 (0.14) [1.64 - 2.81] | -0.16 (0.14) [-1.30 - 0.50] | -0.45, 0.12 | 0.255 | (1.81 - 2.78) [0.88, 3.61] |
| Pyridoxine HCl/Vitamin B6 (mg/kg DW) | 6.17 (0.27) [5.43 - 6.57] | 6.15 (0.27) [4.97 - 8.27] | 0.013 (0.33) [-1.96 - 1.20] | -0.69, 0.71 | 0.969 | (5.30 - 8.22) [2.06, 9.98] |
| Vitamin E (mg/kg DW) | 13.01 (0.40) [12.16 - 14.24] | 12.16 (0.40) [10.15 - 13.64] | 0.84 (0.54) [-0.45 - 2.42] | -0.30, 1.99 | 0.135 | (2.84 - 15.53) [0, 22.61] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-25. Comparison of the Anti-nutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control for Combined Sites in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| **Anti-nutrient** | | | | | | |
| Phytic Acid (% DW) | 0.79 (0.035) [0.63 - 0.89] | 0.77 (0.035) [0.60 - 0.89] | 0.022 (0.043) [-0.16 - 0.27] | -0.067, 0.11 | 0.612 | (0.67 - 0.94) [0.40, 1.12] |
| Raffinose (% DW) | 0.11 (0.013) [0.087 - 0.14] | 0.12 (0.013) [0.097 - 0.15] | -0.0087 (0.0050) [-0.018 - 0.0025] | -0.019, 0.0017 | 0.095 | (0.061 - 0.15) [0, 0.21] |
| **Secondary Metabolite** | | | | | | |
| Ferulic Acid (µg/g DW) | 1923.79 (114.60) [1208.67 - 2352.27] | 1852.11 (114.60) [1088.34 - 2301.59] | 71.68 (160.59) [-852.84 - 788.80] | -249.18, 392.53 | 0.656 | (1011.40 - 2539.86) [0, 4071.51] |
| *p*-Coumaric Acid (µg/g DW) | 137.29 (15.51) [85.52 - 168.18] | 149.45 (15.51) [66.48 - 208.43] | -12.16 (18.53) [-122.91 - 65.49] | -51.13, 26.81 | 0.519 | (84.15 - 259.68) [0, 378.67] |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-26. Comparison of the Additional Secondary Metabolite Composition of Forage from MON 87460 and Conventional Control for Combined-Sites from the 2006/2007 Chile Production Conducted under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
|---|---|---|---|---|---|---|
| Free Proline (% DW) | 0.023 (0.0025) [0.014 - 0.031] | 0.019 (0.0025) [0.012 - 0.024] | 0.0035 (0.0018) [-0.0077 - 0.014] | -0.00026, 0.0073 | 0.066 | (0.0094 - 0.030) [0, 0.042] |
| Abscisic Acid (ppb FW) | 37.03 (8.19) [11.90 - 122.00] | 15.66 (7.97) [10.30 - 21.70] | 21.38 (9.64) [-5.50 - 106.90] | 1.01, 41.75 | 0.040 | (12.70 - 23.80) [1.22, 33.02] |
| Choline (ppm FW) | 137.00 (6.68) [114.00 - 159.00] | 128.77 (6.68) [94.90 - 145.00] | 8.23 (6.28) [-10.00 - 36.00] | -4.99, 21.46 | 0.207 | (111.00 - 154.00) [76.96, 179.64] |
| Glycerol (% DW) | 0.16 (0.0085) [0.11 - 0.21] | 0.14 (0.0085) [0.12 - 0.18] | 0.019 (0.0097) [-0.023 - 0.048] | -0.00090, 0.039 | 0.060 | (0.097 - 0.18) [0.024, 0.25] |
| Glycine Betaine (ppm FW) | 84.24 (9.15) [66.40 - 104.00] | 76.27 (9.15) [55.60 - 91.00] | 7.98 (11.23) [-17.40 - 24.90] | -15.64, 31.59 | 0.486 | (4.46 - 147.00) [0, 271.19] |
| Salicylic Acid (ppm DW) | 0.14 (0.049) [0.072 - 0.21] | 0.19 (0.049) [0.060 - 0.30] | -0.046 (0.045) [-0.15 - 0.060] | -0.14, 0.051 | 0.327 | (0.11 - 0.34) [0, 0.51] |
| Fructose (% DW) | 8.56 (1.15) [6.74 - 10.29] | 9.06 (1.15) [7.63 - 9.80] | -0.50 (0.89) [-1.86 - 0.77] | -2.38, 1.38 | 0.581 | (4.32 - 10.04) [1.20, 14.57] |
| Glucose (% DW) | 9.22 (1.07) [7.31 - 10.43] | 9.68 (1.07) [7.63 - 11.04] | -0.45 (0.96) [-2.26 - 0.87] | -2.48, 1.58 | 0.642 | (4.19 - 11.67) [1.01, 16.70] |
| Sucrose (% DW) | 0.46 (1.07) [0.10 - 1.03] | 0.86 (1.07) [0.094 - 2.35] | -0.40 (1.11) [-1.58 - 0.46] | -2.61, 1.82 | 0.722 | (0.076 - 5.36) [0, 9.76] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-27. Comparison of the Additional Secondary Metabolite Composition of Grain from MON 87460 and Conventional Control for Combined-Sites from the 2006/2007 Chile Production Conducted under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
| Free Proline (% DW) | 0.058 (0.011) [0.029 - 0.090] | 0.055 (0.011) [0.030 - 0.083] | 0.0026 (0.0035) [-0.0036 - 0.010] | -0.0048, 0.0099 | 0.476 | (0.0093 - 0.076) [0, 0.12] |
| Abscisic Acid (ppb FW) | 9.73 (2.00) [3.61 - 20.70] | 11.49 (2.00) [7.24 - 21.90] | -1.76 (2.71) [-10.40 - 8.40] | -7.16, 3.63 | 0.517 | (9.48 - 116.00) [0, 162.21] |
| Choline (ppm FW) | 219.89 (13.09) [181.00 - 255.00] | 235.78 (13.09) [203.00 - 265.00] | -15.89 (10.18) [-48.00 - 33.00] | -37.49, 5.71 | 0.138 | (174.00 - 264.00) [129.07, 327.26] |
| Glycerol (% DW) | 0.023 (0.0035) [0.020 - 0.029] | 0.022 (0.0035) [0.017 - 0.030] | 0.0013 (0.0024) [-0.0017 - 0.0051] | -0.0035, 0.0061 | 0.595 | (0.015 - 0.037) [0, 0.048] |
| Glycine Betaine (ppm FW) | 2.27 (0.33) [1.31 - 3.11] | 2.41 (0.33) [1.32 - 4.19] | -0.14 (0.26) [-1.08 - 0.36] | -0.70, 0.41 | 0.588 | (0.50 - 7.67) [0, 12.03] |
| Salicylic Acid (ppm FW) | 0.088 (0.018) [0.065 - 0.12] | 0.094 (0.018) [0.069 - 0.11] | -0.0060 (0.016) [-0.030 - 0.030] | -0.039, 0.027 | 0.705 | (0.061 - 0.71) [0, 0.95] |
| Fructose (% DW) | 0.44 (0.029) [0.34 - 0.50] | 0.44 (0.029) [0.25 - 0.53] | -0.0074 (0.034) [-0.19 - 0.24] | -0.078, 0.063 | 0.830 | (0.21 - 0.57) [0, 0.87] |
| Glucose (% DW) | 0.46 (0.029) [0.35 - 0.55] | 0.48 (0.029) [0.34 - 0.56] | -0.018 (0.035) [-0.20 - 0.069] | -0.091, 0.054 | 0.607 | (0.23 - 0.54) [0.038, 0.81] |
| Sucrose (% DW) | 1.77 (0.17) [1.47 - 2.49] | 1.82 (0.17) [1.40 - 2.47] | -0.053 (0.076) [-0.33 - 0.085] | -0.21, 0.11 | 0.490 | (1.47 - 2.86) [0.41, 3.46] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-28. Comparison of the Additional Secondary Metabolite Composition of Forage from MON 87460 and Conventional Control for Combined-Sites from the 2006/2007 Chile Production Conducted under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
| Free Proline (% DW) | 0.018 (0.0025) [0.013 - 0.027] | 0.018 (0.0025) [0.011 - 0.025] | -0.00028 (0.0018) [-0.0092 - 0.0069] | -0.0041, 0.0035 | 0.876 | (0.011 - 0.025) [0, 0.036] |
| Abscisic Acid (ppb FW) | 31.60 (7.97) [20.40 - 54.30] | 29.42 (7.97) [15.80 - 56.60] | 2.18 (9.46) [-31.10 - 31.80] | -17.91, 22.27 | 0.820 | (16.00 - 58.50) [0, 94.59] |
| Choline (ppm FW) | 155.11 (6.68) [134.00 - 181.00] | 145.89 (6.68) [136.00 - 163.00] | 9.22 (6.28) [-13.00 - 34.00] | -4.00, 22.45 | 0.159 | (118.00 - 166.00) [66.54, 217.46] |
| Glycerol (% DW) | 0.14 (0.0085) [0.11 - 0.17] | 0.14 (0.0085) [0.12 - 0.17] | -0.0038 (0.0097) [-0.029 - 0.042] | -0.024, 0.016 | 0.697 | (0.10 - 0.19) [0.025, 0.24] |
| Glycine Betaine (ppm FW) | 116.80 (9.15) [73.20 - 138.00] | 119.79 (9.15) [89.60 - 176.00] | -2.99 (11.23) [-64.00 - 32.50] | -26.61, 20.63 | 0.793 | (7.19 - 189.00) [0, 357.15] |
| Salicylic Acid (ppm FW) | 0.21 (0.049) [0.10 - 0.58] | 0.24 (0.049) [0.12 - 0.49] | -0.024 (0.045) [-0.39 - 0.34] | -0.12, 0.073 | 0.607 | (0.12 - 0.47) [0, 0.82] |
| Fructose (% DW) | 11.12 (1.15) [7.77 - 20.17] | 10.91 (1.15) [7.52 - 14.95] | 0.21 (0.89) [-2.41 - 7.53] | -1.67, 2.09 | 0.813 | (7.53 - 14.83) [0.69, 18.60] |
| Glucose (% DW) | 12.31 (1.07) [8.60 - 20.87] | 11.87 (1.07) [8.60 - 15.05] | 0.43 (0.96) [-2.82 - 8.81] | -1.59, 2.46 | 0.655 | (8.11 - 15.87) [1.24, 20.22] |
| Sucrose (% DW) | 2.27 (1.07) [0.10 - 6.36] | 1.88 (1.07) [0.11 - 4.07] | 0.39 (1.11) [-3.62 - 4.83] | -1.82, 2.60 | 0.724 | (0.12 - 4.68) [0, 8.87] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-29. Comparison of the Additional Secondary Metabolite Composition of Grain from MON 87460 and Conventional Control for Combined-Sites from the 2006/2007 Chile Production Conducted under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) [99% Tolerance Int.[2]] |
| Free Proline (% DW) | 0.051 (0.011) [0.029 - 0.076] | 0.058 (0.011) [0.029 - 0.079] | -0.0063 (0.0035) [-0.027 - 0.016] | -0.014, 0.0011 | 0.089 | (0.013 - 0.056) [0, 0.11] |
| Abscisic Acid (ppb FW) | 11.43 (2.00) [8.78 - 17.70] | 13.54 (2.00) [6.79 - 23.90] | -2.11 (2.71) [-15.12 - 7.01] | -7.50, 3.29 | 0.438 | (7.37 - 120.00) [0, 176.41] |
| Choline (ppm FW) | 238.11 (13.09) [191.00 - 308.00] | 241.56 (13.09) [209.00 - 284.00] | -3.44 (10.18) [-45.00 - 76.00] | -25.04, 18.16 | 0.739 | (202.00 - 306.00) [104.72, 381.48] |
| Glycerol (% DW) | 0.030 (0.0035) [0.023 - 0.049] | 0.029 (0.0035) [0.018 - 0.043] | 0.00069 (0.0024) [-0.020 - 0.017] | -0.0041, 0.0055 | 0.776 | (0.019 - 0.045) [0, 0.060] |
| Glycine Betaine (ppm FW) | 2.21 (0.33) [1.52 - 3.24] | 1.99 (0.33) [1.18 - 3.98] | 0.21 (0.26) [-2.22 - 1.68] | -0.34, 0.77 | 0.421 | (0.50 - 11.40) [0, 21.14] |
| Salicylic Acid (ppm FW) | 0.11 (0.018) [0.073 - 0.19] | 0.12 (0.018) [0.084 - 0.15] | -0.0026 (0.016) [-0.074 - 0.060] | -0.036, 0.031 | 0.871 | (0.057 - 0.60) [0, 1.00] |
| Fructose (% DW) | 0.47 (0.029) [0.37 - 0.60] | 0.48 (0.029) [0.38 - 0.63] | -0.011 (0.034) [-0.26 - 0.19] | -0.082, 0.059 | 0.739 | (0.29 - 0.74) [0, 1.12] |
| Glucose (% DW) | 0.48 (0.029) [0.38 - 0.59] | 0.50 (0.029) [0.39 - 0.64] | -0.015 (0.035) [-0.26 - 0.17] | -0.087, 0.058 | 0.671 | (0.32 - 0.77) [0, 1.17] |
| Sucrose (% DW) | 1.63 (0.17) [1.33 - 1.86] | 1.86 (0.17) [1.37 - 2.27] | -0.23 (0.076) [-0.72 - 0.17] | -0.39, -0.069 | 0.008 | (1.41 - 2.19) [0.61, 2.84] |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-30. Literature and ILSI Database Ranges of Components of Corn Forage and Grain**

| Tissue/ Component[1] | Literature Range[2] | ILSI Range[3] |
|---|---|---|
| **Forage** | | |
| **Proximates (% DW)** | | |
| Ash | 2.43-9.64[a]; 2-6.6[b] | 1.527 – 9.638 |
| Carbohydrates | 83.2-91.6[b]; 76.5-87.3[a] | 76.4 – 92.1 |
| Fat, total | 0.35-3.62[b]; 1.42-4.57[a] | 0.296 – 4.570 |
| Moisture (% fw) | 56.5-80.4[a];55.3-75.3[b] | 49.1 – 81.3 |
| Protein | 4.98-11.56[a] | 3.14 – 11.57 |
| | | |
| **Fiber (% DW)** | | |
| Acid detergent fiber (ADF) | 18.3-41.0[b]; 17.5-38.3[a] | 16.13 – 47.39 |
| Neutral detergent fiber (NDF) | 26.4-54.5[b]; 27.9-54.8[a] | 20.29 – 63.71 |
| | | |
| **Minerals (% DW)** | | |
| Calcium | 0.0969-0.3184[b] | 0.0714 – 0.5768 |
| Phosphorous | 0.1367-0.2914[b] | 0.0936 – 0.3704 |
| | | |
| **Grain** | | |
| **Proximates (% DW)** | | |
| Ash | 1.1-3.9[d]; 0.89-6.28[b] | 0.616 – 6.282 |
| Carbohydrates | 77.4-87.2[b]; 82.2-88.1[a] | 77.4 – 89.5 |
| Fat, total | 3.1-5.7[d]; 2.48-4.81[b] | 1.742 – 5.823 |
| Moisture (% FW) | 7-23[d]; 8.18-26.2[b] | 6.1 – 40.5 |
| Protein | 6-12[d]; 9.7-16.1[c] | 6.15 – 17.26 |
| | | |
| **Fiber (% dw)** | | |
| Acid detergent fiber (ADF) | 3.3-4.3[d]; 2.46-11.34[a,b] | 1.82 – 11.34 |
| Neutral detergent fiber (NDF) | 8.3-11.9[d]; 7.58-15.91[b] | 5.59 – 22.64 |
| Total dietary fiber (TDF) | 10.99-11.41[h] | 8.82 – 35.31 |
| | | |
| **Minerals** | | |
| Calcium (% DW) | 0.01-0.1[d] | 0.00127 – 0.02084 |
| Copper (mg/kg DW) | 0.9-10[d] | 0.73 – 18.50 |
| Iron (mg/kg DW) | 1-100[d] | 10.42 – 49.07 |
| Magnesium (% DW) | 0.09-1[d] | 0.0594 – 0.194 |
| Manganese (mg/kg DW) | 0.7-54[d] | 1.69 – 14.30 |
| Phosphorus (% DW) | 0.26-0.75[d] | 0.147 – 0.533 |
| Potassium (% DW) | 0.32-0.72[d] | 0.181 – 0.603 |
| Zinc (mg/kg DW) | 12-30[d] | 6.5 – 37.2 |

**Table E-30 (cont.). Literature and ILSI Database Ranges of Components of Corn Forage and Grain**

| Tissue/ Component[1] | Literature Range[2] | ILSI Range[3] |
|---|---|---|
| **Grain** | | |
| **Amino Acids (% DW)** | | |
| Alanine | N/A | 0.439 – 1.393 |
| Arginine | N/A | 0.119 – 0.639 |
| Aspartic acid | N/A | 0.335 – 1.208 |
| Cystine | N/A | 0.125 – 0.514 |
| Glutamic acid | N/A | 0.965 – 3.536 |
| Glycine | N/A | 0.184 – 0.539 |
| Histidine | N/A | 0.137 – 0.434 |
| Isoleucine | N/A | 0.179 – 0.692 |
| Leucine | N/A | 0.642 – 2.492 |
| Lysine | N/A | 0.172 – 0.668 |
| Methionine | N/A | 0.124 – 0.468 |
| Phenylalanine | N/A | 0.244 – 0.930 |
| Proline | N/A | 0.462 – 1.632 |
| Serine | N/A | 0.235 – 0.769 |
| Threonine | N/A | 0.224 – 0.666 |
| Tryptophan | N/A | 0.0271 – 0.215 |
| Tyrosine | N/A | 0.103 – 0.642 |
| Valine | N/A | 0.266 – 0.855 |
| | | |
| **Fatty Acids** | (% total fat) | (% total fatty acid) |
| 16:0 Palmitic | 7-19[e] | 7.94 – 20.71 |
| 16:1 Palmitoleic | 1[e] | 0.095 – 0.447 |
| 18:0 Stearic | 1-3[e] | 1.02 – 3.40 |
| 18:1 Oleic | 20-46[e] | 17.4 – 40.2 |
| 18:2 Linoleic | 35-70[e] | 36.2 – 66.5 |
| 18:3 Linolenic | 0.8-2[e] | 0.57 – 2.25 |
| 20:0 Arachidic | 0.1-2[e] | 0.279 – 0.965 |
| 20:1 Eicosenoic | - | 0.170 – 1.917 |
| 22:0 Behenic | - | 0.110 – 0.349 |
| | | |
| **Vitamins (mg/kg DW)** | | |
| Folic acid | 0.3[d] | 0.147 – 1.464 |
| Niacin | 9.3-70[d] | 10.37 – 46.94 |
| Vitamin $B_1$ | 3-8.6[e] | 1.26 – 40.00 |
| Vitamin $B_2$ | 0.25-5.6[e] | 0.50 – 2.36 |
| Vitamin $B_6$ | 5.3[d]; 9.6[e] | 3.68 – 11.32 |
| Vitamin E | 3-12.1[e]; 17-47[d] | 1.5 – 68.7 |

**Table E-30 (cont.). Literature and Historical Ranges of Components of Corn Forage and Grain**

| Tissue/ Component[1] | Literature Range[2] | ILSI Range[3] |
|---|---|---|
| **Grain** | | |
| **Anti-nutrients (% DW)** | | |
| Phytic acid | 0.48-1.12[a] | 0.111 – 1.570 |
| Raffinose | 0.08-0.30[e] | 0.020 – 0.320 |
| | | |
| **Secondary Metabolites (µg/g dw)** | | |
| Ferulic acid | 113-1194[f]; 3000[g] | 291.9 – 3885.8 |
| p-Coumaric acid | 22-75[f] | 53.4 – 576.2 |

[1]FW=fresh weight; DW=dry weight; Niacin =Vitamin $B_3$; Vitamin $B_1$ =Thiamine; Vitamin $B_2$ =Riboflavin; Vitamin $B_6$ =Pyridoxine ; N/A = not available as percent dry weight.
[2]Literature range references: [a]Ridley et al., 2002. [b]Sidhu et al., 2000. [c]Jugenheimer, 1976. [d]Watson, 1987. [e]Watson, 1982. [f]Classen et al., 1990. [g]Dowd and Vega, 1996. [h]Choi et al., 1999.
[3]ILSI range is from ILSI CCD, 2006.

Conversions: % DW x $10^4$ = µg/g dw; mg/g dw x $10^3$ = mg/kg DW; mg/100g dw x 10 = mg/kg DW

## E.8. Compositional Analysis for the QUI Site in the Chile 2006/2007 Study

This section presents composition data on forage and grain collected from MON 87460 grown at an individual site (QUI) of the Chile 2006/2007 study. As noted in Section VII.2, this site was excluded from the combined-site data analysis because it did not meet the requirements specified by the intended water-limited conditions.
Table E-31 presents data on applied water and temperatures from the production period for the QUI site.

Evaluation of the overall data set confirmed analyte results were as expected from the respective assays and were similar to reference and published ranges for conventional corn. No unexpected compositional values for any components were observed.

A summary of significant differences ($p<0.05$) between test and control for the compositional data is presented in Table E-32. Mean values, ranges, and statistical analyses data are presented in (Tables E-33 through E-39) for the well-watered treatment and Tables E-40 through E-46) for the water-limited treatment.

A summary of significant differences ($p<0.05$) between test and control for the additional secondary metabolites is presented in Table E-47. Mean values, ranges, and statistical analyses data are presented in (Tables E-48 and E-49) for the well-watered treatment and Tables E-50 and E-51) for the water-limited treatment.

The QUI site results reported below do not impact the conclusion of compositional equivalence of MON 87460 to conventional corn as established in Section VII.

**Table E-31. Monthly Temperature and Monthly Accumulated Water Data for the QUI Site in the Chile 2006/2007 Study**

| Site[1] | Measurement | December | January | February | March | April | May |
|---|---|---|---|---|---|---|---|
| QUI | Accumulated water (in.), well-watered | 1.9 | 10.3 | 8.5 | 9.4 | 1.9 | 0.0 |
| | Accumulated water (in.), water-limited[1, 2] | 1.9 | 10.3 | 1.9 | 5.6 | 1.9 | 0.0 |
| | Avg Max temp (°F) | NA[4] | 83 | 79 | 77 | 74 | 68 |
| | Avg Min temp (°F) | NA[4] | 51 | 51 | 49 | 44 | 37 |
| | Range[3] (°F) | NA[4] | 48 - 92 | 44 - 90 | 41 - 93 | 34 - 92 | 29 – 77 |

[1] Water limitation began at the V10 growth stage which occurred at approximately February 7.
[2] Water limitation ended at the R2 growth stage which occurred at approximately March 13.
[3] The range is the absolute maximum and minimum temperature in each month.
[4] Temperature data are available from January 6 through May 25; planting occurred in late December and early January.
Rainfall did not occur during the production period.

**Table E-32. Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered and Water-Limited Conditions**

| Tissue/Site/ Components (Units)[1] | Mean MON 87460 | Mean Control | Mean Diff (% of Control) | Signif. (p-value) | MON 87460 (Range) | Commercial (Range) |
|---|---|---|---|---|---|---|
| **Forage** | | | | | | |
| **Water-Limited** | | | | | | |
| Moisture (% FW) | 73.53 | 76.57 | -3.96 | 0.019 | (72.40 - 75.30) | (74.30 - 77.30) |
| Protein (% DW) | 5.78 | 6.69 | -13.68 | 0.013 | (5.25 - 6.57) | (5.44 - 6.38) |
| Total Fat (% DW) | 1.44 | 0.82 | 75.70 | 0.049 | (0.58 - 2.34) | (0.58 - 1.42) |
| **Grain** | | | | | | |
| **Well-watered** | | | | | | |
| Moisture (% FW) | 13.63 | 8.48 | 60.83 | <0.001 | (13.10 - 14.2) | (9.41 - 13.70) |
| Magnesium (% DW) | 0.12 | 0.11 | 7.86 | 0.043 | (0.12 - 0.13) | (0.10 - 0.12) |
| Phosphorus (% DW) | 0.32 | 0.30 | 7.63 | 0.047 | (0.31 - 0.34) | (0.27 - 0.33) |
| 16:0 Palmitic (% Total FA) | 11.06 | 11.38 | -2.77 | 0.017 | (10.92 - 11.29) | (9.56 - 12.93) |
| Vitamin E (mg/kg DW) | 11.63 | 9.85 | 18.09 | 0.023 | (11.06 - 12.47) | (2.88 - 8.47) |
| **Water-Limited** | | | | | | |
| Carbohydrates (% DW) | 85.95 | 85.21 | 0.87 | 0.038 | (85.69 - 86.33) | (85.53 - 86.50) |
| Moisture (% FW) | 13.60 | 8.72 | 55.96 | <0.001 | (12.70 - 14.10) | (9.55 - 13.40) |
| Protein (% DW) | 8.69 | 9.24 | -5.90 | 0.043 | (8.24 - 8.99) | (8.32 - 9.67) |
| Neutral Detergent Fiber (% DW | 9.65 | 10.89 | -11.36 | 0.042 | (8.85 - 10.54) | (7.75 - 10.73) |
| Copper (mg/kg DW) | 1.61 | 4.22 | -61.82 | 0.032 | (1.53 - 1.65) | (1.37 - 1.87) |
| Iron (mg/kg DW) | 14.35 | 15.48 | -7.29 | 0.037 | (13.75 - 14.77) | (14.04 - 19.16) |
| Alanine (% DW) | 0.62 | 0.70 | -11.03 | 0.006 | (0.59 - 0.65) | (0.62 - 0.70) |
| Aspartic Acid (% DW) | 0.56 | 0.60 | -7.42 | 0.018 | (0.54 - 0.58) | (0.53 - 0.60) |
| Glutamic Acid (% DW) | 1.60 | 1.76 | -9.04 | 0.020 | (1.50 - 1.66) | (1.53 - 1.76) |
| Isoleucine (% DW) | 0.29 | 0.33 | -10.09 | 0.007 | (0.28 - 0.31) | (0.29 - 0.32) |
| Leucine (% DW) | 1.04 | 1.16 | -10.50 | 0.015 | (0.97 - 1.09) | (0.97 - 1.14) |
| Lysine (% DW) | 0.27 | 0.29 | -5.44 | 0.037 | (0.27 - 0.27) | (0.26 - 0.29) |
| Phenylalanine (% DW) | 0.43 | 0.47 | -8.79 | 0.022 | (0.40 - 0.44) | (0.40 - 0.46) |
| Valine (% DW) | 0.43 | 0.46 | -7.50 | 0.018 | (0.41 - 0.44) | (0.40 - 0.45) |

[1]DW= dry weight; FW=fresh weight, FA= fatty acid.

**Table E-33. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 34.70 (1.57) [31.50 - 39.52] | 32.92 (1.57) [31.44 - 34.55] | 1.78 (2.21) [-3.05 - 8.08] | -2.91, 6.47 | 0.433 | (32.07 - 39.64) |
| Neutral Detergent Fiber (% DW) | 46.04 (1.90) [42.13 - 50.79] | 48.39 (1.90) [43.78 - 52.67] | -2.35 (2.45) [-7.46 - 2.06] | -7.57, 2.87 | 0.353 | (46.84 - 50.22) |
| **Mineral** | | | | | | |
| Calcium (% DW) | 0.31 (0.021) [0.29 - 0.34] | 0.33 (0.021) [0.31 - 0.35] | -0.020 (0.26) [-0.033 - - 0.0051] | -0.075, 0.034 | 0.443 | (0.26 - 0.33) |
| Phosphorus (% DW) | 0.16 (0.010) [0.14 - 0.17] | 0.16 (0.010) [0.13 - 0.17] | 0.0075 (0.014) [-0.021- 0.041] | -0.022, 0.037 | 0.594 | (0.13 - 0.17) |
| **Proximate** | | | | | | |
| Ash (% DW) | 4.13 (0.18) [4.02 - 4.33] | 4.55 (0.18) [4.51 - 4.62] | -0.42 (0.25) [-0.50 - -0.29] | -0.96, 0.13 | 0.123 | (4.73 - 6.65) |
| Carbohydrates (% DW) | 88.78 (0.34) [88.57 - 89.20] | 88.57 (0.34) [88.12 - 88.91] | 0.21 (0.47) [-0.34 – 0.52] | -0.78, 1.20 | 0.665 | (86.88 - 88.82) |
| Moisture (% FW) | 74.43 (0.96) [73.90 - 74.80] | 76.27 (0.96) [75.70 - 76.70] | -1.83 (1.17) [-2.10 - -1.60] | -4.30, 0.64 | 0.135 | (75.10 - 77.90) |
| Protein (% DW) | 5.89 (0.24) [5.63 - 6.10] | 6.12 (0.24) [5.92 - 6.30] | -0.23 (0.34) [-0.51 – 0.18] | -0.93, 0.47 | 0.504 | (5.78 - 6.47) |
| Total Fat (% DW) | 1.20 (0.22) [0.84 - 1.47] | 0.76 (0.22) [0.56 - 1.06] | 0.44 (0.29) [0.29 - 0.61] | -0.18, 1.06 | 0.154 | (0.23 - 0.86) |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-34. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| **Proximates** | | | | | | |
| Ash (% DW) | 1.48 (0.033) [1.39 - 1.54] | 1.44 (0.033) [1.34 - 1.57] | 0.036 (0.046) [-0.18 - 0.20] | -0.061, 0.13 | 0.448 | (1.30 - 1.36) |
| Carbohydrates (% DW) | 85.22 (0.24) [85.00 - 85.65] | 85.41 (0.24) [84.63 - 86.01] | -0.19 (0.33) [-1.01 - 1.02] | -0.89, 0.51 | 0.571 | (86.05 - 86.57) |
| Moisture (% FW) | 13.63 (0.24) [13.10 - 14.20] | 8.48 (0.24) [8.15 - 8.82] | 5.16 (0.34) [4.95 - 5.38] | 4.42, 5.89 | <0.001 | (9.41 - 13.70) |
| Protein (% DW) | 9.23 (0.19) [9.16 - 9.30] | 9.20 (0.19) [8.87 - 9.45] | 0.029 (0.25) [-0.29 - 0.37] | -0.50, 0.56 | 0.909 | (8.35 - 9.23) |
| Total Fat (% DW) | 4.07 (0.14) [3.81 - 4.25] | 3.94 (0.14) [3.71 - 4.35] | 0.13 (0.19) [-0.54 - 0.55] | -0.28, 0.54 | 0.518 | (3.15 - 4.16) |
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 3.58 (0.37) [2.45 - 4.15] | 3.34 (0.37) [2.48 - 3.78] | 0.24 (0.52) [-1.33 - 1.67] | -0.84, 1.32 | 0.652 | (2.83 - 4.02) |
| Neutral Detergent Fiber (% DW) | 10.25 (0.40) [10.01 - 10.61] | 9.12 (0.40) [8.63 - 9.54] | 1.13 (0.57) [0.94 - 1.38] | -0.057, 2.32 | 0.060 | (8.91 - 10.10) |
| Total Dietary Fiber (% DW) | 14.32 (0.58) [13.89 - 14.92] | 13.55 (0.58) [12.96 - 14.26] | 0.77 (0.70) [0.45 - 1.20] | -0.71, 2.25 | 0.286 | (11.76 - 13.56) |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-35. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| Calcium (% DW) | 0.0047 (0.00021) [0.0044 - 0.0051] | 0.0047 (0.00021) [0.0046 - 0.0051] | 0 (0.00022) [-0.00068 - 0.00059] | -0.00049, 0.00047 | 0.966 | (0.0041 - 0.0054) |
| Copper (mg/kg DW) | 1.70 (0.80) [1.54 - 1.79] | 3.78 (0.80) [1.93 - 7.38] | -2.08 (1.14) [-5.61 - -0.25] | -4.46, 0.29 | 0.082 | (1.33 - 1.72) |
| Iron (mg/kg DW) | 16.87 (0.37) [16.32 - 17.59] | 16.20 (0.37) [14.81 - 17.26] | 0.66 (0.50) [0.14 - 1.51] | -0.39, 1.71 | 0.202 | (13.79 - 18.40) |
| Magnesium (% DW) | 0.12 (0.0030) [0.12 - 0.13] | 0.11 (0.0030) [0.11 - 0.12] | 0.0090 (0.0042) [-0.0088 - 0.020] | 0.00028, 0.018 | 0.043 | (0.10 - 0.12) |
| Manganese (mg/kg DW) | 6.46 (0.19) [6.01 - 7.10] | 6.68 (0.19) [5.91 - 7.08] | -0.22 (0.26) [-1.07 - 1.19] | -0.76, 0.33 | 0.421 | (4.92 - 5.28) |
| Phosphorus (% DW) | 0.32 (0.0076) [0.31 - 0.34] | 0.30 (0.0076) [0.28 - 0.33] | 0.023 (0.011) [-0.013 - 0.046] | 0.00032, 0.046 | 0.047 | (0.27 - 0.33) |
| Potassium (% DW) | 0.39 (0.0084) [0.38 - 0.40] | 0.38 (0.0084) [0.36 - 0.39] | 0.014 (0.011) [-0.015 - 0.033] | -0.011, 0.038 | 0.256 | (0.32 - 0.40) |
| Zinc (mg/kg DW) | 18.18 (0.58) [17.01 - 19.35] | 19.09 (0.58) [17.96 - 19.96] | -0.91 (0.78) [-2.32 - 0.22] | -2.54, 0.73 | 0.260 | (16.12 - 18.18) |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-36. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| Alanine (% DW) | 0.67 (0.018) [0.67 - 0.68] | 0.68 (0.018) [0.64 - 0.71] | -0.0098 (0.025) [-0.032 - 0.032] | -0.062, 0.043 | 0.697 | (0.62 - 0.72) |
| Arginine (% DW) | 0.43 (0.011) [0.42 - 0.43] | 0.40 (0.011) [0.39 - 0.42] | 0.023 (0.015) [0.016 - 0.034] | -0.0096, 0.055 | 0.158 | (0.38 - 0.42) |
| Aspartic Acid (% DW) | 0.60 (0.012) [0.59 - 0.60] | 0.58 (0.012) [0.55 - 0.61] | 0.011 (0.017) [-0.014 - 0.042] | -0.025, 0.047 | 0.530 | (0.52 - 0.63) |
| Cystine (% DW) | 0.21 (0.0050) [0.21 - 0.22] | 0.21 (0.0050) [0.20 - 0.21] | 0.0028 (0.0058) [-0.0043 - 0.0089] | -0.0096, 0.015 | 0.639 | (0.19 - 0.21) |
| Glutamic Acid (% DW) | 1.73 (0.045) [1.71 - 1.74] | 1.71 (0.045) [1.61 - 1.76] | 0.014 (0.063) [-0.034 - 0.10] | -0.12, 0.14 | 0.830 | (1.52 - 1.81) |
| Glycine (% DW) | 0.34 (0.0050) [0.34 - 0.34] | 0.33 (0.0050) [0.31 - 0.34] | 0.0097 (0.0068) [0.00028 - 0.026] | -0.0048, 0.024 | 0.175 | (0.32 - 0.35) |
| Histidine (% DW) | 0.29 (0.0053) [0.29 - 0.29] | 0.28 (0.0053) [0.26 - 0.29] | 0.0078 (0.0072) [-0.0014 - 0.022] | -0.0073, 0.023 | 0.292 | (0.24 - 0.28) |
| Isoleucine (% DW) | 0.32 (0.0079) [0.32 - 0.33] | 0.32 (0.0079) [0.31 - 0.33] | 0.0027 (0.011) [-0.0077 - 0.020] | -0.021, 0.026 | 0.813 | (0.28 - 0.32) |

**Table E-36 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| Leucine (% DW) | 1.15 (0.033) [1.14 - 1.15] | 1.14 (0.033) [1.07 - 1.18] | 0.0086 (0.045) [-0.025 - 0.068] | -0.087, 0.10 | 0.851 | (0.99 - 1.17) |
| Lysine (% DW) | 0.29 (0.0050) [0.28 - 0.29] | 0.28 (0.0050) [0.27 - 0.29] | 0.0059 (0.0071) [-0.0043 - 0.020] | -0.0089, 0.021 | 0.413 | (0.27 - 0.30) |
| Methionine (% DW) | 0.17 (0.0045) [0.16 - 0.17] | 0.16 (0.0045) [0.16 - 0.17] | 0.0047 (0.0060) [-0.0072 - 0.011] | -0.0081, 0.017 | 0.447 | (0.16 - 0.18) |
| Phenylalanine (% DW) | 0.46 (0.012) [0.46 - 0.46] | 0.46 (0.012) [0.43 - 0.48] | 0.0050 (0.017) [-0.012 - 0.028] | -0.030, 0.040 | 0.767 | (0.41 - 0.48) |
| Proline (% DW) | 0.88 (0.022) [0.86 - 0.90] | 0.86 (0.022) [0.80 - 0.91] | 0.025 (0.032) [-0.048 - 0.084] | -0.041, 0.090 | 0.445 | (0.74 - 0.85) |
| Serine (% DW) | 0.43 (0.013) [0.42 - 0.44] | 0.43 (0.013) [0.41 - 0.46] | -0.0020 (0.018) [-0.019 - 0.010] | -0.039, 0.035 | 0.912 | (0.39 - 0.46) |
| Threonine (% DW) | 0.31 (0.0071) [0.31 - 0.32] | 0.30 (0.0071) [0.29 - 0.32] | 0.0074 (0.010) [-0.0015 - 0.015] | -0.013, 0.028 | 0.468 | (0.28 - 0.33) |
| Tryptophan (% DW) | 0.051 (0.0013) [0.049 - 0.052] | 0.049 (0.0013) [0.048 - 0.050] | 0.0019 (0.0018) [0.00056 - 0.0031] | -0.0020, 0.0058 | 0.318 | (0.045 - 0.054) |

**Table E-36 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| Tyrosine (% DW) | 0.29 (0.021) [0.27 - 0.30] | 0.28 (0.021) [0.27 - 0.32] | 0.0038 (0.030) [-0.026 - 0.029] | -0.060, 0.067 | 0.901 | (0.24 - 0.30) |
| Valine (% DW) | 0.46 (0.0095) [0.45 - 0.46] | 0.45 (0.0095) [0.42 - 0.47] | 0.0049 (0.013) [-0.014 - 0.035] | -0.023, 0.033 | 0.716 | (0.39 - 0.46) |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-37. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| 16:0 Palmitic (% Total FA) | 11.06 (0.099) [10.92 - 11.29] | 11.38 (0.099) [11.21 - 11.53] | -0.31 (0.12) [-0.41 - -0.24] | -0.57, -0.063 | 0.017 | (9.56 - 12.93) |
| 18:0 Stearic (% Total FA) | 1.83 (0.038) [1.74 - 1.89] | 1.84 (0.038) [1.75 - 1.90] | -0.0084 (0.053) [-0.13 - 0.12] | -0.12, 0.11 | 0.877 | (1.32 - 2.11) |
| 18:1 Oleic (% Total FA) | 20.91 (0.22) [20.27 - 21.55] | 20.88 (0.22) [20.41 - 21.29] | 0.030 (0.27) [-0.67 - 0.50] | -0.56, 0.62 | 0.913 | (20.59 - 31.56) |
| 18:2 Linoleic (% Total FA) | 64.43 (0.26) [63.86 - 64.94] | 64.06 (0.26) [63.58 - 64.86] | 0.36 (0.31) [-0.39 - 1.20] | -0.29, 1.02 | 0.257 | (55.08 - 64.79) |
| 18:3 Linolenic (% Total FA) | 1.21 (0.017) [1.19 - 1.23] | 1.26 (0.017) [1.22 - 1.28] | -0.043 (0.023) [-0.055 - -0.030] | -0.091, 0.0058 | 0.080 | (1.10 - 1.61) |
| 20:0 Arachidic (% Total FA) | 0.30 (0.0063) [0.29 - 0.30] | 0.30 (0.0063) [0.28 - 0.31] | -0.0010 (0.0081) [-0.010 - 0.017] | -0.018, 0.016 | 0.901 | (0.32 - 0.36) |
| 20:1 Eicosenoic (% Total FA) | 0.18 (0.0024) [0.17 - 0.18] | 0.18 (0.0024) [0.17 - 0.18] | -0.0020 (0.0033) [-0.0097 - 0.0047] | -0.0089, 0.0050 | 0.560 | (0.20 - 0.26) |
| 22:0 Behenic (% Total FA) | 0.087 (0.021) [0.060 - 0.14] | 0.11 (0.021) [0.058 - 0.15] | -0.026 (0.029) [-0.086 - 0.081] | -0.086, 0.035 | 0.386 | (0.060 - 0.17) |

[1]FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-38. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| **Analytical Component[1]** | **Test Mean ± S.E.[1] [Range]** | **Control Mean ± S.E. [Range]** | **Mean ± S.E. [Range]** | **95% CI[1] (Lower,Upper)** | **p-Value** | **Commercial (Range)** |
| Folic Acid (mg/kg DW) | 0.29 (0.024) [0.26 - 0.35] | 0.24 (0.024) [0.23 - 0.25] | 0.055 (0.026) [0.027 - 0.099] | -0.0020, 0.11 | 0.057 | (0.28 - 0.30) |
| Niacin (mg/kg DW) | 21.92 (1.34) [20.49 - 23.01] | 23.52 (1.34) [22.48 - 24.71] | -1.60 (1.73) [-2.89 - -0.22] | -5.31, 2.10 | 0.369 | (24.16 - 29.08) |
| Thiamine HCl (mg/kg DW) | 2.89 (0.12) [2.80 - 3.01] | 2.88 (0.12) [2.74 - 2.95] | 0.017 (0.17) [-0.063 - 0.060] | -0.33, 0.37 | 0.917 | (2.19 - 3.59) |
| Vitamin B2 (mg/kg DW) | 2.17 (0.22) [1.77 - 2.87] | 2.14 (0.22) [1.86 - 2.57] | 0.034 (0.31) [-0.69 - 1.01] | -0.62, 0.69 | 0.913 | (1.96 - 2.40) |
| Vitamin B6 (mg/kg DW) | 5.64 (0.21) [5.32 - 6.05] | 5.40 (0.21) [5.36 - 5.44] | 0.24 (0.30) [-0.073 - 0.61] | -0.39, 0.87 | 0.430 | (5.37 - 5.80) |
| Vitamin E (mg/kg DW) | 11.63 (0.53) [11.06 - 12.47] | 9.85 (0.53) [8.30 - 10.97] | 1.78 (0.71) [1.08 - 2.76] | 0.27, 3.29 | 0.023 | (2.88 - 8.47) |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-39. Comparison of the Anti-nutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| **Anti-nutrient** | | | | | | |
| Phytic Acid (% DW) | 0.76 (0.042) [0.65 - 0.83] | 0.71 (0.042) [0.68 - 0.73] | 0.046 (0.060) [-0.034 - 0.11] | -0.079, 0.17 | 0.450 | (0.57 - 0.71) |
| Raffinose (% DW) | 0.096 (0.0064) [0.089 - 0.11] | 0.093 (0.0064) [0.082 - 0.10] | 0.0031 (0.0090) [-0.010 - 0.024] | -0.016, 0.022 | 0.738 | (0.029 - 0.095) |
| **Secondary Metabolite** | | | | | | |
| Ferulic Acid (µg/g DW) | 1988.05 (116.42) [1910.24 - 2097.90] | 1904.98 (116.42) [1704.17 - 2072.82] | 83.07 (158.06) [-27.70 - 251.85] | -250.94, 417.09 | 0.606 | (1263.58 - 2704.49) |
| p-Coumaric Acid (µg/g DW) | 201.09 (10.65) [180.56 - 213.29] | 176.29 (10.65) [159.49 - 188.64] | 24.81 (14.34) [21.06 - 28.71] | -5.32, 54.93 | 0.100 | (119.71 - 286.21) |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-40. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 29.22 (1.57) [26.90 - 32.72] | 30.72 (1.57) [28.45 - 33.32] | -1.50 (2.21) [-3.49 - -0.40] | -6.19, 3.20 | 0.508 | (28.33 - 32.37) |
| Neutral Detergent Fiber (% DW) | 41.55 (1.90) [38.01 - 44.93] | 45.54 (1.90) [42.49 - 48.44] | -4.00 (2.45) [-10.43 - -0.77] | -9.22, 1.22 | 0.123 | (37.80 - 47.14) |
| **Mineral** | | | | | | |
| Calcium (% DW) | 0.27 (0.021) [0.24 - 0.31] | 0.30 (0.021) [0.29 - 0.30] | -0.028 (0.027) [-0.051 - 0.013] | -0.085, 0.030 | 0.316 | (0.23 - 0.32) |
| Phosphorus (% DW) | 0.15 (0.010) [0.13 - 0.16] | 0.14 (0.010) [0.13 - 0.15] | 0.0082 (0.014) [-0.0045 - 0.025] | -0.021, 0.037 | 0.561 | (0.14 - 0.17) |
| **Proximate** | | | | | | |
| Ash (% DW) | 3.82 (0.18) [3.61 - 4.09] | 4.10 (0.18) [3.86 - 4.53] | -0.28 (0.25) [-0.92 - 0.18] | -0.82, 0.27 | 0.291 | (4.80 - 5.14) |
| Carbohydrates (% DW) | 88.97 (0.34) [88.28 - 89.83] | 88.38 (0.34) [87.74 - 89.09] | 0.58 (0.47) [-0.81 - 2.08] | -0.41, 1.57 | 0.232 | (87.43 - 89.00) |
| Moisture (% FW) | 73.53 (0.96) [72.40 - 75.30] | 76.57 (0.96) [74.40 - 78.60] | -3.03 (1.17) [-6.20 - -1.40] | -5.50, -0.56 | 0.019 | (74.30 - 77.30) |
| Protein (% DW) | 5.78 (0.24) [5.25 - 6.57] | 6.69 (0.24) [6.25 - 7.47] | -0.91 (0.34) [-1.96 - 0.32] | -1.62, -0.21 | 0.013 | (5.44 - 6.38) |
| Total Fat (% DW) | 1.44 (0.22) [0.58 - 2.34] | 0.82 (0.22) [0.77 - 0.88] | 0.62 (0.29) [-0.31 - 1.58] | 0.00006, 1.24 | 0.049 | (0.58 - 1.42) |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-41. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| **Proximates** | | | | | | |
| Ash (% DW) | 1.39 (0.033) [1.38 - 1.40] | 1.43 (0.033) [1.39 - 1.48] | -0.039 (0.046) [-0.085 - -0.0024] | -0.14, 0.058 | 0.409 | (1.21 - 1.50) |
| Carbohydrates (% DW) | 85.95 (0.24) [85.69 - 86.33] | 85.21 (0.24) [84.92 - 85.65] | 0.74 (0.33) [0.18 - 1.41] | 0.042, 1.44 | 0.038 | (85.53 - 86.50) |
| Moisture (% FW) | 13.60 (0.24) [12.70 - 14.10] | 8.72 (0.24) [8.53 - 8.89] | 4.88 (0.34) [4.17 - 5.26] | 4.15, 5.61 | <0.001 | (9.55 - 13.40) |
| Protein (% DW) | 8.69 (0.19) [8.24 - 8.99] | 9.24 (0.19) [9.20 - 9.28] | -0.54 (0.25) [-1.05 - -0.24] | -1.07, -0.018 | 0.043 | (8.32 - 9.67) |
| Total Fat (% DW) | 3.97 (0.14) [3.80 - 4.06] | 4.13 (0.14) [3.71 - 4.41] | -0.16 (0.19) [-0.36 - 0.088] | -0.56, 0.25 | 0.429 | (3.46 - 3.97) |
| **Fiber** | | | | | | |
| Acid Detergent Fiber (% DW) | 3.14 (0.37) [2.91 - 3.57] | 3.58 (0.37) [3.10 - 3.93] | -0.43 (0.52) [-0.98 - 0.47] | -1.51, 0.65 | 0.412 | (2.85 - 4.42) |
| Neutral Detergent Fiber (% DW) | 9.65 (0.40) [8.85 - 10.54] | 10.89 (0.40) [10.32 - 11.70] | -1.24 (0.57) [-1.47 - -1.08] | -2.42, -0.049 | 0.042 | (7.75 - 10.73) |
| Total Dietary Fiber (% DW) | 13.81 (0.58) [13.02 - 14.43] | 14.17 (0.58) [13.26 - 15.15] | -0.36 (0.70) [-1.18 - 0.33] | -1.84, 1.12 | 0.612 | (11.74 - 14.40) |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-42. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| Calcium (% DW) | 0.0045 (0.00021) [0.0041 - 0.0049] | 0.0044 (0.00021) [0.0042 - 0.0046] | 0.00012 (0.00022) [-0.00022 - 0.00030] | -0.00036, 0.00060 | 0.595 | (0.0038 - 0.0055) |
| Copper (mg/kg DW) | 1.61 (0.80) [1.53 - 1.65] | 4.22 (0.80) [2.17 - 7.76] | -2.61 (1.14) [-6.23 - -0.52] | -4.99, -0.24 | 0.032 | (1.37 - 1.87) |
| Iron (mg/kg DW) | 14.35 (0.37) [13.75 - 14.77] | 15.48 (0.37) [15.20 - 15.70] | -1.13 (0.50) [-1.45 - -0.79] | -2.18, -0.075 | 0.037 | (14.04 - 19.16) |
| Magnesium (% DW) | 0.11 (0.0030) [0.11 - 0.11] | 0.11 (0.0030) [0.11 - 0.12] | -0.0013 (0.0042) [-0.010 - 0.0052] | -0.010, 0.0075 | 0.767 | (0.11 - 0.11) |
| Manganese (mg/kg DW) | 5.84 (0.19) [5.66 - 6.07] | 6.07 (0.19) [5.94 - 6.17] | -0.23 (0.26) [-0.51 - -0.037] | -0.77, 0.32 | 0.397 | (4.59 - 5.18) |
| Phosphorus (% DW) | 0.30 (0.0076) [0.29 - 0.30] | 0.29 (0.0076) [0.28 - 0.32] | 0.00040 (0.011) [-0.025 - 0.019] | -0.022, 0.023 | 0.970 | (0.26 - 0.31) |
| Potassium (% DW) | 0.38 (0.0084) [0.38 - 0.39] | 0.38 (0.0084) [0.38 - 0.38] | 0.0033 (0.011) [-0.0023 - 0.0089] | -0.021, 0.028 | 0.774 | (0.31 - 0.38) |
| Zinc (mg/kg DW) | 18.17 (0.58) [17.33 - 18.67] | 19.13 (0.58) [17.86 - 20.77] | -0.96 (0.78) [-2.10 - -0.26] | -2.60, 0.67 | 0.231 | (15.65 - 19.27) |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-43. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| Alanine (% DW) | 0.62 (0.018) [0.59 - 0.65] | 0.70 (0.018) [0.68 - 0.72] | -0.077 (0.025) [-0.093 - -0.049] | -0.13, -0.025 | 0.006 | (0.62 - 0.70) |
| Arginine (% DW) | 0.40 (0.011) [0.38 - 0.40] | 0.41 (0.011) [0.38 - 0.45] | -0.015 (0.015) [-0.063 - 0.027] | -0.047, 0.018 | 0.352 | (0.34 - 0.42) |
| Aspartic Acid (% DW) | 0.56 (0.012) [0.54 - 0.58] | 0.60 (0.012) [0.59 - 0.61] | -0.045 (0.017) [-0.058 - -0.027] | -0.081, -0.0086 | 0.018 | (0.53 - 0.60) |
| Cystine (% DW) | 0.20 (0.0050) [0.19 - 0.21] | 0.21 (0.0050) [0.20 - 0.22] | -0.0097 (0.0058) [-0.029 - 0.0015] | -0.022, 0.0026 | 0.112 | (0.19 - 0.22) |
| Glutamic Acid (% DW) | 1.60 (0.045) [1.50 - 1.66] | 1.76 (0.045) [1.72 - 1.79] | -0.16 (0.063) [-0.22 - -0.10] | -0.29, -0.027 | 0.020 | (1.53 - 1.76) |
| Glycine (% DW) | 0.33 (0.0050) [0.33 - 0.34] | 0.34 (0.0050) [0.33 - 0.35] | -0.0088 (0.0068) [-0.018 - -0.0014] | -0.023, 0.0057 | 0.218 | (0.31 - 0.33) |
| Histidine (% DW) | 0.27 (0.0053) [0.26 - 0.28] | 0.29 (0.0053) [0.28 - 0.29] | -0.013 (0.0072) [-0.018 - -0.0080] | -0.028, 0.0020 | 0.084 | (0.23 - 0.27) |
| Isoleucine (% DW) | 0.29 (0.0079) [0.28 - 0.31] | 0.33 (0.0079) [0.33 - 0.33] | -0.033 (0.011) [-0.047 - -0.020] | -0.056, -0.0099 | 0.007 | (0.29 - 0.32) |

**Table E-43 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| Leucine (% DW) | 1.04 (0.033) [0.97 - 1.09] | 1.16 (0.033) [1.15 - 1.17] | -0.12 (0.045) [-0.18 - -0.074] | -0.22, -0.026 | 0.015 | (0.97 - 1.14) |
| Lysine (% DW) | 0.27 (0.0050) [0.27 - 0.27] | 0.29 (0.0050) [0.28 - 0.30] | -0.016 (0.0071) [-0.033 - -0.0028] | -0.030, -0.00096 | 0.037 | (0.26 - 0.29) |
| Methionine (% DW) | 0.17 (0.0045) [0.16 - 0.17] | 0.17 (0.0045) [0.16 - 0.18] | -0.0027 (0.0060) [-0.022 - 0.016] | -0.016, 0.010 | 0.658 | (0.17 - 0.19) |
| Phenylalanine (% DW) | 0.43 (0.012) [0.40 - 0.44] | 0.47 (0.012) [0.46 - 0.48] | -0.041 (0.017) [-0.057 - -0.022] | -0.076, -0.0063 | 0.022 | (0.40 - 0.46) |
| Proline (% DW) | 0.81 (0.022) [0.76 - 0.84] | 0.87 (0.022) [0.84 - 0.91] | -0.062 (0.032) [-0.11 - -0.0054] | -0.13, 0.0039 | 0.063 | (0.71 - 0.86) |
| Serine (% DW) | 0.41 (0.013) [0.39 - 0.43] | 0.44 (0.013) [0.42 - 0.46] | -0.030 (0.018) [-0.044 - -0.020] | -0.067, 0.0074 | 0.109 | (0.37 - 0.43) |
| Threonine (% DW) | 0.30 (0.0071) [0.29 - 0.30] | 0.31 (0.0071) [0.30 - 0.33] | -0.017 (0.010) [-0.028 - -0.011] | -0.038, 0.0035 | 0.098 | (0.27 - 0.31) |
| Tryptophan (% DW) | 0.052 (0.0013) [0.048 - 0.055] | 0.050 (0.0013) [0.048 - 0.051] | 0.0019 (0.0018) [-0.00035 - 0.0038] | -0.0019, 0.0058 | 0.304 | (0.048 - 0.052) |

**Table E-43 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| Tyrosine (% DW) | 0.26 (0.021) [0.22 - 0.30] | 0.27 (0.021) [0.20 - 0.32] | -0.0032 (0.030) [-0.10 - 0.097] | -0.067, 0.060 | 0.915 | (0.13- 0.28) |
| Valine (% DW) | 0.43 (0.0095) [0.41 - 0.44] | 0.46 (0.0095) [0.46 - 0.47] | -0.034 (0.013) [-0.049 - -0.020] | -0.062, -0.0065 | 0.018 | (0.40 - 0.45) |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-44. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| 16:0 Palmitic (% Total FA) | 11.35 (0.099) [11.21 - 11.45] | 11.60 (0.099) [11.48 - 11.72] | -0.25 (0.12) [-0.51 - -0.080] | -0.50, 0.0021 | 0.051 | (9.61 - 12.95) |
| 18:0 Stearic (% Total FA) | 1.93 (0.038) [1.84 - 2.01] | 1.89 (0.038) [1.84 - 1.92] | 0.035 (0.053) [-0.0075 - 0.10] | -0.079, 0.15 | 0.527 | (1.39 - 2.21) |
| 18:1 Oleic (% Total FA) | 21.26 (0.22) [20.92 - 21.89] | 21.03 (0.22) [20.83 - 21.29] | 0.23 (0.27) [-0.33 - 1.06] | -0.36, 0.82 | 0.412 | (21.04 - 31.63) |
| 18:2 Linoleic (% Total FA) | 63.60 (0.26) [62.77 - 64.18] | 63.64 (0.26) [63.46 - 63.85] | -0.043 (0.31) [-1.07 - 0.57] | -0.70, 0.61 | 0.890 | (54.81 - 65.11) |
| 18:3 Linolenic (% Total FA) | 1.23 (0.017) [1.22 - 1.24] | 1.21 (0.017) [1.18 - 1.23] | 0.019 (0.023) [0.0040 - 0.041] | -0.029, 0.067 | 0.414 | (1.14 - 1.60) |
| 20:0 Arachidic (% Total FA) | 0.32 (0.0063) [0.30 - 0.33] | 0.31 (0.0063) [0.29 - 0.31] | 0.012 (0.0081) [-0.0081 - 0.034] | -0.0054, 0.029 | 0.168 | (0.32 - 0.35) |
| 20:1 Eicosenoic (% Total FA) | 0.18 (0.0024) [0.18 - 0.18] | 0.19 (0.0024) [0.18 - 0.19] | -0.0057 (0.0033) [-0.015 - 0.0041] | -0.013, 0.0013 | 0.103 | (0.20 - 0.25) |
| 22:0 Behenic (% Total FA) | 0.14 (0.021) [0.13 - 0.15] | 0.14 (0.021) [0.12 - 0.18] | 0.0034 (0.029) [-0.035 - 0.032] | -0.057, 0.064 | 0.906 | (0.063 - 0.16) |

[1]FA – fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-45. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| Folic Acid (mg/kg DW) | 0.27 (0.024) [0.23 - 0.31] | 0.31 (0.024) [0.24 - 0.39] | -0.032 (0.026) [-0.11 - 0.025] | -0.089, 0.026 | 0.251 | (0.27 - 0.36) |
| Niacin (mg/kg DW) | 24.28 (1.34) [20.00 - 28.64] | 23.37 (1.34) [21.92 - 24.71] | 0.91 (1.73) [-1.92 - 3.93] | -2.79, 4.62 | 0.605 | (24.60 - 28.88) |
| Thiamine HCl (mg/kg DW) | 2.97 (0.12) [2.86 - 3.14] | 2.85 (0.12) [2.74 - 2.95] | 0.12 (0.17) [-0.088 - 0.29] | -0.23, 0.47 | 0.468 | (2.88 - 3.66) |
| Vitamin B2 (mg/kg DW) | 2.15 (0.22) [1.79 - 2.42] | 2.11 (0.22) [1.96 - 2.27] | 0.036 (0.31) [-0.49 - 0.46] | -0.62, 0.69 | 0.908 | (1.91 - 2.75) |
| Vitamin B6 (mg/kg DW) | 5.43 (0.21) [5.41 - 5.46] | 5.51 (0.21) [5.25 - 5.71] | -0.075 (0.30) [-0.29 - 0.22] | -0.71, 0.56 | 0.804 | (4.19 - 6.07) |
| Vitamin E (mg/kg DW) | 11.52 (0.53) [10.34 - 12.37] | 10.57 (0.53) [10.05 - 10.94] | 0.95 (0.71) [-0.40 - 2.32] | -0.56, 2.46 | 0.201 | (5.20 - 9.95) |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-46. Comparison of the Anti-nutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E. [1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| **Anti-nutrient** | | | | | | |
| Phytic Acid (% DW) | 0.78 (0.042) [0.75 - 0.80] | 0.69 (0.042) [0.63 - 0.74] | 0.093 (0.060) [0.052 - 0.17] | -0.032, 0.22 | 0.136 | (0.55 - 0.73) |
| Raffinose (% DW) | 0.10 (0.0064) [0.092 - 0.11] | 0.091 (0.0064) [0.081 - 0.11] | 0.0094 (0.0090) [-0.010 - 0.028] | -0.0094, 0.028 | 0.308 | (0.029 - 0.069) |
| **Secondary Metabolite** | | | | | | |
| Ferulic Acid (µg/g DW) | 2079.28 (116.42) [2034.88 - 2119.13] | 1986.45 (116.42) [1898.80 - 2066.25] | 92.82 (158.06) [40.58 - 185.01] | -241.19, 426.84 | 0.564 | (1503.59 - 2078.52) |
| p-Coumaric Acid (µg/g DW) | 192.51 (10.65) [181.40 - 201.40] | 189.88 (10.65) [175.61 - 202.72] | 2.62 (14.34) [-21.32 - 25.79] | -27.50, 32.75 | 0.856 | (127.14 - 277.33) |

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

**Table E-47. Summary of Significant Differences in Additional Secondary Metabolite Composition (p<0.05) Comparing MON 87460 to the Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered and Water-Limited Conditions**

| Tissue/Site/ Components (Units)[1] | Mean MON 87460 | Mean Control | Mean Diff (% of Control) | Signif. (p-value) | MON 87460 (Range) | Commercial (Range) |
|---|---|---|---|---|---|---|
| **Forage** | | | | | | |
| **Well-watered** | | | | | | |
| Fructose (% DW) | 5.30 | 6.95 | -23.77 | 0.005 | (4.40 - 6.31) | (6.50 - 8.09) |
| Glucose (% DW) | 6.09 | 7.73 | -21.18 | 0.005 | (5.22 - 6.96) | (7.93 - 8.97) |
| **Water-Limited** | | | | | | |
| Glucose (% DW) | 6.10 | 7.53 | -18.94 | 0.012 | (5.55 - 6.81) | (6.72 - 7.58) |
| **Grain** | | | | | | |
| **Well-watered** | | | | | | |
| Abscisic Acid (ppb FW) | 10.80 | 18.93 | -42.94 | 0.001 | (9.41 - 12.60) | (23.00 - 35.80) |
| Glucose (% DW) | 0.47 | 0.57 | -17.65 | 0.021 | (0.46 - 0.48) | (0.41 - 0.64) |
| Sucrose (% DW) | 1.81 | 2.22 | -18.67 | 0.002 | (1.76 - 1.85) | (2.07 - 2.74) |
| **Water-Limited** | | | | | | |
| Abscisic Acid (ppb FW) | 8.04 | 23.13 | -65.24 | <0.001 | (7.02 - 8.66) | [(9.30 - 30.500 |
| Sucrose (% DW) | 1.84 | 2.10 | -12.24 | 0.040 | (1.67 - 2.08) | (2.12 - 2.59) |

[1]DW= dry weight; FW=fresh weight.

**Table E-48. Comparison of the Additional Secondary Metabolite Content in Forage from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| Free Proline (% DW) | 0.020 (0.0017) [0.017 - 0.022] | 0.018 (0.0017) [0.017 - 0.019] | 0.0019 (0.0023) [-0.0015 - 0.0042] | -0.0030, 0.0069 | 0.422 | (0.014 - 0.015) |
| Abscisic Acid (ppb FW) | 15.70 (4.35) [14.60 - 16.90] | 12.53 (4.35) [10.50 - 14.00] | 3.17 (5.90) [1.50 - 6.40] | -9.39, 15.72 | 0.599 | (13.00 - 19.80) |
| Choline (ppm FW) | 127.67 (6.53) [121.00 - 135.00] | 120.00 (6.53) [111.00 - 133.00] | 7.67 (8.71) [-6.00 - 24.00] | -10.78, 26.11 | 0.391 | (111.00 - 115.00) |
| Glycerol (% DW) | 0.14 (0.0084) [0.14 - 0.14] | 0.14 (0.0084) [0.12 - 0.16] | 0.0019 (0.011) [-0.026 - 0.028] | -0.021, 0.025 | 0.864 | (0.12 - 0.18) |
| Glycine Betaine (ppm FW) | 63.53 (5.24) [58.40 - 69.80] | 57.83 (5.24) [51.60 - 62.80] | 5.70 (6.24) [-4.40 - 10.80] | -7.73, 19.13 | 0.376 | (49.80 - 64.20) |
| Salicylic Acid (ppm FW) | 0.098 (0.040) [0.088 - 0.11] | 0.16 (0.040) [0.091 - 0.21] | -0.058 (0.057) [-0.12 - 0.020] | -0.18, 0.061 | 0.321 | (0.075 - 0.15) |
| Fructose (% DW) | 5.30 (0.46) [4.40 - 6.31] | 6.95 (0.46) [6.22 - 7.44] | -1.65 (0.51) [-2.26 - -0.88] | -2.74, -0.57 | 0.005 | (6.50 - 8.09) |
| Glucose (% DW) | 6.09 (0.52) [5.22 - 6.96] | 7.73 (0.52) [6.99 - 8.15] | -1.64 (0.50) [-2.05 - -1.10] | -2.71, -0.57 | 0.005 | (7.93 - 8.97) |
| Sucrose (% DW) | 3.80 (1.31) [2.32 - 4.74] | 2.53 (1.31) [2.08 - 2.93] | 1.27 (1.74) [-0.25 - 2.25] | -2.41, 4.95 | 0.475 | (0.11 - 3.77) |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

**Table E-49. Comparison of the Additional Secondary Metabolite Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| Free Proline (% DW) | 0.072 (0.0030) [0.068 - 0.076] | 0.076 (0.0030) [0.075 - 0.078] | -0.0041 (0.0039) [-0.0091 - 0.0016] | -0.012, 0.0042 | 0.313 | (0.048 - 0.059) |
| Abscisic Acid (ppb FW) | 10.80 (1.71) [9.41 - 12.60] | 18.93 (1.71) [17.60 - 20.30] | -8.13 (2.05) [-8.50 - -7.70] | -12.48, -3.78 | 0.001 | (23.00 - 35.80) |
| Choline (ppm FW) | 210.67 (12.86) [206.00 - 219.00] | 227.33 (12.86) [213.00 - 248.00] | -16.67 (17.84) [-29.00 - -6.00] | -54.25, 20.92 | 0.363 | (194.00 - 265.00) |
| Glycerol (% DW) | 0.019 (0.0019) [0.018 - 0.022] | 0.019 (0.0019) [0.016 - 0.024] | 0.00018 (0.0025) [-0.0057 - 0.0061] | -0.0051, 0.0054 | 0.944 | (0.018 - 0.025) |
| Glycine Betaine (ppm FW) | 1.89 (0.17) [1.69 - 2.08] | 1.75 (0.17) [1.61 - 1.82] | 0.14 (0.23) [0.080 - 0.26] | -0.35, 0.64 | 0.548 | (0.50 - 3.34) |
| Salicylic Acid (ppm FW) | 0.092 (0.026) [0.076 - 0.12] | 0.12 (0.026) [0.095 - 0.15] | -0.032 (0.030) [-0.043 - -0.019] | -0.099, 0.035 | 0.317 | (0.069 - 0.32) |
| Fructose (% DW) | 0.46 (0.031) [0.44 - 0.48] | 0.54 (0.031) [0.54 - 0.54] | -0.081 (0.040) [-0.10 - -0.067] | -0.17, 0.0034 | 0.058 | (0.41 - 0.65) |
| Glucose (% DW) | 0.47 (0.031) [0.46 - 0.48] | 0.57 (0.031) [0.53 - 0.60] | -0.10 (0.039) [-0.12 - -0.071] | -0.18, -0.017 | 0.021 | (0.41 - 0.64) |
| Sucrose (% DW) | 1.81 (0.083) [1.76 - 1.85] | 2.22 (0.083) [2.10 - 2.38] | -0.41 (0.12) [-0.53 - -0.30] | -0.66, -0.17 | 0.002 | (2.07 - 2.74) |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

**Table E-50. Comparison of the Additional Secondary Metabolite Content in Forage from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| | | | Difference (Test minus Control) | | | |
|---|---|---|---|---|---|---|
| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
| Free Proline (% DW) | 0.024 (0.0017) [0.019 - 0.027] | 0.020 (0.0017) [0.018 - 0.021] | 0.0040 (0.0023) [-0.0020 - 0.0076] | -0.00095, 0.0089 | 0.106 | (0.012 - 0.027) |
| Abscisic Acid (ppb FW) | 17.10 (4.35) [13.80 - 21.40] | 11.56 (4.35) [9.98 - 14.70] | 5.54 (5.90) [3.82 - 6.70] | -7.01, 18.09 | 0.362 | (14.10 - 28.70) |
| Choline (ppm FW) | 127.67 (6.53) [116.00 - 139.00] | 117.00 (6.53) [101.00 - 134.00] | 10.67 (8.71) [0 - 27.00] | -7.78, 29.11 | 0.238 | (115.00 - 119.00) |
| Glycerol (% DW) | 0.17 (0.0084) [0.15 - 0.20] | 0.15 (0.0084) [0.14 - 0.16] | 0.022 (0.011) [0.0014 - 0.052] | -0.0017, 0.045 | 0.067 | (0.14 - 0.17) |
| Glycine Betaine (ppm FW) | 51.17 (5.24) [42.20 - 61.10] | 52.97 (5.24) [41.90 - 66.60] | -1.80 (6.24) [-5.50 - 0.30] | -15.23, 11.63 | 0.777 | (46.60 - 64.00) |
| Salicylic Acid (ppm FW) | 0.098 (0.040) [0.073 - 0.15] | 0.11 (0.040) [0.078 - 0.18] | -0.017 (0.057) [-0.035 - -0.0042] | -0.14, 0.10 | 0.769 | (0.077 - 0.41) |
| Fructose (% DW) | 5.48 (0.46) [5.00 - 5.97] | 6.53 (0.46) [6.33 - 6.68] | -1.05 (0.51) [-1.68 - -0.61] | -2.14, 0.030 | 0.055 | (5.68 - 6.42) |
| Glucose (% DW) | 6.10 (0.52) [5.55 - 6.81] | 7.53 (0.52) [7.51 - 7.56] | -1.43 (0.50) [-1.96 - -0.70] | -2.49, -0.36 | 0.012 | (6.72 - 7.58) |
| Sucrose (% DW) | 2.33 (1.31) [1.14 - 4.26] | 3.06 (1.31) [1.49 - 5.56] | -0.72 (1.74) [-3.96 - 2.14] | -4.40, 2.96 | 0.682 | (0.095 - 4.24) |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

**Table E-51. Comparison of the Additional Secondary Metabolite Content in Grain from MON 87460 and Conventional Control from the QUI Site in Chile during 2006/2007 under Water-Limited Conditions**

| Analytical Component[1] | Test Mean ± S.E.[1] [Range] | Control Mean ± S.E. [Range] | Difference (Test minus Control) Mean ± S.E. [Range] | 95% CI[1] (Lower,Upper) | p-Value | Commercial (Range) |
|---|---|---|---|---|---|---|
| Free Proline (% DW) | 0.068 (0.0030) [0.064 - 0.075] | 0.073 (0.0030) [0.067 - 0.078] | -0.0045 (0.0039) [-0.012 - 0.0076] | -0.013, 0.0038 | 0.268 | (0.044 - 0.067) |
| Abscisic Acid (ppb FW) | 8.04 (1.71) [7.02 - 8.66] | 23.13 (1.71) [22.50 - 23.90] | -15.09 (2.05) [-16.88 - -13.84] | -19.44, -10.74 | <0.001 | (19.30 - 30.50) |
| Choline (ppm FW) | 231.00 (12.86) [209.00 - 265.00] | 234.00 (12.86) [212.00 - 262.00] | -3.00 (17.84) [-53.00 - 53.00] | -40.59, 34.59 | 0.868 | (175.00 - 235.00) |
| Glycerol (% DW) | 0.019 (0.0019) [0.018 - 0.020] | 0.018 (0.0019) [0.014 - 0.024] | 0.0016 (0.0025) [-0.0032 - 0.0042] | -0.0036, 0.0069 | 0.520 | (0.017 - 0.021) |
| Glycine Betaine (ppm FW) | 2.09 (0.17) [1.99 - 2.18] | 2.10 (0.17) [1.63 - 2.44] | -0.010 (0.23) [-0.34 - 0.36] | -0.50, 0.48 | 0.966 | (0.50 - 2.91) |
| Salicylic Acid (ppm FW) | 0.094 (0.026) [0.087 - 0.10] | 0.14 (0.026) [0.11 - 0.18] | -0.048 (0.030) [-0.080 - -0.019] | -0.11, 0.019 | 0.143 | (0.055 - 0.38) |
| Fructose (% DW) | 0.50 (0.031) [0.41 - 0.57] | 0.48 (0.031) [0.45 - 0.52] | 0.022 (0.040) [-0.038 - 0.054] | -0.063, 0.11 | 0.586 | (0.38 - 0.66) |
| Glucose (% DW) | 0.52 (0.031) [0.45 - 0.57] | 0.48 (0.031) [0.47 - 0.52] | 0.031 (0.039) [-0.021 - 0.063] | -0.053, 0.11 | 0.449 | (0.38 - 0.66) |
| Sucrose (% DW) | 1.84 (0.083) [1.67 - 2.08] | 2.10 (0.083) [1.96 - 2.18] | -0.26 (0.12) [-0.51 - -0.086] | -0.50, -0.013 | 0.040 | (2.12 - 2.59) |

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

## Appendix F. Phenotypic and Agronomic Assessment: Individual Site Results from 2006 and 2007 Field Studies

### F.1. Materials

The materials for phenotypic, agronomic, and plant pest assessment studies are included in Tables F-2 – F-7, and planting information for each site is reported in Tables F-8 – F-13. The presence or absence of the MON 87460 insert in the test and control materials was confirmed by event-specific PCR analyses.

### F.2. Test Sites

#### F.2.1. 2006 and 2007 U.S. Field Studies under Well-Watered Conditions

Field trials included eight test sites that were established as well watered locations in 2006: (site codes in parenthesis): Jefferson Co., IA (IA1), Benton, Co., IA (IA2), Stark Co., IL (IL1), Warren Co., IL (IL2), Boone Co., IN (IN1), Parke Co., IN (IN2), Pawnee Co., KS (KS) and York Co., NE (Tables F-1, F-2, and F-8); and nine sites in 2007 (site codes in parenthesis): Jefferson County, IA (IA1); Van Horne County, IA ( IA2); Stark County, IL ( IL1); Warren County, IL (IL2); Clinton County, IL (IL3); Boone County, IN (IN); York County, NE (NE); Fayette County, OH (OH); and Berks County, PA (Tables F-1, F-3, and F-9).

#### F.2.2. 2006 and 2007 U.S. and Chilean Field Studies Established with Well-Watered and Water-Limited Treatments

In 2006/2007, a Chile field study was established at four sites: Calera de Tango, Region Metropolitana de Santiago (CT); Colina, Region Metropolitana de Santiago (CL); Lumbreras, Region Metropolitana de Santiago (LUM); and Quillota, Region de Valparaiso (QUI) (Tables F-1, F-4, and F-10)

In 2007, two U.S. field studies were established. Study-1 had two sites: Sutter County, CA (CA) and Carson, County, TX (Tables F-1, F-5, and F-11). Study-2 had three sites: Pawnee County, KS (KS); York County Nebraska (NE); and Carson County, TX (Tables F-1, F-6, and F-12).

#### F.2.3. 2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices

In 2006, five sites where water was managed according to local agronomic practices were established (site codes in parenthesis): Benton County, IA (IAE); Greene County, IA (IAW); Stark County, IL (IL); Parke County, IN (IN); and York County, NE (Tables F-1, F-7, and F-13).

## F.3. Statistical Analyses

Statistical analyses were performed by the Monsanto Statistics Technology Center. SAS was used for analysis and contrast statements in SAS were used to compare the test to the control. The level of statistical significance was 5% ($\alpha = 0.05$). No statistical comparisons were made between the test and reference substances. The reference range, defined as the minimum and maximum mean values, was determined from the references among the sites. The means of the test and control substance and the results of the statistical analyses are reported. Standard error was calculated from the raw data. Each test and control mean has its own individual standard deviation and a different standard error. The characteristics reported for each substance type include: seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture percentage, test weight, insect abundance, European corn borer and corn earworm damage, and yield.

## F.4. Individual Field Study Results

### F.4.1. 2006 U.S. Field Study under Well-Watered Conditions

The results of the 14 phenotypic or agronomic characteristics evaluated at eight well-watered sites in 2006 are presented in Table F-14. In the individual site analyses, no differences between MON 87460 and the control were detected for seedling vigor, days to 50% pollen shed, days to 50% silking, ear height, plant height, dropped ears, grain moisture, or test weight at any location. A total of seven out of 96 site × characteristic comparisons were significantly different between MON 87460 and the control. The differences were distributed among six of the 14 phenotypic characteristics. MON 87460 had a higher early stand count than the control at IL1 (104.3 vs. 86.0) and a lower early stand count than the control at NE (102.0 vs. 114.3). MON 87460 had lower stay green (less green tissue) than the control at IA2 (4.0 vs. 5.0). MON 87460 had more stalk lodged plants than the control at NE (9.0 vs. 5.0) and more root lodged plants than the control at IL1 (23.7 vs. 2.7). MON 87460 had a higher final stand than the control at IL1 (94.0 vs. 77.3). Yield was lower for MON 87460 compared to the control at KS (159.4 vs. 182.3).

The differences detected in the individual site analyses for early stand count, stay green, stalk lodging, final stand, and yield were not detected in the combined-site analysis (Table VIII-4). Thus, the differences detected for these phenotypic characteristics in the by-site analysis were not indicative of a consistent trend in the data and are unlikely to be biologically meaningful in terms of increased weed potential of MON 87460 compared to the control (Figure VIII-1, Step 2). The results of this study support a conclusion that the introduction of the drought tolerance trait did not unexpectedly alter the assessed phenotypic characteristics of MON 87460 compared to conventional corn. Thus, the results support a conclusion of no increased pest potential or significant environmental impact of MON 87460 compared to conventional corn.

### F.4.2. 2007 U.S. Field Study under Well-Watered Conditions

The results of the 14 phenotypic or agronomic characteristics evaluated at nine well-watered sites in 2007 are presented in Table F-15. In the individual analysis, no differences between MON 87460 and the control were detected for seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant height, root lodged plants, final stand count, test weight, and yield. Eight differences were detected in the individual site analysis in the 2007 trials spanning four assessments. Stay green values were higher for MON 87460 vs. the control (less green tissue) than the control at IA2 (5.7 vs. 5.0), at IL1 (6.7 vs. 5.3), and at IL3 (4.0 vs. 3.0, respectively at each site). Dropped ears were greater for MON 87460 compared to the control at PA (1.3 vs. 0.0 respectively). MON 87460 had more stalk lodged plants than the control at OH (3.7 vs. 1.0) and at PA (2.3 vs. 0.3, respectively at each site). MON 87460 was higher in grain moisture than the control at IA2 (18.0 vs. 17.1, respectively), but was lower in grain moisture than the control at IL3 (13.0 vs. 14.7, respectively). Differences in stay green, dropped ears, stalk lodged plants, and grain moisture were not observed in the combined-site analysis reported in Table VIII-5). Thus, the differences detected for these phenotypic characteristics are not indicative of a consistent response associated with the trait and are unlikely to be biologically meaningful in terms of increased pest potential of MON 87460 compared to the control (Figure VIII-1, Step 2). The results of this study support a conclusion that the introduction of the drought tolerance trait did not unexpectedly alter the assessed phenotypic characteristics of MON 87460 compared to conventional corn and a conclusion of no increased pest potential or significant environmental impact of MON 87460 compared to conventional corn under well watered conditions.

### F.4.3. 2006/2007 Chilean Field Study Established with Well-Watered and Water-Limited Treatments

The results of the 14 phenotypic or agronomic characteristics evaluated at three sites in Chile for the well-watered treatment in 2006/2007 are presented in Table F-16. In the individual site analysis of the well-watered treatment, no significant differences were detected at the CL, CT, and LUM sites for any measured characteristic supporting the hypothesis of no statistical differences between MON 87460 and the control under well-watered conditions.

The results of the 14 phenotypic or agronomic characteristics evaluated at three sites in Chile for the water-limited treatment in 2006/2007 are presented in Table F-17. Data for a fourth site, QUI, are reported in Appendix G. In the individual site analysis of the water-limited treatment, no significant differences were detected between MON 87460 and the control at the CL, CT, and LUM sites for seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, and test weight. Ear height and plant height were higher for MON 87460 compared to the control at the CL site (61.0 vs. 51.4 in; 97.2 vs. 79.3 in, respectively). Yield was higher for MON 87460 than the control for the CT site (160.9 vs. 123.7 bu/A, respectively). The differences detected in ear height and plant height were not detected when the data were pooled across all three sites (Table VIII-7). Thus, the

differences detected for these phenotypic characteristics are not indicative of a consistent response associated with the trait and are unlikely to be biologically meaningful in terms of increased pest potential of MON 87460 compared to the control. (Figure VIII-1, Step 2). However, the difference in yield detected at the CT site is consistent with the expected yield benefit of this trait and was also detected in the combined-site analysis (Table VIII-7). Furthermore, statistical differences in ear height and plant height are expected differences between MON 87460 and the control under water-limited conditions (Figure VIII-2).

The results of this study support a conclusion of no increased pest potential or significant environmental impact for MON 87460 when grown under well-watered or water-limited conditions in the field.

### F.4.4. 2007 U.S. Field Study-1 Established with Well-Watered and Water-Limited Treatments

The results of the 14 phenotypic or agronomic characteristics evaluated at two sites with the well-watered treatment in Study-1 in 2007 are presented in Table F-18. In the individual-site analysis, no differences between MON 87460 and control were detected for seedling vigor, days to 50% pollen shed, days to 50% silking, ear height, dropped ears, root lodged plants, final stand count, grain moisture, and test weight (TX site only) in the well-watered treatment. Five comparisons between MON 87460 and the control were significantly different in the individual-site analysis under well-watered conditions. At the CA site, MON 87460 had higher early stand count than the control at (75.8 vs. 70.0 plants), MON 87460 had more stalked lodged plants than the control (0.5 vs. 0.0 plants) and MON 87460 had more yield than the control (239.7 vs. 181.4 bu/acre). At the TX site, MON 87460 had a lower stay green value (more green tissue) than the control (1.8 vs. 3.5), and MON 87460 was shorter than the control (70.4 vs. 75.1 in.). At the CA site, mean values for MON 87460 for early stand count (75.8 plants) and stalk lodged plants (0.5 plants) are within the range of reference hybrid values (Table VIII-8). Therefore, the differences in early stand count and stalk lodged plants are unlikely to be biologically meaningful in terms of increased weed potential of MON 87460 compared to the control (Figure VIII-1, Step 3). The differences reported in Table F-18 at CA for yield of MON 87460 (239.7 bushel/acre), and at TX for stay green (1.8 rating) and for plant height (70.4 inches) are not interpreted as characteristics that would confer an increase in weediness potential. The differences detected for yield, stay green and plant height were not detected in the combined-site analysis for the well-watered treatment (Table VIII-8). Therefore, the differences detected in the individual site analysis are unlikely to be adverse and do not contribute to a biological or environmental change for MON 87460 in terms of pest potential.

The results of the 14 phenotypic or agronomic characteristics evaluated at two sites with the water-limited treatment in Study-1 in 2007 are presented in Table F-19. In the individual-site analysis, no differences between MON 87460 and the control were detected for seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants,

final stand count, grain moisture, and test weight in the water-limited treatment). Two comparisons between MON 87460 and the control were significantly different in the individual-site analysis under water-limited conditions. At the CA site, MON 87460 had more yield than the control (188.3 vs. 148.1 bu/acre, respectively). At the TX site, MON 87460 had a lower stay green value (more green tissue) than the control (1.7 vs. 4.3 respectively). The difference detected in stay green is not interpreted as a characteristic that would confer an increase in weediness potential. Furthermore, the difference in stay green was not detected in the combined-site analysis for plants in the water-limited treatment (Table VIII-9). Therefore, the difference detected in the individual site analysis for stay green is likely not adverse and does not contribute to a biological or environmental change for MON 87460 in terms of pest potential.

### F.4.5. 2007 U.S. Field Study-2 Established with Well-Watered and Water-Limited Treatments

The results of the 14 phenotypic or agronomic characteristics evaluated at three sites with the well-watered treatment in Study-2 in 2007 are presented in Table F-20. In the individual-site analysis, no differences between MON 87460 and control were detected for seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, test weight, and yield in the well-watered treatment. Three comparisons between MON 87460 and the control were significantly different in the individual-site analysis under well-watered conditions. MON 87460 had a higher stay green rating at NE (4.7 vs. 4.0) and a lower stay green rating at TX (6.3 vs. 8.0, respectively) than the control. MON 87460 was higher in grain moisture than the control at KS (13.7 vs. 12.4, respectively). The differences detected in stay green and grain moisture are within the range of reference hybrid values (Table VIII-12). Therefore, the differences in stay green and grain moisture are unlikely to be biologically meaningful in terms of increased weed potential of MON 87460 compared to the control (Figure VIII-1, Step 3).

Results from the water-limited treatment are presented in Section VIII.C.2 and Appendix G. TX was the only site that met the inclusion criteria (Section VIII.C, Table VIII-3). Data for the water-limited treatment at TX are presented in Section VIII.C.2.2, Table VIII-11. Data for the water-limited treatment at KS and NE are presented in Appendix G, Table G-3.

The results of this study support a conclusion that MON 87460 was not unexpectedly altered for the assessed phenotypic characteristics compared to conventional corn under water-limited conditions. Thus, the results support a conclusion of no increased pest potential or significant environmental impact of MON 87460 compared to a conventional control under well-watered or water-limited conditions.

### F.4.6. 2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices

The results of the 14 phenotypic or agronomic characteristics evaluated at five sites where water was managed by local agronomic practices in 2006 are presented in Table F-21. In the individual site analysis, no differences were detected between MON 87460 and the control at any site for early stand count, days to 50% pollen shed, stay green rating, ear height, plant height, dropped ears, final stand count, grain moisture, test weight, and yield. A total of four differences were detected across four different characteristics. At the NE site, MON 87460 exhibited lower vigor than the control (7.0 vs. 8.0) and greater days to 50% silking (65.0 vs. 64.0). At the IN site MON 87460 exhibited more stalk lodged plants and root lodged plants than the control (8.3 vs. 4.7 plants, and 3.3 vs. 0.3 plants, respectively). The differences detected in vigor, days to 50% silking, stalk lodging and root lodging were not detected when the data were pooled across all five sites (Table VIII-12). Thus, the differences detected for these phenotypic characteristics are not indicative of a consistent response associated with the trait and are unlikely to be biologically meaningful in terms of increased weed potential under well-watered conditions.

**Table F-1. Field Phenotypic Evaluation Sites for MON 87460 during 2006 and 2007**

| Water Management | Study Year | Location | Site Code |
|---|---|---|---|
| Well-watered | 2006 | Jefferson Co.. Iowa | IA1 |
| (randomized complete block design) | | Benton Co., Iowa | IA2 |
| | | Stark Co., Illinois | IL1 |
| | | Warren Co., Illinois | IL2 |
| | | Boone Co., Indiana | IN1 |
| | | Parke Co., Indiana | IN2 |
| | | Pawnee Co., Kansas | KS |
| | | York Co., Nebraska | NE |
| (randomized complete block design) | 2007 | Jefferson Co., Iowa | IA1 |
| | | Van Horne Co., Iowa | IA2 |
| | | Stark Co., Illinois | IL1 |
| | | Warren Co., Illinois | IL2 |
| | | Clinton Co., Illinois | IL3 |
| | | Boone Co., Indiana | IN |
| | | York Co., Nebraska | NE |
| | | Fayette Co., Ohio | OH |
| | | Berks Co., Pennsylvania | PA |
| Well-watered and water-limited | 2006/2007 | Calera de Tango, Chile[1] | CT |
| (strip-plot design) | | Colina, Chile[1] | CL |
| | | Lumbreras, Chile[1] | LUM |
| | | Quillota, Chile[1] | QUI |
| (split-plot design) | 2007 Study-1 | Sutter Co., California | CA |
| | | Carson Co., Texas | TX |
| (strip-plot design) | 2007 Study-2 | Pawnee Co., Kansas | CA |
| | | York Co., Nebraska | NE |
| | | Carson Co., Texas | TX |
| Typical agronomic practices, | 2006 | Benton Co., Iowa | IAE |
| (rainfed and normal irrigation) | | Greene Co., Iowa | IAW |
| (randomized complete block design) | | Stark Co., Illinois | IL |
| | | Parke Co., Indiana | IN |
| | | York Co., Nebraska | NE |

**Table F-2. Test, Control, and Reference Substances in the 2006 U.S. Field Study under Well-Watered Conditions**

| Corn Hybrid Names[1] | Seed Types | Site |
|---|---|---|
| MON 87460 | Test | All |
| H1548126 | Control | All |
| DKC 61-42 | Reference | IA1, IA2 |
| DKC 60-15 | Reference | IA1, IA2 |
| DKC 63-78 | Reference | IA1, IA2 |
| 33N29 | Reference | IA1, IA2 |
| 33K39 | Reference | IL1, IL2 |
| M-3744 | Reference | IL1, IL2 |
| M-3765 | Reference | IL1, IL2 |
| RX715AR | Reference | IL1, IL2 |
| 631 MF-B17 | Reference | IN1, IN2 |
| 33N56 | Reference | IN1, IN2 |
| H8920 | Reference | IN1, IN2 |
| RX715AR | Reference | IN1, IN2 |
| S-2721 | Reference | KS |
| 32B33 | Reference | KS |
| 33H25 | Reference | KS |
| H8991 | Reference | KS |
| G-8424 | Reference | NE |
| NC+4822 | Reference | NE |
| 34N43 | Reference | NE |
| DKC 61-50 | Reference | NE |

[1]Test and control substance names are a Monsanto Regulatory ID. Reference substance names are commercial corn hybrid designations.

**Table F-3. Test, Control, and Reference Substances in the 2007 U.S. Field Study under Well-Watered Conditions**

| Corn Hybrid Names[1] | Seed Types | Site |
|---|---|---|
| MON 87460 | Test | All |
| DM1718 | Control | All |
| DKC 57-83 | Reference | IL2, IL3, OH, PA |
| DKC 60-15 | Reference | IA1, IL1, IL3, OH |
| G-8424 | Reference | IA1, IA2, IL2, IN |
| RX 715 | Reference | IA1, IA2, IL2, IL3 |
| DKC 61-42 | Reference | IA2, IN, NE, PA |
| DKC 61-50 | Reference | IA2, IL1 |
| H-8920 | Reference | IL1,NE |
| RX754RR | Reference | OH, PA |
| DKC 63-78 | Reference | IL1, IL3, IN, PA |
| Pioneer 33H25 | Reference | IA1, IN, NE |
| DKC 64-27 | Reference | IL2, NE, OH |

[1]Test and control substance names are a Monsanto Regulatory ID. Reference substance names are commercial corn hybrid designations.

**Table F-4. Test, Control, and Reference Corns used in the 2006/2007 Chilean Field Study Established with Well-Watered and Water-Limited Treatments**

| Corn Hybrid Names[1] | Seed Types | Site |
|---|---|---|
| MON 87460 | Test | All Sites |
| DM1718 | Control | All Sites |
| 33N09 | Reference | CT |
| 33K39 | Reference | CT |
| BT 6613 | Reference | CT |
| DKC63-78 | Reference | CT |
| 33D11 | Reference | CL |
| BT 6011 | Reference | CL |
| Garst 8424 | Reference | CL |
| DKC62-30 | Reference | CL |
| 33N29 | Reference | LUM |
| Garst 8445 | Reference | LUM |
| DKC61-50 | Reference | LUM |
| RX 715 | Reference | LUM |
| 34N43 | Reference | QUI |
| BT 6610 | Reference | QUI |
| Garst 8545 | Reference | QUI |
| DKC60-15 | Reference | QUI |

[1]Test and control substance names are a Monsanto Regulatory ID. Reference substance names are commercial corn hybrid designations.

**Table F-5. Study-1, Test, Control, and Reference Corns used in the 2007 U.S. Field Studies Established with Well-Watered and Water-Limited Treatments**

| Corn Hybrid Names[1] | Seed Types | Site Code |
|---|---|---|
| MON 87460 | Test | CA, TX |
| DM1718 | Control | CA, TX |
| DKC63-78 | Reference | CA, TX |
| RX715 | Reference | CA |
| RX754RR2 | Reference | CA |
| DKC61-50 | Reference | CA |
| 33H25 | Reference | TX |
| DKC61-42 | Reference | TX |
| DKC57-83 | Reference | TX |

[1]Test and control substance names are a Monsanto Regulatory ID. Reference substance names are commercial corn hybrid designations.

**Table F-6. Study-2, Test, Control, and Reference Corns used in the 2007 U.S. Field Studies Established with Well-Watered and Water-Limited Treatments**

| Corn Hybrid Names[1] | Substance Type | Phenotype | Sites |
|---|---|---|---|
| MON 87460 | Test | drought tolerant | All |
| DM 1718 | Control | conventional | All |
| Burrus 645 | Reference | conventional | KS |
| DKC63-78 | Reference | conventional | KS |
| 33H25 | Reference | conventional | KS |
| RX 772 | Reference | conventional | KS |
| 33D11 | Reference | conventional | NE |
| DKC62-30 | Reference | conventional | NE |
| BT 6613 | Reference | conventional | NE |
| Garst 8445 | Reference | conventional | NE |
| SC 1085 | Reference | conventional | TX |
| DKC57-01 | Reference | conventional | TX |
| RX 708 | Reference | conventional | TX |
| Garst 8545 | Reference | conventional | TX |

[1] Test and control substance names are a Monsanto Regulatory ID. Reference substance names are commercial corn hybrid designations

**Table F-7. Test, Control, and Reference Substances in the 2006 U.S. Field Study under Typical Agronomic Conditions**

| Corn Hybrid Names[1] | Seed Types | Site |
|---|---|---|
| MON 87460 | Test | All Sites |
| H1548126 | Control | All Sites |
| DKC 61-42 | Reference | IAE |
| DKC 60-15 | Reference | IAE |
| DKC 63-78 | Reference | IAE |
| H8991 | Reference | IAW |
| DKC 61-50 | Reference | IAW |
| 33N29 | Reference | IAW |
| 33K39 | Reference | IL |
| M-3744 | Reference | IL |
| M-3765 | Reference | IL |
| BT-6512 | Reference | IN |
| B-625 | Reference | IN |
| B-645 | Reference | IN |
| G-8424 | Reference | NE |
| NC+4822 | Reference | NE |
| 34N43 | Reference | NE |

[1]Test and control substance names are a Monsanto Regulatory ID. Reference substance names are commercial corn hybrid designations.

**Table F-8. Test Site Locations, Planting Information, Soil Description, and Cropping History in a 2006 Field Study under Well-Watered Conditions**

| Study site[1] | Planting date (m/dd/yy) | Planting rate (seeds/row)[2] | Planting depth (in) | Plot size (ft) | Reps[3] | Soil series; organic matter; soil pH | 2005 crop | 2004 crop |
|---|---|---|---|---|---|---|---|---|
| IA1 | 5/26/06 | 40 | 1.5 | 10 x 20 | 3 | Silty clay loam; 3.6%; 7.7 | Soybean | Corn |
| IA2 | 5/19/06 | 40 | 2.0 | 10 x 20 | 3 | Silty clay loam; 3-4%; 6.2 | Fallow | Corn |
| IL1 | 5/25/06 | 60 | 1.5 | 20 x 30 | 3 | Plano silt loam; 3.5%; 6.2 | Corn | Corn |
| IL2 | 5/23/06 | 40 | 2.0 | 10 x 20 | 3 | Muscatine silt loam; 3.6%; 6.1 | Soybean | Corn |
| IN1 | 5/23/06 | 40 | 1.5-1.8 | 10 x 20 | 3 | Silt loam; 2.1%; 6.9 | Corn | Soybean |
| IN2 | 6/06/06 | 40 | 1.5 | 10 x 20 | 3 | Reesville silt loam; 2.0%; 6.9 | Corn | Corn |
| KS | 5/17/06 | 60 | 1.5 | 20 x 30 | 3 | Lubbock silt Loam; 1.2%; 7.9 | Sorghum | Soybean |
| NE | 5/13/06 | 60 | 1.5 | 20 x 30 | 3 | Hastings silt loam; 3.0%; 6.6 | Soybean | Soybean |

[1] Study sites: IA1 = Jefferson County, IA; IA2 = Benton, County, IA; IL1 = Stark County, IL; IL2 = Warren County, IL; IN1 = Boone County, IN; IN2 = Parke County, IN; KS = Pawnee County, KS, NE = York County, NE.
[2] After seedling vigor and early stand count data were collected at each site, all plots were thinned to a uniform density.
[3] Reps = replications.

**Table F-9. Test Site Locations, Planting Information, Soil Description, and Cropping History in 2007 U.S. Field Studies under Well-Watered Conditions**

| Study Site[1] | Planting date (m/dd/yy) | Planting rate[2] (seed/row) | Planting depth (in) | Plot size (ft) | Reps[3] | Soil series; organic matter (%); and pH | 2006 crop | 2005 crop |
|---|---|---|---|---|---|---|---|---|
| IA1 | 5/11/07 | 60 | 1.8 | 10 x 30 | 3 | Tainter-Mahska silty clay loam; 3.9%; 6.5 | Soybean | Corn |
| IA2 | 5/15/07 | 40 | 2.0 | 10 x 20 | 3 | Tama silty clay loam; 2.8%; 6.3 | Soybean | Corn |
| IL1 | 5/16/07 | 60 | 1.8 | 10-13 x 30 | 3 | Sawmill silt loam; 3.4%; 6.3 | Corn | Corn |
| IL2 | 5/17/07 | 40 | 1.8 | 10 x 20 | 3 | Sable silty clay loam; 6%; 5.6-7.3 | Fallow | Soybean |
| IL3 | 5/17/07 | 40 | 1.5 | 10 x 20 | 3 | Cisne silt loam; 1.5-2.0%; 6.5-7.0 | Corn | Wheat/Soybean |
| IN | 5/16/07 | 60 | 1.5 | 10 x 30 | 3 | Crosby silt loam; 2.1%; 6.9 | Corn | Soybean |
| NE | 5/11/07 | 40 | 2.0 | 10 x 20 | 3 | Hastings silt loam; 3.0%; 6.1 | Soybean | Corn |
| OH | 5/21/07 | 40 | 1.5 | 10 x 20 | 3 | Millsdale silt loam; 2.0%; 7.1 | Corn | Soybean |
| PA | 5/22/07 | 40 | 1.5 | 10 x 25 | 3 | Philo Atkin silt loam; 2.4%; 5.8 | Fallow | Tomato |

[1] Study sites: IA1 = Jefferson County, IA; IA2 = Van Horne County, IA; IL1 = Wyoming County, IL; IL2 = Monmouth County, IL; IL3 = Carlyle County, IL; IN = Sheridan County, IN; NE = York County, NE; OH = Fayette County, OH; PA = Hamburg County, PA.
[2] After seedling vigor and early stand count data were collected at each site, all plots were thinned to a uniform density.
[3] Reps = replications.

**Table F-10. Test Site Locations, Planting Information, Soil Description, and Cropping History in a 2006/2007 Chilean Field Study Established with Well-Watered and Water-Limited Treatments**

| Study site [1] | Planting date (mm/dd/yy) | Planting rate (seeds/row)[2] | Planting depth (in) | Plot size (ft) | Reps[3] | Soil series; organic matter; pH | 2005-2006 crop | 2004-2005 crop |
|---|---|---|---|---|---|---|---|---|
| CL | 12/19/06 | 42 | 2 | 16.4 x 19.7 | 3 | Clay; 3 – 5%; 5.5 – 6.5 | Table grapes | Table grapes |
| CT | 12/15/06 | 42 | 2 | 16.4 x 19.7 | 3 | Clay; 4 – 5%; 7 – 9 | Oats | Table grapes |
| LUM | 12/15/06 | 42 | 2 | 16.4 x 19.7 | 3 | Sandy clay; 3 – 5%; 5.5 – 6.5 | Pumpkins | Corn |
| QUI† | 12/16/06 | 42 | 2 | 16.4 x 19.7 | 3 | Clay; 3 – 5% ; 5.5 – 6.5 | Swiss chard, cilantro, beets | Swiss chard, cilantro, beets |

[1] Study sites: CL = Colina; CT = Calera de Tango; LUM = Lumbreras; QUI = Quillota. All sites were managed as well-watered and water-limited locations.
†Site QUI did not meet defined non-stress / stress criteria. Data are reported in **Appendix G**.
[2] After seedling vigor and early stand count data were collected, all plots were thinned to approximately 40 plants per row.
[3] Reps = replications.

**Table F-11. Study-1, Test Site Locations, Planting Information, Soil Description, and Cropping History in 2007 U.S. Field Studies Established with Well-Watered and Water-Limited Treatments**

| Study Site[1] | Planting date (m/dd/yy) | Planting rate (seeds/row)[2] | Planting depth (in) | Plot size (ft) | Reps[3] | Soil series; organic matter; pH | 2006 crop | 2005 crop |
|---|---|---|---|---|---|---|---|---|
| CA | 5/22/07 | 40 | 1.0-2.0 | 10 x 20 | 4 | Subaco clay loam; 1.9%; 7.0 | Fallow | Fallow |
| TX | 5/31/07 | 60 | 0.5-0.8 | 20 x 30 | 4 | Pullman loam; 2.3%; 7.2 | Sorghum | Wheat |

[1] Study sites: CA = Sutter County, CA; TX = Carson, County, TX
[2] After seedling vigor and early stand count data were collected at each site, all plots were thinned to a uniform density.
[3] Reps = replications.

**Table F-12. Study-2, Test Site Locations, Planting Information, Soil Description, and Cropping History in 2007 U.S. Field Studies Established with Well-Watered and Water-Limited Treatments**

| Study Site[1] | Planting date (m/dd/yy) | Planting rate (seeds/row)[2] | Planting depth (in) | Plot size (ft) | Reps[3] | Soil Series; organic matter; pH | 2006 crop | 2005 crop |
|---|---|---|---|---|---|---|---|---|
| KS | 5/12/07 | 35 | 1.5 | 20 × 20 | 3 | Farnum silt loam; 3.1%; 7.8 | wheat | alfalfa |
| NE | 5/03/07 | 35 | 2 | 15 × 20 | 3 | Hastings silt loam; 3%; 6.3 | soybean | soybean |
| TX | 5/30/07 | 35 | 0.5 | 20 × 20 | 3 | Pullman silty clay loam; 2.2%: 7.1 | cotton | wheat |

[1]Study sites: KS – Pawnee County, KS; NE = York County, NE; and TX = Carson County, TX.
[2]After seedling vigor and early stand count data were collected, all plots at each site were thinned to a uniform density.
[3]Reps = replications

**Table F-13. Test Site Locations, Planting Information, Soil Description, and Cropping History in a 2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices**

| Study site[1] | Planting date (mm/dd/yy) | Planting rate (seeds/row)[2] | Planting depth (in) | Plot size (ft) | Reps[3] | Soil series; organic matter; pH | 2005 crop | 2004 crop |
|---|---|---|---|---|---|---|---|---|
| IAE | 5/19/06 | 34 | 2.0 | 20 x 15 | 3 | Silty clay loam; 3.5%; 6.2 | Fallow | Corn |
| IAW | 5/16/06 | 34 | 2.0 | 20 x 15 | 3 | Loam; 3 – 4%; 5.6 – 7.3 | Soybeans | Corn |
| IL | 5/23/06 | 34 | 1.8 | 20 x 15 | 3 | Plano silt loam; 3.5%; 6.2 | Field corn | Field corn |
| IN | 6/06/06 | 34 | 1.4 | 20 x 15 | 3 | Silt loam; 2.0%; 6.9 | Corn | Corn |
| NE | 5/12/06 | 34 | 1.5 | 20 x 15 | 3 | Silt loam; 3.0%; 6.6 | Soybeans | Soybeans |

[1] All sites were managed according to local agronomic practices. Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; NE = York County, NE.
[2] After seedling vigor and early stand count data were collected, all plots were thinned to a uniform density.
[3] Reps = replications.

**Table F-14. Phenotypic Comparison of MON 87460 to the Control at Each Site in the 2006 U.S. Field Study Under Well-Watered Conditions**

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seedling vigor (0=9 scale) | | Early stand count (#/plot)[1] | | Days to 50% pollen shed | | Days to 50% silking | | Stay green (0-9 scale) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IA1 | 8.0 ± 0.00 | 8.0 ± 0.00 | 74.7 ± 1.45 | 72.0 ± 2.89 | 55.7 ± 0.67 | 57.0 ± 0.58 | 56.0 ± 0.58 | 55.3 ± 1.20 | 3.3 ± 0.67 | 2.7 ± 0.33 |
| IA2 | 7.0 ± 0.00 | 7.0 ± 0.00 | 68.7 ± 1.33 | 70.7 ± 2.03 | 65.0 ± 0.00 | 65.0 ± 0.00 | 63.0 ± 0.00 | 63.0 ± 0.00 | 4.0* ± 0.00 | 5.0 ± 0.00 |
| IL1 | 9.0 ± 0.00 | 9.0 ± 0.00 | 104.3* ± 0.88 | 86.0 ± 7.51 | 65.7 ± 0.33 | 66.0 ± 0.58 | 63.0 ± 0.00 | 63.3 ± 0.33 | 4.0 ± 0.00 | 4.0 ± 0.00 |
| IL2 | 7.7 ± 0.33 | 7.3 ± 0.33 | - | - | 57.3 ± 0.33 | 57.7 ± 0.67 | 58.0 ± 0.00 | 58.0 ± 0.58 | 4.3 ± 0.33 | 4.0 ± 0.00 |
| IN1 | 4.3 ± 0.33 | 5.0 ± 0.00 | 62.7 ± 9.21 | 65.3 ± 4.98 | 65.0 ± 0.00 | 65.0 ± 0.00 | 63.0 ± 0.00 | 63.0 ± 0.00 | 2.0 ± 0.58 | 2.3 ± 0.33 |
| IN2 | 8.3 ± 0.33 | 8.0 ± 0.00 | 77.3 ± 1.33 | 75.7 ± 0.88 | 57.3 ± 0.33 | 57.0 ± 0.00 | 56.3 ± 0.33 | 56.0 ± 0.58 | 4.7 ± 0.33 | 4.7 ± 0.33 |
| KS | 5.7 ± 0.33 | 6.0 ± 0.00 | 104.0 ± 4.04 | 108.7 ± 1.20 | 57.0 ± 0.58 | 56.7 ± 0.33 | 57.3 ± 0.67 | 56.7 ± 0.33 | 4.7 ± 0.33 | 5.0 ± 0.00 |
| NE | 7.3 ± 0.33 | 7.3 ± 0.33 | 102.0* ± 0.58 | 114.3 ± 1.33 | 63.0 ± 0.00 | 62.7 ± 0.33 | 63.0 ± 0.00 | 63.3 ± 0.33 | 6.0 ± 0.00 | 5.0 ± 0.58 |

S.E. = standard error

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Dashes indicate that data are missing or excluded from statistical analysis.

**Table F-14 (continued). Phenotypic Comparison of MON 87460 to the Control at Each Site in the 2006 U.S. Field study under Well-Watered Conditions**

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ear height (in) | | Plant height (in) | | Dropped ears (#/plot) | | Stalk lodged plants (#/plot) | | Root lodged plants (#/plot) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IA1 | 50.1 ± 0.52 | 49.7 ± 1.29 | 96.5 ± 0.47 | 97.9 ± 1.44 | 0.0 ± 0.00 | 0.0 ± 0.00 | 7.0 ± 0.58 | 8.3 ± 3.18 | 0.0[2] ± 0.00 | 0.0 ± 0.00 |
| IA2 | 53.1 ± 0.35 | 54.2 ± 0.53 | 107.1 ± 1.33 | 106.4 ± 0.60 | 0.7 ± 0.33 | 0.0 ± 0.00 | 3.7 ± 0.33 | 3.7 ± 0.67 | 3.7 ± 1.33 | 4.7 ± 1.67 |
| IL1 | 51.7 ± 0.64 | 53.3 ± 0.07 | 99.1 ± 0.99 | 98.1 ± 1.46 | 0.0 ± 0.00 | 0.0 ± 0.00 | 1.0 ± 0.58 | 3.3 ± 1.86 | 23.7* ± 5.21 | 2.7 ± 1.45 |
| IL2 | 52.3 ± 2.60 | 49.6 ± 2.75 | 100.8 ± 1.29 | 100.5 ± 2.95 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0[2] ± 0.00 | 0.0 ± 0.00 |
| IN1 | 49.9 ± 2.23 | 49.9 ± 2.49 | 94.9 ± 6.06 | 102.5 ± 2.31 | 1.0 ± 1.00 | 0.7 ± 0.33 | 2.0 ± 1.00 | 2.0 ± 0.58 | 0.0[2] ± 0.00 | 0.0 ± 0.00 |
| IN2 | 46.8 ± 2.71 | 46.3 ± 1.22 | 95.8 ± 2.25 | 97.7 ± 2.79 | 0.7 ± 0.67 | 0.3 ± 0.33 | 5.0 ± 0.58 | 6.7 ± 0.88 | 2.3 ± 1.20 | 3.7 ± 0.67 |
| KS | 37.8 ± 0.83 | 36.4 ± 2.21 | 78.7 ± 0.97 | 82.5 ± 3.16 | 1.0 ± 0.58 | 1.0 ± 0.58 | 46.7 ± 8.82 | 36.7 ±3.18 | 0.0[2] ± 0.00 | 0.0 ± 0.00 |
| NE | 55.1 ± 0.82 | 54.4 ± 0.92 | 104.7 ± 1.64 | 104.7 ± 1.57 | 0.0 ± 0.00 | 0.3 ± 0.33 | 9.0* ± 1.15 | 5.0 ± 1.53 | 15.0 ± 8.39 | 1.3 ± 0.88 |

S.E. = standard error

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[2]Not statistically analyzed due to lack of variation.

**Table F-14 (continued). Phenotypic Comparison of MON 87460 to the Control at Each Site in the 2006 U.S. Field Study under Well-Watered Conditions**

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Final stand count (#/plot) | | Grain moisture (%) | | Test weight (lbs/bu) | | Yield (bu/a) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IA1 | 71.3 ± 1.76 | 67.0 ± 5.00 | 14.0 ± 0.21 | 14.5 ± 0.50 | 60.6 ± 3.27 | 62.3 ± 3.35 | 172.7 ± 8.13 | 182.9 ± 7.02 |
| IA2 | 62.0 ± 0.58 | 62.3 ± 0.33 | 13.5 ± 0.03 | 13.5 ± 0.21 | 57.7 ± 0.88 | 57.7 ± 0.67 | 173.9 ± 10.39 | 174.9 ± 6.43 |
| IL1 | 94.0* ± 1.00 | 77.3 ± 0.67 | 18.9 ± 0.23 | 18.9 ± 0.18 | 52.7 ± 1.20 | 53.3 ± 0.33 | 19.0[4] ± 5.49 | 22.3[4] ± 4.50 |
| IL2 | 62.0 ± 1.53 | 62.0 ± 1.15 | 17.9 ± 0.21 | 17.6 ± 0.15 | 57.5 ± 0.17 | 57.7 ± 0.12 | 184.0 ± 9.98 | 181.0 ± 15.59 |
| IN1 | 52.3 ± 3.28 | 55.3 ± 3.18 | 19.3 ± 0.23 | 20.3 ± 0.52 | 58.9 ± 0.67 | 59.3 ± 0.25 | 127.1 ± 3.31 | 113.6 ± 6.71 |
| IN2 | 53.0 ± 2.65 | 51.3 ± 2.91 | 20.4 ± 0.45 | 20.7 ± 0.61 | 56.0[3] ± 0.00 | 56.0[3] ± 0.00 | 161.2 ± 17.33 | 144.5 ± 6.96 |
| KS | 87.3 ± 2.91 | 86.0 ± 2.00 | 13.1 ± 0.59 | 13.0 ± 0.62 | 56.4 ± 0.26 | 56.2 ± 0.37 | 159.4* ± 6.78 | 182.3 ± 3.98 |
| NE | 92.0 ± 1.73 | 94.3 ± 0.67 | 17.8 ± 0.38 | 17.4 ± 0.31 | 59.0 ± 0.06 | 59.1 ± 0.20 | 168.8 ± 2.33 | 169.5 ± 4.47 |

S.E. = standard error.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[3]Values are used for IN2 due to equipment malfunction.

[4]Yield response at IL1 negatively impacted by heat stress during pollination.

**Table F-15. Phenotypic Comparison of MON 87460 to the Control at Each Site in the 2007 U.S. Field Study under Well-Watered Conditions**

| Study Site[1] | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seedling vigor (1-9 scale) | | Early stand count (#/plot) | | Days to 50% pollen shed | | Days to 50% silking | | Stay green (1-9 scale) | |
| | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IA1 | 1.0 ± 0.00 | 1.0 ± 0.00 | 118.0 ± 1.15 | 117.0 ± 1.53 | 61.3 ± 0.33 | 61.0 ± 0.58 | 59.7 ± 0.67 | 60.0 ± 1.00 | 5.7 ± 0.33 | 5.0 ± 0.00 |
| IA2 | 1.0[2] ± 0.00 | 1.0 ± 0.00 | 78.3 ± 1.45 | 79.7 ± 0.33 | 59.7 ± 0.33 | 59.3 ± 0.33 | 57.0 ± 0.00 | 57.0 ± 0.00 | 5.7* ± 0.33 | 5.0 ± 0.00 |
| IL1 | 2.0[2] ± 0.00 | 2.0 ± 0.00 | 113.3 ± 1.45 | 112.3 ± 1.33 | 64.7 ± 0.33 | 65.0 ± 0.00 | 63.0[2] ± 0.00 | 63.0 ± 0.00 | 6.7* ± 0.33 | 5.3 ± 0.33 |
| IL2 | 4.3 ± 0.67 | 4.0 ± 1.00 | 77.7 ± 1.76 | 78.0 ± 2.31 | 61.0 ± 0.58 | 60.7 ± 0.33 | 60.3 ± 0.33 | 60.3 ± 0.33 | - | - |
| IL3 | 3.7 ± 0.33 | 4.0 ± 0.00 | 78.3 ± 1.20 | 78.3 ± 1.20 | 58.0 ± 0.58 | 58.0 ± 0.00 | 56.7 ± 0.33 | 57.0 ± 0.00 | 4.0* ± 0.00 | 3.0 ± 0.58 |
| IN | 5.3 ± 0.33 | 4.7 ± 0.33 | 111.0 ± 3.51 | 118.7 ± 4.06 | 77.0[2] ± 0.00 | 77.0 ± 0.00 | 75.0[2] ± 0.00 | 75.0 ± 0.00 | 9.0 ± 0.00 | 9.0 ± 0.00 |
| NE | 4.3 ± 0.33 | 5.3 ± 0.33 | 68.0 ± 2.52 | 72.0 ± 1.00 | 61.7 ± 0.33 | 61.3 ± 0.33 | 61.7 ± 0.33 | 61.3 ± 0.33 | 6.7 ± 0.33 | 6.3 ± 0.33 |
| OH | 1.0 ± 0.00 | 1.3 ± 0.33 | 70.0 ± 1.15 | 77.0 ± 0.58 | 63.3 ± 0.88 | 63.3 ± 1.20 | 63.0 ± 1.15 | 63.0 ± 1.15 | 5.0 ± 1.15 | 5.7 ± 1.67 |
| PA | 4.7 ± 0.33 | 4.0 ± 0.00 | 69.7 ± 3.18 | 72.0 ± 3.06 | 65.3 ± 0.67 | 64.7 ± 0.33 | 65.3 ± 0.67 | 64.0 ± 0.00 | 5.0 ± 0.58 | 3.7 ± 0.33 |

S.E. = standard error.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Dashes (-) indicate that data are missing or excluded from statistical analysis.

[2] Not statistically analyzed due to lack of variation.

**Table F-15 (continued). Phenotypic Comparison of MON 87460 to the Control at Each Site in the 2007 U.S. Field Study under Well-Watered Conditions**

| Study | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ear height (in) | | Plant height (in) | | Dropped ears (#/plot) | | Stalk lodged plants (#/plot) | | Root lodged plants (#/plot) | |
| Site[1] | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IA1 | 42.1 ± 0.53 | 42.5 ± 0.64 | 93.9 ± 1.14 | 93.5 ± 2.21 | 0.0 ± 0.00 | 0.0 ± 0.00 | 1.0 ± 0.58 | 0.7 ± 0.33 | 0.7 ± 0.67 | 0.0 ± 0.00 |
| IA2 | 45.0 ± 0.53 | 44.1 ± 0.55 | 93.5 ± 0.35 | 94.7 ± 0.87 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.3 ± 0.33 | 1.0 ± 1.00 | 0.7 ± 0.67 | 0.3 ± 0.33 |
| IL1 | 60.1 ± 1.39 | 59.0 ± 0.62 | 121.8 ± 1.65 | 124.3 ± 1.97 | 0.0[2] ± 0.00 | 0.0 ± 0.00 | 6.3 ± 1.76 | 6.0 ± 2.52 | 0.0 ± 0.00 | 0.7 ± 0.33 |
| IL2 | - | - | - | - | - | - | - | - | - | - |
| IL3 | 50.3 ± 1.23 | 47.1 ± 0.59 | 100.9 ± 0.55 | 100.1 ± 0.55 | 1.7 ± 0.33 | 2.7 ± 0.33 | 2.3 ± 0.67 | 3.0 ± 0.00 | 3.0 ± 1.00 | 3.3 ± 0.33 |
| IN | 41.3 ± 1.18 | 37.4 ± 1.30 | 94.2 ± 1.33 | 85.9 ± 0.75 | 0.3 ± 0.33 | 0.7 ± 0.33 | 10.7 ± 1.20 | 7.3 ± 1.45 | 0.0[2] ± 0.00 | 0.0 ± 0.00 |
| NE | 54.2 ± 1.45 | 54.0 ± 0.31 | 108.2 ± 2.47 | 106.6 ± 0.76 | 0.3 ± 0.33 | 0.0 ± 0.00 | 1.3 ± 0.67 | 1.0 ± 0.58 | 0.0[2] ± 0.00 | 0.0 ± 0.00 |
| OH | 39.7 ± 0.64 | 35.8 ± 0.87 | 81.1 ± 2.68 | 78.7 ± 2.66 | 0.7 ± 0.67 | 0.3 ± 0.33 | 3.7* ± 0.88 | 1.0 ± 0.58 | 0.0[2] ± 0.00 | 0.0 ± 0.00 |
| PA | 41.3 ± 0.57 | 41.5 ± 0.71 | 86.4 ± 2.40 | 90.9 ± 1.57 | 1.3* ± 0.33 | 0.0 ± 0.00 | 2.3* ± 0.33 | 0.3 ± 0.33 | 0.0[2] ± 0.00 | 0.0 ± 0.00 |

S.E. = standard error.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Dashes (-) indicate that data are missing or excluded from statistical analysis.

[2] Not statistically analyzed due to lack of variation.

**Table F-15 (continued). Phenotypic Comparison of MON 87460 to the Control at each Site in the 2007 U.S. Field Study under Well-Watered Conditions**

| Study Site[1] | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Final stand count (#/plot) | | Grain moisture (%) | | Test weight (lbs/bu) | | Yield (bu/a) | |
| | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IA1 | 81.7 ± 4.10 | 83.3 ± 2.73 | 11.8 ± 0.23 | 12.4 ± 0.10 | 53.9 ± 0.35 | 54.5 ± 0.48 | 200.2 ± 3.67 | 202.4 ± 6.33 |
| IA2 | 64.7 ± 0.88 | 66.0 ± 1.15 | 18.0* ± 0.23 | 17.1 ± 0.25 | 57.3 ± 0.44 | 57.0 ± 0.58 | 206.1 ± 6.91 | 209.6 ± 4.04 |
| IL1 | 96.0 ± 0.00 | 95.7 ± 0.33 | 21.1 ± 1.12 | 22.4 ± 1.32 | 57.3 ± 0.48 | 56.1 ± 0.98 | 155.4 ± 15.93 | 136.5 ± 8.01 |
| IL2 | - | - | - | - | - | - | - | - |
| IL3 | 61.7 ± 1.86 | 64.3 ± 2.03 | 13.0* ± 0.50 | 14.7 ± 0.95 | 56.1 ± 0.24 | 56.4 ± 0.42 | 144.7 ± 6.75 | 148.1 ± 9.40 |
| IN | 85.3 ± 1.86 | 89.7 ± 3.33 | 18.5 ± 0.98 | 18.6 ± 0.38 | 48.0 ± 2.11 | 53.3 ± 0.57 | 136.9 ± 5.24 | 120.9 ± 17.65 |
| NE | 61.7 ± 0.88 | 63.0 ± 0.58 | 18.5 ± 0.40 | 19.4 ± 0.95 | 58.2 ± 0.20 | 58.0 ± 0.35 | 156.4 ± 4.00 | 156.5 ± 5.77 |
| OH | 62.0 ± 1.00 | 62.0 ± 1.53 | 15.1 ± 0.63 | 15.3 ± 0.64 | 51.5 ± 0.59 | 51.9 ± 0.05 | 137.0 ± 11.72 | 131.9 ± 7.02 |
| PA | 60.7 ± 3.53 | 61.0 ± 1.00 | 22.6 ± 0.29 | 23.1 ± 0.29 | 54.5 ± 0.29 | 54.0 ± 0.00 | 162.2 ± 7.96 | 179.2 ± 9.21 |

S.E. = standard error.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Dashes (-) indicate that data are missing or excluded from statistical analysis.

[2] Not statistically analyzed due to lack of variation.

**Table F-16. Phenotypic Comparison of MON 87460 to the Control at Each Site in a 2006/2007 Chilean Field Study Established with Well-Watered and Water-Limited Treatments in 2006/2007**

**Well-Watered Treatment**

| | | CL Mean ± S.E. | | CT Mean ± S.E. | | LUM Mean ± S.E. | |
|---|---|---|---|---|---|---|---|
| **Phenotypic characteristic** | **Units** | **MON 87460** | **Control** | **MON 87460** | **Control** | **MON 87460** | **Control** |
| Seedling vigor | 0-9 scale[3] | 5.3 ± 0.33 | 4.3 ± 0.33 | 5.0 ± 0.00 | 5.0 ± 0.00 | 4.3 ± 0.33 | 4.7 ± 0.33 |
| Early stand count | #/plot | 74.0 ± 3.51 | 66.0 ± 3.06 | 75.7 ± 2.60 | 77.7 ± 2.33 | 78.7 ± 1.20 | 75.3 ± 4.91 |
| Days to 50% pollen shed | Days | 65.0 ± 2.31 | 64.0 ± 1.15 | 64.3 ± 0.88 | 65.0 ± 0.58 | 71.0 ± 1.53 | 71.0 ± 0.58 |
| Days to 50% silking | Days | 64.0 ± 1.53 | 64.0 ± 1.00 | 63.0 ± 1.00 | 63.3 ± 0.33 | 68.7 ± 0.67 | 68.7 ± 0.67 |
| Stay green | 0-9 scale[4] | 0.7 ± 0.33 | 1.0 ± 0.00 | 3.7 ± 0.33 | 4.3 ± 1.33 | 3.0 ± 0.58 | 3.3 ± 0.67 |
| Ear height | in | 64.7 ± 3.52 | 59.1 ± 1.67 | 53.9 ± 2.00 | 53.5 ± 0.57 | 49.2 ± 3.95 | 45.9 ± 1.57 |
| Plant height | in | 110.3 ± 4.47 | 104.3 ± 2.61 | 99.5 ± 2.38 | 100.8 ± 1.81 | 93.4 ± 4.76 | 92.0 ± 2.04 |
| Dropped ears[2] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| Stalk lodged plants[2] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| Root lodged plants[2] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| Final stand count | #/plot | 74.0 ± 3.46 | 68.7 ± 2.33 | 74.7 ± 2.96 | 77.3 ± 2.03 | 77.0 ± 0.58 | 76.0 ± 3.79 |
| Grain moisture | % moisture | 12.1 ± 0.33 | 11.9 ± 0.47 | 14.0 ± 1.21 | 13.9 ± 1.07 | 18.3 ± 0.64 | 19.8 ± 0.87 |
| Test weight | lbs/bu | 56.2 ± 0.17 | 54.8 ± 0.44 | 58.7 ± 0.44 | 58.7 ± 0.83 | 54.5 ± 0.58 | 54.0 ± 1.15 |
| Yield | bu/a | 213.0 ± 16.59 | 195.6 ± 4.80 | 240.7 ± 8.68 | 258.8 ± 7.85 | 208.5 ± 9.39 | 205.7 ± 2.82 |

S.E. = standard error

Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).

[1] Study sites: CL = Colina; CT = Calera de Tango; LUM = Lumbreras.

[2] No statistical comparisons were made for this rating due to lack of variability in the data. The test was considered effectively not different from the control because the test and control mean values were identical.

[3] Seedling vigor rating scale: 0 = dead and 9 = above average vigor.

[4] Stay green rating scale: 0 = entire plant is dried and 9 = entire plant is green.

**Table F-17 Phenotypic Comparison of MON 87460 to the Control at Each Site in a 2006/2007 Chilean Field Study Established with Well-Watered and Water-Limited Treatments**

**Water-Limited Treatment**

| | | CL Mean ± S.E. | | CT Mean ± S.E. | | LUM Mean ± S.E. | |
|---|---|---|---|---|---|---|---|
| **Phenotypic characteristic** | **Units** | **MON 87460** | **Control** | **MON 87460** | **Control** | **MON 87460** | **Control** |
| Seedling vigor | 0-9 scale[3] | 5.0 ± 0.58 | 5.0 ± 0.00 | 5.3 ± 0.33 | 4.7 ± 0.33 | 4.7 ± 0.67 | 4.7 ± 0.67 |
| Early stand count | #/plot | 75.7 ± 1.33 | 73.0 ± 3.79 | 77.7 ± 0.88 | 76.0 ± 1.00 | 77.0 ± 2.08 | 78.0 ± 0.58 |
| Days to 50% pollen shed | Days | 65.0 ± 1.53 | 65.3 ± 0.67 | 65.3 ± 0.33 | 65.3 ± 0.33 | 72.0 ± 1.53 | 73.7 ± 1.45 |
| Days to 50% silking | Days | 64.3 ± 0.88 | 62.7 ± 0.33 | 63.7 ± 0.33 | 64.0 ± 0.58 | 74.0 ± 0.58 | 73.7 ± 0.33 |
| Stay green | 0-9 scale[4] | 1.3 ± 0.33 | 1.7 ± 0.67 | 5.7 ± 0.33 | 5.7 ± 0.33 | 6.0 ± 0.58 | 6.7 ± 0.33 |
| Ear height | in | 61.0* ± 3.56 | 51.4 ± 4.55 | 44.9 ± 11.41 | 43.2 ± 10.76 | 38.1 ± 1.39 | 40.9 ± 3.41 |
| Plant height | in | 97.2* ± 3.19 | 79.3 ± 10.73 | 87.5 ± 13.21 | 84.5 ± 12.94 | 67.0 ± 0.56 | 70.4 ± 4.64 |
| Dropped ears[2] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| Stalk lodged plants[2] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| Root lodged plants[2] | #/plot | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| Final stand count | #/plot | 74.3 ± 1.20 | 71.3 ± 1.20 | 78.0 ± 0.58 | 76.3 ± 2.03 | 77.7 ± 2.03 | 77.7 ± 1.20 |
| Grain moisture | % moisture | 14.6 ± 0.75 | 16.3 ± 0.68 | 15.5 ± 0.30 | 15.6 ± 1.40 | 28.5 ± 3.93 | 31.9 ± 6.51 |
| Test weight | lbs/bu | 57.0 ± 0.00 | 55.7 ± 0.67 | 60.5 ± 0.50 | 60.5 ± 1.26 | 52.5 ± 1.04 | 51.8 ± 0.44 |
| Yield | bu/a | 96.9 ± 32.09 | 65.6 ± 30.99 | 160.9* ± 18.60 | 123.7 ± 18.05 | 85.8 ± 8.36 | 70.8 ± 9.55 |

S.E. = standard error.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Study sites: CL = Colina; CT = Calera de Tango; LUM = Lumbreras.

[2] No statistical comparisons were made for this rating due to lack of variability in the data. The test was considered effectively not different from the control because the test and control mean values were identical.

[3] Seedling vigor rating scale: 0 = dead and 9 = above average vigor.

[4] Stay green rating scale: 0 = entire plant is dried and 9 = entire plant is green.

**Table F-18. Study-1, Phenotypic Comparison of MON 87460 to the Control at Each Site in 2007 Field Studies Established with Well-Watered and Water-Limited Treatments**

**Well-Watered Treatment**

| | Phenotypic Characteristic, (units), Means ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seedling vigor (1-9 scale) | | Early stand count (#/plot)[§] | | Days to 50% pollen shed | | Days to 50% silking | | Stay green (1-9 scale) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| CA | 5.0[1] ± 0.00 | 5.0 ±0.00 | 75.8* ± 0.25 | 70.0 ± 2.48 | 58.0 ± 0.41 | 57.8 ± 0.25 | 60.0 ± 0.00 | 59.8± 0.63 | 2.5 ± 0.65 | 2.8± 0.48 |
| TX | 1.0 ± 0.00 | 1.0 ± 0.00 | 105.0 ± 2.12 | 106.5± 4.35 | 63.5 ± 0.50 | 64.0 ±0.58 | 57.0 ± 0.00 | 57.0± 0.00 | 1.8* ± 0.25 | 3.5± 0.29 |

| | Phenotypic Characteristic, (units), Means ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ear height (in) | | Plant height (in) | | Dropped ears (#/plot) | | Stalk lodged plants (#/plot) | | Root lodged plants (#/plot) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| CA | 51.4 ± 1.01 | 48.1± 0.89 | 100.5 ± 2.50 | 96.8 ±1.34 | 0.0[1] ± 0.00 | 0.0 ± 0.00 | 0.5* ± 0.29 | 0.0 ± 0.00 | 0.5 ± 0.29 | 1.3 ± 0.63 |
| TX | 33.3 ± 0.63 | 34.6 ± 1.39 | 70.4* ± 1.51 | 75.1± 1.77 | 0.0 ± 0.00 | 0.3 ± 0.25 | 0.0 ± 0.00 | 0.3 ± 0.25 | 0.0[1] ± 0.00 | 0.0 ± 0.00 |

| | Phenotypic Characteristic, (units), Means ± S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Final stand count (#/plot)[§] | | Grain moisture (%) | | Test weight (lbs/bu) | | Yield (bu/a) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| CA | 63.5 ± 0.50 | 63.0 ± 0.41 | 14.6 ± 0.28 | 14.2 ± 0.31 | - | - | 239.7* ± 7.34 | 181.4 ± 19.21 |
| TX | 96.0 ± 0.00 | 94.8 ± 1.25 | 14.8 ± 0.07 | 15.0 ± 0.20 | 58.4 ± 0.96 | 57.8 ± 0.63 | 215.4 ± 6.24 | 219.1 ± 5.22 |

S.E. = standard error. *Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$). [§] Note: For CA, the row length was 20 feet; for TX, rows were 30 feet in length. [1]Not statistically analyzed due to lack of variation. Test weight data not provided from CA site.

**Table F-19. Study-1, Phenotypic Comparison of MON 87460 to the Control at Each Site in 2007 Field Studies Established with Well-Watered and Water-Limited Treatments**

**Water-Limited Treatment**

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seedling vigor (1-9 scale) | | Early stand count (#/plot)[§] | | Days to 50% pollen shed | | Days to 50% silking | | Stay green (1-9 scale) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| CA | 5.0[1] ± 0.00 | 5.0 ± 0.00 | 73.5 ± 2.53 | 74.0 ± 2.65 | 58.3 ± 0.25 | 58.8 ± 0.48 | 60.8 ± 0.25 | 60.0 ± 1.00 | 4.0 ± 0.41 | 4.0 ± 0.00 |
| TX | 1.0 ± 0.00 | 1.0 ± 0.00 | 101.0 ± 2.08 | 107.5 ± 2.18 | 64.3 ±0.67 | 64.0 ± 0.58 | 57.0 ± 0.00 | 57.0 ± 0.00 | 1.7* ± 0.33 | 4.3 ± 0.75 |

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ear height (in) | | Plant height (in) | | Dropped ears (#/plot) | | Stalk lodged plants (#/plot) | | Root lodged plants (#/plot) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| CA | 52.0 ± 0.57 | 50.7 ± 1.91 | 97.5 ± 0.87 | 95.3 ± 1.34 | 0.0[1] ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 4.8 ± 3.47 | 1.5 ± 0.87 |
| TX | 27.9 ± 0.69 | 29.7 ± 0.26 | 62.6 ± 0.55 | 62.2 ± 0.73 | 1.0 ± 0.58 | 1.5 ± 0.5 | 1.3 ± 0.88 | 1.5 ± 0.65 | 0.0[1] ± 0.00 | 0.0 ± 0.00 |

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Final stand count (#/plot)[§] | | Grain moisture (%) | | Test weight (lbs/bu) | | Yield (bu/a) | |
| Site | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| CA | 62.8 ± 0.75 | 63.3 ± 0.63 | 15.2 ± 0.49 | 14.7 ± 0.40 | - | - | 188.3* ± 31.49 | 148.1 ± 28.69 |
| TX | 96.0 ± 0.00 | 96.0 ± 0.00 | 14.0 ± 0.10 | 14.4 ± 0.07 | 57.6 ± 0.74 | 56.7 ± 0.72 | 186.0 ± 2.98 | 173.1 ± 0.70 |

S.E. = standard error. *Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$). [§]Note: For CA, the row length was 20 feet; for TX, rows were 30 feet in length. [1]Not statistically analyzed due to lack of variation. -Test weight data not provided from CA site.

**Table F-20. Study-2, Phenotypic Comparison of MON 87460 to the Control at Each Site in 2007 U.S. Field Studies Established with Well-Watered and Water-Limited Treatments**

**Well-Watered Treatment[1]**

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seedling vigor (0-9 scale) | | Early stand count (#/plot) | | Days to 50% pollen shed | | Days to 50% silking | | Stay green (0-9 scale) | |
| Site | Test | Control | Test | Control | Test | Control | Test | Control | Test | Control |
| KS | 2.7 ± 0.33 | 3.0 ± 0.58 | 68.7 ± 1.86 | 65.3 ± 1.33 | 63.3 ± 0.88 | 62.3 ± 0.33 | 62.0 ± 1.15 | 62.0 ± 0.00 | 6.3 ± 0.33 | 8.0 ± 0.00 |
| NE | 2.7 ± 0.33 | 2.3 ± 0.33 | 46.3 ± 0.88 | 52.3 ± 3.18 | 66.3 ± 0.33 | 66.7 ± 0.33 | 65.7 ± 0.33 | 66.0 ± 0.58 | 4.7* ± 0.33 | 4.0 ± 0.00 |
| TX | 1.7 ± 0.67 | 1.3 ± 0.33 | 52.3 ± 9.28 | 60.0 ± 5.29 | 61.0 ± 0.00 | 61.0 ± 0.00 | 59.0 ± 0.00 | 59.0 ± 0.00 | 6.3* ± 0.33 | 8.0 ± 0.00 |

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ear height (in) | | Plant height (in) | | Dropped ears (#/plot) | | Stalk lodged plants (#/plot) | | Root lodged plants (#/plot) | |
| Site | Test | Control | Test | Control | Test | Control | Test | Control | Test | Control |
| KS | 45.7 ± 1.83 | 44.2 ± 1.21 | 90.6 ± 1.94 | 88.8 ± 2.43 | 1.0 ± 0.58 | 0.7 ± 0.67 | 3.0 ± 0.00 | 4.7 ± 0.88 | 2.7 ± 1.67 | 15.0 ± 15.00 |
| NE | 42.9 ± 0.13 | 40.7 ± 0.66 | 87.8 ± 1.33 | 88.6 ± 1.06 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.7 ± 0.33 | 1.0 ± 0.58 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| TX | 25.1 ± 2.45 | 27.8 ± 1.51 | 74.1 ± 2.53 | 74.2 ± 1.92 | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.3 ± 0.33 |

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Final stand count (#/plot) | | Grain moisture (%) | | Test weight (lbs/bu) | | Yield (bu/a) | |
| Site | Test | Control | Test | Control | Test | Control | Test | Control |
| KS | 59.3 ± 2.73 | 61.3 ± 0.33 | 13.7* ± 0.03 | 12.4 ± 0.09 | 61.4 ± 0.04 | 61.7 ± 0.04 | 137.2 ± 2.17 | 110.8 ± 7.20 |
| NE | 47.7 ± 2.03 | 50.3 ± 1.76 | 15.4 ± 0.25 | 15.7 ± 0.19 | 60.5 ± 0.15 | 60.3 ± 0.12 | 189.4 ± 6.89 | 202.8 ± 7.54 |
| TX | 51.0 ± 8.5 | 56.7 ± 3.33 | 12.7 ± 0.34 | 12.9 ± 0.12 | 59.6 ± 0.25 | 60.0 ± 0.19 | 250.3 ± 15.58 | 255.1 ± 35.19 |

S.E. = standard error.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

Note: No comparisons were made for Root lodged plants at the NE site, and Days to 50% pollen shed, Days to 50% silking, and Stalk lodged plants at the TX site due to a lack of variability.

[1]Water-limited data for the TX site that met the inclusion criteria are presented in Section VIII.C.2, Table VIII-13. Data for KS and NE are reported in Appendix G, Table G-3.

**Table F-21. Phenotypic Comparison of MON 87460 to the Control at Each Site in a 2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices**

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seedling vigor [2] | | Early stand count (#/plot) | | Days to 50% pollen shed | | Days to 50% silking [3] | | Stay green | |
| Site[1] | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IAE | 8.0 ± 0.00 | 8.0 ± 0.00 | 62.3 ± 0.33 | 63.0 ± 1.15 | 64.3 ± 0.33 | 63.7 ± 0.33 | 63.0 ± 0.00 | 61.0 ± 0.00 | 4.3 ± 0.33 | 5.0 ± 0.58 |
| IAW | 7.0 ± 0.00 | 7.0 ± 0.58 | 63.0 ± 1.00 | 59.0 ± 5.51 | 64.0 ± 1.00 | 64.3 ± 0.67 | 63.7 ± 0.67 | 64.3 ± 0.67 | 0.7 ± 0.33 | 0.3 ± 0.33 |
| IL | 8.0 ± 0.00 | 8.0 ± 0.00 | 65.0 ± 2.00 | 62.0 ± 1.15 | 63.3 ± 0.33 | 64.0 ± 0.58 | 62.0 ± 0.00 | 62.0 ± 0.00 | 5.0 ± 0.00 | 5.0 ± 0.00 |
| IN | 8.0 ± 0.00 | 8.0 ± 0.00 | 64.7 ± 1.45 | 60.7 ± 0.67 | 59.3 ± 0.33 | 59.3 ± 0.33 | 57.3 ± 0.33 | 57.3 ± 0.33 | 2.7 ± 0.33 | 2.7 ± 0.33 |
| NE | 7.0* ± 0.00 | 8.0 ± 0.00 | 67.3 ± 0.88 | 66.7 ± 0.88 | 64.3 ± 0.33 | 64.0 ± 0.00 | 65.0* ± 0.00 | 64.0 ± 0.00 | 6.0 ± 0.58 | 5.7 ± 0.33 |

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ear height (in) | | Plant height (in) | | Dropped ears (#/plot) [4] | | Stalk lodged plants (#/plot) | | Root lodged plants (#/plot) [5] | |
| Site[1] | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IAE | 54.1 ± 1.41 | 52.7 ± 1.46 | 108.3 ± 0.44 | 108.1 ± 0.18 | 0.0 ± 0.00 | 0.0 ± 0.00 | 2.3 ± 0.67 | 2.3 ± 0.67 | 5.7 ± 1.33 | 4.7 ± 0.67 |
| IAW | 38.6 ± 0.81 | 37.7 ± 1.87 | 97.5 ± 0.74 | 97.9 ± 1.05 | 0.0 ± 0.00 | 0.0 ± 0.00 | 11.3 ± 1.20 | 13.7 ± 5.24 | 0.7 ± 0.33 | 0.7 ± 0.67 |
| IL | 40.5 ± 1.05 | 40.1 ± 0.70 | 88.9 ± 1.43 | 89.2 ± 0.52 | 0.0 ± 0.00 | 0.0 ± 0.00 | 2.7 ± 0.88 | 2.0 ± 1.53 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| IN | 46.1 ± 0.47 | 46.4 ± 1.29 | 94.7 ± 0.35 | 94.4 ± 0.76 | 0.0 ± 0.00 | 0.3 ± 0.33 | 8.3* ± 0.67 | 4.7 ± 1.86 | 3.3* ± 0.88 | 0.3 ± 0.33 |
| NE | 48.6 ± 1.03 | 47.4 ± 1.62 | 99.5 ± 1.27 | 96.6 ± 2.50 | 0.7 ± 0.67 | 0.3 ± 0.33 | 2.7 ± 0.33 | 3.0 ± 1.53 | 0.7 ± 0.33 | 0.0 ± 0.00 |

S.E. = standard error. *Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; NE = York County, NE.

[2] Lack of variance prevented statistical analysis of seedling vigor at the IAE, IL, and IN sites.

[3] Lack of variance prevented statistical analysis of days to 50% silking at the IAE and IN sites.

[4] Lack of variance prevented statistical analysis of dropped ears at the IAE, IAW, and IL sites.

[5] Lack of variance prevented statistical analysis of root lodged plants at the IL site.

**Table F-21 (continued). Phenotypic Comparison of MON 87460 to the Control at Each Site in a 2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices**

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Final stand count (#/plot) | | Grain moisture (%) | | Test weight (lbs/bu) | | Yield (bu/a) | |
| Site[1] | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control |
| IAE | 60.3 ± 0.67 | 58.7 ± 0.88 | 16.1 ± 0.50 | 15.6 ± 0.19 | 56.7 ± 0.33 | 57.0 ± 0.58 | 187.8 ± 5.34 | 180.9 ± 5.72 |
| IAW | 53.3 ± 2.33 | 52.7 ± 3.53 | 15.9 ± 0.64 | 16.4 ± 0.23 | 50.7 ± 0.00 | 48.8 ± 0.94 | 154.9 ± 5.32 | 138.5 ± 15.26 |
| IL | 58.7 ± 1.45 | 59.3 ± 0.33 | 20.6 ± 0.91 | 20.0 ± 2.40 | 56.2 ± 0.45 | 56.1 ± 0.99 | 52.0 ± 3.69 | 35.8 ± 18.00 |
| IN | 59.0 ± 0.58 | 57.0 ± 1.73 | 18.3 ± 0.31 | 19.4 ± 0.36 | 54.2 ± 0.32 | 53.6 ± 0.12 | 146.8 ± 5.20 | 161.9 ± 10.18 |
| NE | 58.0 ± 1.00 | 59.3 ± 0.33 | 16.8 ± 0.45 | 17.1 ± 0.61 | 58.6 ± 0.36 | 58.7 ± 0.09 | 191.3 ± 3.27 | 179.8 ± 15.64 |

S.E. = standard error.

Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).

[1] Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; NE = York County, NE.

## Appendix G. Phenotypic Data from U.S. and Chile Field Sites Excluded from Combined-Site Analyses

This section contains data that were excluded from the combined-site analyses discussed in Section VIII.C. All data reported here are for sites that did not meet the well-watered and water-limited inclusion criteria described in Section VIII.C (Table VIII-3).

*Site Code, Test Location, and Year*
Site Code QUI, Chile, 2006/2007

**Table G-1. Individual Site Analysis of Phenotypic Characteristics of MON 87460 to the Control for the QUI Site in Chile during 2006/2007 under Well-Watered Conditions**

| | Mean ± S.E. | |
|---|---|---|
| **Phenotypic Characteristic** | **MON 87460** | **Control** |
| **Seedling vigor** | 5.7 ± 0.33 | 5.0 ± 0.00 |
| **Early stand count (#/plot)** | 73.0 ± 4.04 | 73.7 ± 4.06 |
| **Days to 50% pollen shed** | 69.3 ± 0.33 | 70.0 ± 1.00 |
| **Days to 50% silking** | 69.0 ± 0.00 | 68.3 ± 1.20 |
| **Stay green** | 3.0* ± 0.00 | 4.7 ± 0.88 |
| **Ear height (in)** | 68.3 ± 1.98 | 66.6 ± 3.99 |
| **Plant height (in)** | 110.8 ± 1.35 | 109.8 ± 4.03 |
| **Dropped ears (#/plot)** [1] | 0.0 ± 0.00 | 0.0 ± 0.00 |
| **Stalk lodged plants (#/plot)** [1] | 0.0 ± 0.00 | 0.0 ± 0.00 |
| **Root lodged plants (#/plot)** [1] | 0.0 ± 0.00 | 0.0 ± 0.00 |
| **Final stand count (#/plot)** | 76.7 ± 2.03 | 75.0 ± 2.65 |
| **Grain moisture (%)** | 13.6 ± 0.84 | 14.1 ± 0.50 |
| **Test weight (lbs/bu)** | 57.3 ± 0.33 | 58.2 ± 1.01 |
| **Yield (bu/ac)** | 200.4 ± 5.75 | 217.8 ± 5.10 |

S.E. = standard error.
*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).
[1] No statistical comparisons were made for this rating due to lack of variability in the data. The test was considered effectively not different from the control because the test and control mean values were identical.

**Table G-2. Individual Site Analysis of Phenotypic Characteristics of MON 87460 to the Control for the QUI site in Chile During 2006/2007 under Water-Limited Conditions**

| | Mean ± S.E. | |
|---|---|---|
| **Phenotypic characteristic** | **MON 87460** | **Control** |
| **Seedling vigor** | 6.0* ± 0.00 | 4.7 ± 0.33 |
| **Early stand count (#/plot)** | 81.0 ± 1.53 | 78.7 ± 1.67 |
| **Days to 50% pollen shed** | 66.0* ± 0.58 | 70.7 ± 0.67 |
| **Days to 50% silking** | 64.7* ± 0.33 | 68.3 ± 1.20 |
| **Stay green** | 2.3 ± 0.33 | 3.7 ± 0.33 |
| **Ear height (in)** | 72.1 ± 2.46 | 67.1 ± 0.39 |
| **Plant height (in)** | 116.9 ± 4.97 | 112.4 ± 2.79 |
| **Dropped ears (#/plot)[1]** | 0.0 ± 0.00 | 0.0 ± 0.00 |
| **Stalk lodged plants (#/plot) [1]** | 0.0 ± 0.00 | 0.0 ± 0.00 |
| **Root lodged plants (#/plot) [1]** | 0.0 ± 0.00 | 0.0 ± 0.00 |
| **Final stand count (#/plot)** | 81.0 ± 1.53 | 78.7 ± 1.76 |
| **Grain moisture (%)** | 14.5* ± 0.82 | 12.5 ± 0.90 |
| **Test weight (lbs/bu)** | 57.2 ± 0.83 | 57.5 ± 0.29 |
| **Yield (bu/ac)** | 199.0 ± 14.60 | 202.0 ± 3.06 |

S.E. = standard error.
*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).
[1] No statistical comparisons were made for this rating due to lack of variability in the data. The test was considered effectively not different from the control because the test and control mean values were identical.

*Site Codes, Test Location, and Year*
KS (Pawnee Co.) and NE (York Co.), 2007

**Table G-3. U.S. 2007 Study-2: Water-Limited Treatment – Phenotypic Comparison of MON 87460 to the Control at Each Site**

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seedling vigor (0-9 scale) | | Early stand count (#/20 ft plot) | | Days to 50% pollen shed | | Days to 50% silking | | Stay green (0-9 scale) | |
| Site | Test | Control | Test | Control | Test | Control | Test | Control | Test | Control |
| KS | 2.3 ± 0.33 | 2.7 ± 0.33 | 68.3 ± 1.33 | 66.3 ± 2.73 | 62.3 ± 0.33 | 62.0 ± 0.00 | 62.0 ± 0.00 | 62.0 ± 0.00 | 6.0 ± 1.00 | 6.7 ± 0.67 |
| NE | 2.0 ± 0.00 | 2.3 ± 0.33 | 50.3 ± 3.18 | 47.7 ± 4.26 | 66.3 ± 0.33 | 66.7 ± 0.67 | 65.3 ± 0.33 | 66.3 ± 0.88 | 5.0* ± 0.00 | 4.0 ± 0.00 |

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ear height (in) | | Plant height (in) | | Dropped ears (#/plot) | | Stalk lodged plants (#/plot) | | Root lodged plants (#/plot) | |
| Site | Test | Control | Test | Control | Test | Control | Test | Control | Test | Control |
| KS | 43.3 ± 0.93 | 44.3 ± 1.39 | 88.7 ± 1.73 | 89.7 ± 1.51 | 1.3 ± 0.88 | 0.3 ± 0.33 | 2.3 ± 0.88 | 6.0 ± 2.00 | 0.7 ± 0.67 | 2.7 ± 1.76 |
| NE | 41.6 ± 1.10 | 41.1 ± 0.79 | 87.4 ± 1.25 | 86.5 ± 1.16 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 ± 0.00 |

| | Phenotypic Characteristic, (units), Mean ± S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Final stand count (#/plot) | | Grain moisture (%) | | Test weight (lbs/bu) | | Yield (bu/a) | |
| Site | Test | Control | Test | Control | Test | Control | Test | Control |
| KS | 61.0 ± 2.52 | 60.0 ± 4.00 | 13.7 ± 0.06 | 12.6 ± 0.09 | 61.4 ± 0.04 | 61.5 ± 0.13 | 111.9 ± 6.25 | 121.1 ± 1.99 |
| NE | 45.0 ± 1.15 | 45.3 ± 3.53 | 15.4 ± 0.12 | 15.9 ± 0.35 | 60.6 ± 0.06 | 60.3 ± 0.23 | 183.3 ± 5.48 | 183.0 ± 8.44 |

S.E. = standard error.
*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).
Note: No comparisons were made for root lodged plants at the NE site, and days to 50% pollen shed, days to 50% silking, and stalk lodged plants at the TX site due to a lack of variability.

## Appendix H. Environmental Interactions Data for Individual Field Sites in U.S. and Chile Field Studies during 2006 and 2007

Environmental interaction evaluations were conducted as part of the plant characterization studies for MON 87460, and will be used as part of the plant pest risk assessment. The environmental interactions evaluation included the collection and comparison of abiotic stressor data, disease damage data, arthropod damage data, and arthropod pest and beneficial data.

### H.1. Materials and Methods for Disease and Insect Assessments

The plots at all sites were qualitatively evaluated at least four times for differential response to naturally occurring environmental stressors during the growing season. During each observation, each plot was evaluated for the severity of symptoms caused by three arthropod, three disease, and three abiotic stressors that commonly occur at the study sites. With a few exceptions, these stressors were predetermined by the individual site Principal Investigators (PIs) based on their experience. The environmental stressors evaluated were not artificially induced and could vary between sites. Plots were rated on the 0 – 9 rating scale of increasing symptomology described below but the results were reported as categorical (none, slight, moderate, or severe).

| | | |
|---|---|---|
| 0 | = | none (no symptoms observed) |
| 1 – 3 | = | slight (symptoms observed, not detrimental to plant growth and development) |
| 4 – 6 | = | moderate (intermediate between slight and severe) |
| 7 – 9 | = | severe (symptoms observed, detrimental to plant growth and development) |

#### H.1.1. Stalk and Ear/Kernel Rot Assessment

Stalk Rot Disease

At harvest, incidence of stalk rot disease from 5 representative plants per plot was assessed. The stalk of each plant was cut longitudinally and examined for shredded and discolored pith tissue. The following 0 – 9 rating scale was be used:

0 = none (no symptoms observed)
1 – 3 = slight (symptoms observed not detrimental to stalk quality)
4 – 6 = moderate (intermediate between slight and severe)
7 – 9 = severe (symptoms observed, detrimental to stalk quality)

Ear and Kernel Rot Disease

At harvest, incidence of ear and kernel rot disease from 5 representative ears (one per plant) per plot was assessed. The husks were pulled back so the ear and kernels could be examined for infection. The following 0 – 9 rating scale was used:
0 = none (no symptoms observed)
1 – 3 = slight (symptoms observed, not detrimental to grain quality)
4 – 6 = moderate (intermediate between slight and severe)
7 – 9 = severe (symptoms observed, detrimental to grain quality)

### H.1.2. Quantitative Assessment: European Corn Borer and Corn Earworm Damage

European corn borer, and corn earworm damage ratings were performed at three well-watered sites in 2006, at three well-watered sites in 2007, and a single site in TX with well-watered and water-limited treatments (Study-2) in 2007.

European corn borer damage was evaluated at harvest by examining five non-systematically selected plants. If damage was present, the number of live larvae, number of galleries, number of entry/exit holes were counted, and the length of galleries in each stalk was measured.

Corn earworm damage was evaluated at harvest by examining ears from ten non-systematically selected plants. For each ear, the following damage rating scale was used:

0 = No visible corn earworm damage
1 = Silk shows evidence of feeding, feeding on the ear is < 0.5 inches
2 = Corn earworm feeding to 0.5 in. beyond the ear tip
3 = Corn earworm feeding to 1.0 in. beyond the ear tip
4 = Corn earworm feeding to 1.5 in. beyond the ear tip
5 = Corn earworm feeding to 2.0 in. beyond the ear tip
6 = Corn earworm feeding to 2.5 in. beyond the ear tip
7 = Corn earworm feeding to 3.0 in. beyond the ear tip
8 = Corn earworm feeding to 3.5 in. beyond the ear tip
9 = Corn earworm feeding to 4.0 in. or greater beyond the ear tip

### H.1.3. Quantitative Assessment: Arthropod Collection

Arthropod collections were performed at three well-watered sites in 2006, at three well-watered sites in 2007, and a single site in TX with well-watered and water-limited treatments (Study-2) in 2007.
Arthropods were collected four times during the growing season at the following intervals:

Collection 1: V2 – V4
Collection 2: V10 – V15
Collection 3: VT – R3
Collection 4: R6

Arthropods were collected using yellow sticky traps. The sticky traps were placed at the approximate midpoint between the ground level and the top of the plant canopy. Once the main ear was visible, the sticky traps were deployed at the approximate corn ear level for the remainder of the arthropod collections. The sticky traps were deployed for approximately seven days. The sticky traps were sent to the Department of Entomology at the University of Arkansas in Fayetteville, AR for arthropod identification and enumeration. Up to six of the most abundant pest and up to six of the most abundant beneficial arthropods were determined for each collection at each site. These arthropods were then enumerated across all plots (i.e., one sticky trap per plot) from a given collection at each site. The arthropods assessed often varied between collections due to differences in seasonal activity.

### H.1.4. Abiotic, Disease, and Arthropod Assessments

In the two years of field studies for evaluation of phenotypic and agronomic characteristics of MON 87460, observational data on the presence of and differential response to biotic (insects, diseases) and abiotic (drought, wind, nutrient deficiency etc.) stressors were also collected to examine the environmental interactions of MON 87460 compared with those of the conventional control corn. The observed stressors were "natural" (i.e., no artificial infestation or interference was used). Therefore, the same stressors were not necessarily observed at each field site. A summary of environmental interactions data are presented in Section VIII. Individual site data are reported in this appendix.

Environmental interactions were assessed qualitatively, and for selected sites, insect interactions data were collected quantitatively. Observation of plant interactions with insect pests and diseases, and plant responses to abiotic stressors were collected from each of the 33 field site locations in 2006 and 2007. The purpose of these evaluations was to assess whether plant-insect or plant-disease interactions, or plant response to abiotic stressors of MON 87460 were altered compared to the conventional control corn. For the plant-insect interactions, plant-disease interactions, and plant responses to abiotic stressors, the reported values represent the range of ratings observed across the three or four replications at each site. MON 87460 and the control were considered qualitatively different in response to a stressor if the ratings between MON 87460 and the control corn did not overlap across all three or four replications for that particular stressor (e.g. "none" rating vs. "slight-moderate" rating). The ratings observed among the commercial reference hybrids provide qualitative assessment data common to the crop for each stressor assessed.

## H.2. Statistical Analysis

<u>2006 and 2007 U.S. Field Studies under Well-Watered Conditions</u>

An analysis of variance was conducted according to a randomized complete block design with three replications using SAS® (SAS Version 9.2, SAS Institute, Inc. 2008) for the corn earworm damage, European corn borer damage, and the arthropod abundance. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). The test substance was compared to the control substance at each site (individual-site analysis)

for the corn earworm damage, European corn borer damage, and arthropod abundance. Additionally, the corn earworm damage and European corn borer damage data were pooled across sites (combined-site analysis) for a statistical comparison of the test and control substances. The test substance was not statistically compared to the reference substances, and the reference range was calculated from the minimum and maximum mean values observed in the references.

U.S. 2007 Field Study (Study-1) Established with Well-Watered and Water-Limited Treatments – TX Site

An analysis of variance was conducted according to a split-plot design with four replications using SAS®. The whole plot factor was the irrigation treatment and the subplot factor was substance with the experimental unit consisting of one plot. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). The test substance was compared to the control substance within irrigation treatments for the corn earworm damage, European corn borer damage, and arthropod abundance. The test substance was not statistically compared to the reference substances, and the reference range was calculated from the minimum and maximum mean values observed in the references.

**H.3. Results**

*2006 U.S. Field Study under Well-Watered Conditions*

Environmental interaction data were collected from eight field trials in 2006 and nine in 2007 that were established as well-watered locations. Six sites provided quantitative insect assessments across both years. For quantitative insect sites, sticky traps were deployed for insect identification and enumeration. Quantitative insect damage assessments were made at all locations.

Plant Response to Abiotic Stressors

No differences in plant response to abiotic stressors were detected between MON 87460 and the control for cold, compaction, drought, flood, frost, green snap, hail, heat, mineral toxicity, nutrient deficiency, or wind evaluated using the observational severity scale at the IA1, IA2, IL1, IL2, IN1, IN2, KS, or NE sites (Table H-1).

Disease Damage

No differences in disease damage were detected between MON 87460 and the control for anthracnose, an external assessment for ear rot, ear and kernel rot assessment after husk removal, eyespot, *Fusarium*, gray leaf spot, leaf blight, northern corn leaf blight, northern leaf spot, *Pythium*, rust, seedling blight, smut, southern leaf blight, stalk rot, Stewarts wilt, or yellow leaf blight evaluated using the observational severity scale at the IA1, IA2, IL1, IL2, IN1, IN2, KS, or NE sites (Table H-2).

Arthropod Damage

No differences in damage were detected between MON 87460 and the control for aphids, armyworms, corn earworm, corn rootworms, cutworms, European corn

borers, flea beetles, grape colaspis, grasshoppers, Japanese beetles, leafhoppers, seedcorn maggots, southwestern corn borers, spider mites, white grubs, or wireworms evaluated using the observational severity scale at the IA1, IA2, IL1, IL2, IN1, IN2, KS, or NE sites (Table H-3).

Corn Earworm and European Corn Borer Damage

No statistical differences were detected between MON 87460 and the control for corn earworm damage using the adapted Widstrom (1967) scale in the combined-site or individual-site analyses at the IL1, KS, and NE sites (Tables H-4 and H-5). No statistical differences were detected between MON 87460 and the control for European corn borer damage including: number of entry/ exit holes; number of galleries; and length of galleries (in.) in the ear shank and the stalk, in the combined-site or individual-site analyses at the IL1, KS, and NE sites (Tables H-4 and H-5).

Arthropod Abundance

In the individual site analysis, no statistical differences were detected between MON 87460 and the control for the following pest and beneficial arthropods at the IL1, KS, and NE sites: delphacid planthoppers, grasshoppers, leafhoppers, northern corn rootworm beetles, sap beetles, southern corn rootworm beetles, western corn rootworm beetles, brown lacewings, green lacewings, lady bird beetles, macro-parasitic hymenopterans (parasitoids), nabids, minute pirate bugs, and spiders (Tables H-6 and H-7). Aphid abundance was statistically lower at the NE site at collection 1 (0.0 vs. 3.3 per trap) and statistically higher at the IL1 site at collection 3 (70.7 vs. 63.3 per trap) for MON 87460 compared to the control out of 12 collections at three sites (Table H-6). Corn flea beetle abundance was statistically lower at the NE site at collection 2 (0.0 vs. 1.3 corn flea beetles per trap) for MON 87460 compared to the control out of nine collections (Table H-6). Micro-parasitic hymenopteran abundance was statistically lower at the KS site for collections 2 and 3 (30.0 vs. 42.3 per trap and 13.0 vs. 23.3 per trap, respectively) for MON 87460 compared to the control out of 12 collections (Table H-7). However, no consistent statistical differences for aphids, corn flea beetles, or micro-parasitic hymenopterans were detected across sites or arthropod collections.

Plant Response to Abiotic Stressors

In an assessment of abiotic stress response, no differences in plant response to abiotic stressors were observed between MON 87460 and the control treatments for cold, compaction, drought, flood, frost, hail, heat, mineral toxicity, nutrient deficiency, or wind evaluated using the observational severity scale at the IA1, IA2, IL1, IL2, IL3, IN, NE, OH or PA sites (Table H-8).

Disease Damage

In an assessment of disease damage, no differences in disease damage were observed between MON 87460 and the control for anthracnose, an external assessment for ear rot, ear rot assessment after husk removal, an external assessment for stalk rot, stalk rot assessment by splitting the stalks, eyespot, *Fusarium*, gray leaf spot, leaf blight, northern corn leaf blight, *Pythium*, seedling blight, root rot, rust, smut, or Stewart's wilt evaluated using the observational severity scale at the at the IA1, IA2, IL1, IL2, IL3, IN, NE, OH, or PA sites (Table H-9).

Arthropod Damage

In an assessment of arthropod damage, no differences in damage were observed between MON 87460 and the control for aphids, leafhoppers, thrips, grasshoppers, armyworms, corn earworm larvae, cutworms, billbugs, flea beetles, grape colaspis adults, Japanese beetles, northern corn rootworm, western corn rootworm beetles, white grubs, wireworms, or seedcorn maggots evaluated using the observational severity scale at the IA1, IA2, IL1, IL2, IL3, IN, NE, OH, or PA sites (Table H-10). One difference was observed between MON 87460 and the control for European corn borer at the IA1 site during the fourth observation (moderate vs. slight). However, the observed incidence of European corn borer damage was not detected at other sites or observations.

Additionally, no statistical differences were detected between MON 87460 and the control for corn earworm damage using the adapted Widstrom (1967) scale in the combined-site analysis (Table H-11). No statistical differences were detected between MON 87460 and the control for European corn borer damage including: number of live larvae; number of entry/ exit holes; number of galleries; and length of galleries (in.) in the stalk in the combined-site analysis (Table H-11). However, number of live larvae and the length of galleries (in) in the stalk were significantly different between MON 87460 and the control at the IL1 site in the individual site analysis (Table H-12). The differences detected in the individual site analysis were not significant in the combined-site analysis and; therefore, not indicative of a consistent trend in the data.

Arthropod Abundance

No statistical differences were detected between MON 87460 and the control for the following pest and beneficial arthropods at the IA1, IL1 and IN sites: aphids,

delphacid planthoppers, grasshoppers, leafhoppers, corn flea beetles, Northern corn rootworms, sap beetles, southern corn rootworm beetles, western corn rootworm beetles, brown lacewings, green lacewings, lady bird beetles, macro-parasitic hymenopterans (parasitoids), *Orius*, and spiders (Tables H-13 and H-14). Micro-parasitic hymenopterans (parasitoids) abundance was statistically lower at the IN site for collection 3 (48.7 vs. 118.3 per trap) for MON 87460 compared to the control and was outside of the reference range (Table H-14). However, no consistent statistical differences between MON 87460 and the control for micro-parasitic hymenopterans (parasitoids) abundance were detected across sites or arthropod collections.

*2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices*

Abiotic Stressors

No differences in plant response to abiotic stressors were detected between MON 87460 and the control for cold, compaction, flood, frost, hail, heat, nutrient deficiency, or wind using the observational severity scale at the IAE, IAW, IL, IN, and NE sites (Table H-15).

Disease Damage

No differences in disease damage were detected between MON 87460 and the control for anthracnose, eyespot, ear rot, *Fusarium*, gray leaf spot, leaf blight, northern corn leaf blight, northern leaf spot, *Pythium*, root rot, rust, seedling blight, southern leaf blight, smut, stalk rot, or Stewarts wilt evaluated using the observational severity scale at the IAE, IAW, IL, IN, or NE sites (Table H-16).

Arthropod Damage

No differences in arthropod damage were detected between MON 87460 and the control for aphids, armyworms, billbugs, corn earworms, corn rootworm beetles, cutworms, European corn borers, flea beetles, seedcorn maggots, southwestern corn borers, western bean cutworms, white grubs, and wireworms using the observational severity scale at the IAW, IL, IN, and NE sites. Grasshopper damage was lower for MON 87460 compared to the control (none vs. slight) at the IAE site at Observation 3. However, the detected difference was within the range of the references. Additionally, no differences were detected between MON 87460 and the control for grasshopper damage using the observational severity scale for Observations 2 and 4 at the IAE site or at any observation time at the other sites (Table H-17). These results support the conclusion that the introduction of the drought tolerance trait did not unexpectedly alter MON 87460 compared to conventional corn based on the assessed environmental interactions.

*2006/2007 Chilean Field Study Established with Well-Watered and Water-Limited Treatments*

Three locations were included in the combined-site based on site inclusion criteria (Section VIII.C, Figure VIII-3).

Abiotic Stressors
No differences in plant response to abiotic stressors were detected between MON 87460 and the control for cold, frost, hail, heat, nitrogen deficiency, or wind damage in the well-watered or water-limited treatments using the observational severity scale at the CL, CT, and LUM sites (Table H-18).

Disease Damage
No differences in disease damage were detected between MON 87460 and the control for ear rot, *Fusarium*, gray leaf spot, leaf blight, northern corn leaf blight, root rot, rust, seedling blight, smut, or stalk rot in the well-watered or water-limited treatments using the observational severity scale at the CL, CT, and LUM sites (Table H-19).

Arthropod Damage
No differences in arthropod damage were detected between MON 87460 and the control for aphids, seed corn maggots, thrips, and wireworms in the well-watered or water-limited treatments using the observational severity scale at the CL, CT, and LUM sites (Table H-20).

*2007 U.S. Field Studies Established with Well-Watered and Water-Limited Treatments*

In 2007, two separate field studies were conducted in the U.S. where sites were established with well-watered and water-limited treatments. In Study-1, two sites were established (CA, TX) and both met the inclusion criteria (Section VIII.C, Figure VIII-3). In Study-2, three sites were established (KS, NE, TX), but only one site (TX) met the inclusion criteria. The two trials had different experimental designs which precluded a combined-study analysis.

Study-1 – Abiotic Stress Response
In assessment of abiotic stress response in the well-watered and water-limited treatments, no differences in plant response to abiotic stressors were observed between MON 87460 and the control treatments for drought, hail, heat, heavy thunderstorm, nutrient deficiency, or wind evaluated using the observational severity scale at the CA and TX sites (Table H-21).

Study-1 – Disease Damage
In assessment of disease damage in the well-watered and water-limited treatments, no differences in disease damage were observed between MON 87460 and the control for ear rot assessment after husk removal, *Fusarium*, gray mold, maize dwarf mosaic

virus, rust, seedling blight, smut, stalk rot, or southern leaf blight evaluated using the observational severity scale at the CA and TX sites (Table H-22).

Study-1 – Arthropod Damage

In assessment of arthropod damage in the well-watered and water-limited treatments, no differences in damage were observed between MON 87460 and the control for aphids, armyworms, western corn rootworm beetles, cutworms, European corn borers, grasshoppers, leafhoppers, southwestern corn borers, or spider mites evaluated using the observational severity scale at the CA and TX sites (Table H-23).

Additionally, no statistical differences were detected between MON 87460 and the control for corn earworm damage using the adapted Widstrom (1967) scale at the TX site under well-watered and water-limited treatments (Table H-24). No statistical differences were detected between MON 87460 and the control for European corn borer damage including: number of live larvae, number of entry/ exit holes; number of galleries; and length of galleries (in.) in the stalk at the TX site under well-watered and water-limited treatments (Table H-24).

Study-1 – Arthropod Abundance

In an assessment of arthropod damage under the well-watered treatment, no statistical differences were detected between MON 87460 and the control for the following pest and beneficial arthropods at the TX site: aphids, delphacid planthoppers, grasshoppers, leafhoppers, corn flea beetles, sap beetles, southern corn rootworm beetles, western corn rootworm beetles, green lacewings, lady bird beetles, macro-parasitic hymenopterans (parasitoids), micro-parasitic hymenopterans (parasitoids), *Orius*, big-eyed bugs, and spiders (Tables H-25 and H-26). Brown lacewing abundance was statistically higher at collection 3 (0.5 vs. 0.0 per trap) for MON 87460 compared to the control, but was within the range of the references (Table H-26).

In an assessment of arthropod damage under the water-limited treatment, no statistical differences were detected between MON 87460 and the control for the following pest and beneficial arthropods at the TX site: aphids, delphacid planthoppers, grasshoppers, leafhoppers, corn flea beetles, sap beetles, western corn rootworm beetles, green lacewings, lady bird beetles, macro-parasitic hymenopterans (parasitoids), micro-parasitic hymenopterans (parasitoids), *Orius*, big-eyed bugs, and spiders (Tables H-25 and H-26). Southern corn rootworm beetle abundance was statistically higher at collection 4 (2.5 vs. 0.8 per trap) for MON 87460 compared to the control and was outside of the reference range (Table H-25). However, differences between MON 87460 and the control for southern corn rootworm abundance were not detected for other arthropod collections.

Study-2 – Abiotic Stress Response

In assessment of abiotic stress response in the well-watered treatment, no differences in plant response to abiotic stressors were observed between MON 87460 and the control treatments for drought, hail, heat, water logging, or wind evaluated using the observational severity scale at the NE, KS and TX sites (Table H-27).

In assessment of abiotic stress response under water-limited treatment, no differences in plant response to abiotic stressors were observed between MON 87460 and the control treatments for drought, hail, heat, or wind evaluated using the observational severity scale at the TX site (Table H-27).

Study-2 – Disease Damage

In assessment of disease damage in the well-watered treatment, no differences in disease damage were observed between MON 87460 and the control for crazy top, ear rot assessment after husk removal, Goss's wilt, grey leaf spot, grey mold, leaf blight, maize dwarf mosaic virus, northern corn leaf blight, rust, seedling blight, smut, stalk rot assessment by splitting the stalk, or southern corn leaf blight evaluated using the observational severity scale at NE, KS and TX sites (Table H-28).

In assessment of disease damage in the water-limited treatment, no differences in disease damage were observed between MON 87460 and the control for ear rot assessment after husk removal, grey mold, maize dwarf mosaic virus, northern corn leaf blight, rust, seedling blight, stalk rot assessment by splitting the stalk, or southern corn leaf blight evaluated using the observational severity scale at the TX site (Table H-28)

Study-2 – Arthropod Damage

In an assessment of arthropod damage in the well-watered treatment, no differences in arthropod damage were observed between MON 87460 and the control for aphids, leafhoppers, grasshoppers, armyworms, corn earworms, cutworms, European corn borers, southwestern corn borers, corn rootworms, wireworms, leafrollers or mites using the observational severity scale at the NE, KS, and TX sites (Table H-29)

In an assessment of arthropod damage in the water-limited treatment, no differences in arthropod damage were observed between MON 87460 and the control for aphids, grasshoppers, armyworms, corn earworms, cutworms, European corn borers, southwestern corn borers, corn rootworms using the observational severity scale at the TX site (Table H-29)

## H.4. Summary

The results of the environmental interactions evaluation for MON 87460 supports the conclusion that the introduction of the drought tolerance trait did not unexpectedly alter MON 87460 compared to conventional corn. The lack of differences in plant response to abiotic stressors, disease damage, arthropod damage, and arthropod pest and beneficial

abundance indicate that the introduction of the drought tolerance trait is unlikely to be biologically meaningful in terms of increased pest potential.

## H.5. References

Widstrom, N.W. 1967. An evaluation for measuring corn earworm injury. Journal of Economic Entomology. 60:791-794.

**Table H-1. Abiotic Stressor Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in a 2006 U.S. Field Study under Well-Watered Conditions**

| Abiotic stressor | Number of observations across all sites [∞] (IA1, IA2, IL1, IL2, IN1, IN2, KS, NE) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 70 | 70 |
| **Cold** | 3 | 3 |
| **Compaction (includes soil compaction)** | 4 | 4 |
| **Drought** | 3 | 3 |
| **Flood (includes excessive water)** | 6 | 6 |
| **Frost** | 2 | 2 |
| **Green Snap [1]** | 1 | 1 |
| **Hail** | 13 | 13 |
| **Heat** | 6 | 6 |
| **Mineral toxicity** | 1 | 1 |
| **Nutrient deficiency** | 6 | 6 |
| **Wind** | 25 | 25 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a randomized complete block with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site codes are as follows: IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Stark County, IL; IL2 = Warren County, IL; IN1 = Boone County, IN; NE = York County, NE; KS = Pawnee County, KS

[1] Observations occurred between the V15 – VT growth stages across all plots

**Table H-2. Disease Damage Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in a 2006 U.S. Field Study under Well-Watered Conditions**

| Disease | Number of observations across all sites [∞] (IA1, IA2, IL1, IL2, IN1, IN2, KS, NE) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 112 | 112 |
| **Anthracnose** | 3 | 3 |
| **Ear rot** [1] | 1 | 1 |
| **Ear and kernel rot** [2] | 8 | 8 |
| **Eyespot** | 4 | 4 |
| ***Fusarium*** [3] | 3 | 3 |
| **Gray leaf spot** | 22 | 22 |
| **Leaf blight** | 1 | 1 |
| **Northern corn leaf blight** [4] | 12 | 12 |
| **Northern leaf spot** | 2 | 2 |
| ***Pythium*** | 4 | 4 |
| **Rust** [5] | 18 | 18 |
| **Seedling blight** | 3 | 3 |
| **Smut** | 4 | 4 |
| **Southern leaf blight** | 5 | 5 |
| **Stalk rot** [6,7] | 8 | 8 |
| **Stalk rot** [8] | 8 | 8 |
| **Stewarts wilt** | 4 | 4 |
| **Yellow leaf blight** | 2 | 2 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a randomized complete block with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site codes are as follows: IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Stark County, IL; IL2 = Warren County, IL; IN1 = Boone County, IN; NE = York County, NE; KS = Pawnee County, KS

[1] Ear rot assessed externally by observing the outside of the ear only; [2] Ear and kernel rot assessed by peeling back the husk and evaluating the ear and kernels; [3] *Fusarium* was rated as *Gibberella*/*Fusarium* at the IN1 site; [4] Includes northern leaf blight; [5] Includes leaf rust, common rust, and common/southern rust; [6] Includes charcoal stalk rot ; [7] Stalk rot assessed externally by observing the outside of the stalk only ; [8] Stalk rot assessed by splitting the stalks of 5 non-systematically selected plants and evaluating the disease

**Table H-3. Arthropod Damage Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in a 2006 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Number of observations across all sites [∞] (IA1, IA2, IL1, IL2, IN1, IN2, KS, NE) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 98 | 98 |
| **Aphids (includes corn leaf aphids)** | 12 | 12 |
| **Leafhoppers** | 2 | 2 |
| **Armyworms (includes fall armyworms)** | 4 | 4 |
| **Corn earworms [1]** | 6 | 6 |
| **Cutworms (includes black cutworms)** | 5 | 5 |
| **European corn borers [2]** | 25 | 25 |
| **Southwestern corn borers** | 1 | 1 |
| **Grasshoppers** | 6 | 6 |
| **Corn rootworms [3]** | 18 | 18 |
| **Flea beetles (includes corn flea beetles)** | 3 | 3 |
| **Japanese beetles** | 4 | 4 |
| **Grape colaspis** | 4 | 4 |
| **Wireworms** | 3 | 3 |
| **White grubs** | 1 | 1 |
| **Spider mites (include mites)** | 2 | 2 |
| **Seedcorn maggots** | 2 | 2 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a randomized complete block with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site codes are as follows: IA1 = Jefferson County, IA; IA2 = Benton County, IA; IL1 = Stark County, IL; IL2 = Warren County, IL; IN1 = Boone County, IN; NE = York County, NE; KS = Pawnee County, KS

[1] External observation, ear husks were not pulled back for evaluation ; [2] External observation; [3] Includes western corn rootworms, northern corn rootworms, and southern corn rootworms

**Table H-4. Quantitative Assessment for Corn Earworm and European Corn Borer Damage to MON 87460 Compared to the Control and References in a 2006 U.S. Field Study under Well-Watered Conditions**

| Pest | Damage Assessment | MON 87460 | Control | Reference Range[1] |
|---|---|---|---|---|
| **Corn earworm** | Mean ± SE of 5 ears (0 – 9 rating scale) | 1.7 ± 0.84 | 1.8 ± 0.93 | 0.0 – 5.20 |
| **European corn borer** | Mean ± SE # of ear shank entry/exit holes of 5 plants | 0.3 ± 0.18 | 0.3 ± 0.16 | 0.0 – 2.27 |
| **European corn borer** | Mean ± SE # of ear shank galleries per plant of 5 plants | 0.2 ± 0.09 | 0.2 ± 0.10 | 0.0 – 0.93 |
| **European corn borer** | Mean ± SE ear shank gallery length per plant of 5 plants (in.) | 0.2 ± 0.11 | 0.2 ± 0.11 | 0.0 – 0.93 |
| **European corn borer** | Mean ± SE # of stalk entry/exit holes of 5 plants | 1.9 ± 0.91 | 1.7 ± 0.65 | 0.07 – 6.53 |
| **European corn borer** | Mean ± SE # of stalk galleries per plant of 5 plants | 1.1 ± 0.40 | 1.0 ± 0.28 | 0.07 – 2.53 |
| **European corn borer** | Mean ± SE stalk gallery length per plant of 5 plants (in.) | 4.9 ± 2.37 | 3.3 ± 1.67 | 0.03 – 15.33 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).
[1] Reference range (the minimum and maximum values of the reference means).

**Table H-5. Quantitative Assessment for Corn Earworm and European Corn Borer Damage to MON 87460 Compared to the Control and References at the IL1, KS, and NE Sites in a 2006 U.S. Field Study under Well-Watered Conditions**

| Pest Arthropod | Damage Assessment | Site | MON 87460 | Control |
|---|---|---|---|---|
| Corn earworm | Mean ± SE of 5 ears (0 – 9 rating scale) | IL1 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| | Mean ± SE of 5 ears (0 – 9 rating scale) | KS | 5.0 ± 0.31 | 5.3 ± 0.96 |
| | Mean ± SE of 5 ears (0 – 9 rating scale) | NE | 0.0 ± 0.00 | 0.0 ± 0.00 |
| European corn borer | Mean ± SE # of ear shank entry/exit holes of 5 plants | IL1 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| | Mean ± SE # of ear shank entry/exit holes of 5 plants | KS | 1.0 ± 0.20 | 0.8 ± 0.31 |
| | Mean ± SE # of ear shank entry/exit holes of 5 plants | NE | 0.0 ± 0.00 | 0.0 ± 0.00 |
| European corn borer | Mean ± SE # of ear shank galleries per plant of 5 plants | IL1 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| | Mean ± SE # of ear shank galleries per plant of 5 plants | KS | 0.5 ± 0.07 | 0.5 ± 0.13 |
| | Mean ± SE # of ear shank galleries per plant of 5 plants | NE | 0.0 ± 0.00 | 0.0 ± 0.00 |
| European corn borer | Mean ± SE ear shank gallery length per plant of 5 plants (in.) | IL1 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| | Mean ± SE ear shank gallery length per plant of 5 plants (in.) | KS | 0.6 ± 0.12 | 0.7 ± 0.10 |
| | Mean ± SE ear shank gallery length per plant of 5 plants (in.) | NE | 0.0 ± 0.00 | 0.0 ± 0.00 |
| European corn borer | Mean ± SE # of stalk entry/exit holes of 5 plants | IL1 | 0.5 ± 0.18 | 1.0 ± 0.64 |
| | Mean ± SE # of stalk entry/exit holes of 5 plants | KS | 4.9 ± 1.75 | 3.8 ± 1.15 |
| | Mean ± SE # of stalk entry/exit holes of 5 plants | NE | 0.3 ± 0.13 | 0.3 ± 0.13 |
| European corn borer | Mean ± SE # of stalk galleries per plant of 5 plants | IL1 | 0.5 ± 0.18 | 0.7 ± 0.37 |
| | Mean ± SE # of stalk galleries per plant of 5 plants | KS | 2.4 ± 0.70 | 1.9 ± 0.37 |
| | Mean ± SE # of stalk galleries per plant of 5 plants | NE | 0.3 ± 0.13 | 0.3 ± 0.07 |
| European corn borer | Mean ± SE stalk gallery length per plant from 5 plants (in.) | IL1 | 0.6 ± 0.31 | 0.8 ± 0.45 |
| | Mean ± SE stalk gallery length per plant from 5 plants (in.) | KS | 14.0 ± 2.45 | 9.0 ± 3.00 |
| | Mean ± SE stalk gallery length per plant from 5 plants (in.) | NE | 0.2 ± 0.08 | 0.2 ± 0.05 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).
[1] Quantitative insect locations.

**Table H-6. Arthropod Pest Abundance on MON 87460 Compared to the Control and References at the IL1, KS, and NE Sites in a 2006 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Site | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Aphids | IL1 | 7.3 ± 1.67 | 9.3 ± 0.67 | 7.7 – 10.3 | 25.7 ± 1.76 | 23.0 ± 4.51 | 18.0 – 27.3 | 70.7 ± 8.35 | 63.3 ± 13.84 | 42.7 – 69.7 | 9.3 ± 0.33 | 3.0 ± 0.58 | 5.0 – 23.7 |
| | KS | 9.0 ± 1.53 | 12.3 ± 2.33 | 13.7– 19.3 | 4.0 ± 2.08 | 5.0 ± 1.53 | 4.0 – 7.3 | 5.3* ± 2.19 | 13.0 ± 1.00 | 1.7 – 5.7 | 3.7 ± 1.67 | 3.0 ± 0.58 | 1.7 – 2.0 |
| | NE | 0.0* ± 0.00 | 3.3 ± 0.33 | 0.7 – 2.7 | 3.0 ± 1.00 | 3.7 ± 0.88 | 2.7 – 6.3 | 76.3 ± 36.68 | 40.0 ± 14.22 | 33.0 – 62.7 | 1.0 ± 0.00 | 1.3 ± 0.88 | 1.0 – 2.7 |
| Corn flea beetles | IL1 | – | – | – | 23.3 ± 5.81 | 16.7 ± 3.18 | 15.0 – 25.0 | 6.0 ± 2.00 | 4.3 ± 1.33 | 4.7 – 7.0 | – | – | – |
| | KS | 5.3 ± 2.33 | 7.3 ± 0.88 | 2.0 – 6.7 | 10.3 ± 3.28 | 15.0 ± 2.08 | 12.3 – 17.7 | 1.7 ± 0.33 | 1.3 ± 0.33 | 0.7 – 1.7 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 1.0 |
| | NE | – | – | – | 0.0 ± 0.00* | 1.3 ± 0.33 | 0.0 – 1.0 | 0.7 ± 0.67 | 1.3 ± 0.88 | 0.0 – 1.0 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 1.0 |
| Delphacid planthoppers | IL1 | 0.0† ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.0 | 1.0 ± 0.58 | 1.0 ± 0.58 | 0.3 – 2.0 | – | – | – | – | – | – |
| Grasshoppers | KS | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – | – | – | – |
| | NE | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – | – | – | – |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.

Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not enumerated at this observation and site.

† p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-6 (Continued). Arthropod Pest Abundance on MON 87460 Compared to the Control and References at the IL1, KS, and NE Sites in a 2006 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Site | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Leafhoppers | KS | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.0 – 1.0 | 0.3 ± 0.33 | 1.0 ± 0.00 | 0.3 – 1.7 | 0.3 ± 0.33 | 1.0 ± 0.58 | 0.0 – 1.0 | 1.0 ± 1.00 | 0.7 ± 0.33 | 0.3 – 1.7 |
| | NE | – | – | – | 0.0 ± 0.00[†] | 0.0 ± 0.00 | 0.0 – 0.0 | – | – | – | 3.7 ± 0.33 | 3.0 ± 1.53 | 3.0 – 5.3 |
| Northern corn rootworms | IL1 | – | – | – | 2.0 ± 1.15 | 2.3 ± 0.33 | 2.3 – 3.7 | 2.7 ± 0.67 | 2.3 ± 2.33 | 1.3 – 2.3 | – | – | – |
| Sap beetles | IL1 | 0.7 ± 0.67 | 0.7 ± 0.33 | 0.3 – 1.7 | – | – | – | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.3 – 1.0 | – | – | – |
| | KS | 1.0 ± 1.00 | 0.3 ± 0.33 | 0.0 – 0.7 | – | – | – | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.0 – 0.3 | – | – | – |
| | NE | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.3 ± 0.33 | 0.7 ± 0.67 | 0.3 – 1.0 |
| Southern corn rootworms | IL1 | 0.0[†] ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.00 | – | – | – | – | – | – | – | – | – |
| | KS | – | – | – | – | – | – | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.0 – 0.3 | – | – | – |
| | NE | – | – | – | – | – | – | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not enumerated at this observation and site.
[†] p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-6 (Continued). Arthropod Pest Abundance on MON 87460 Compared to the Control and References at the IL1, KS, and NE Sites in a 2006 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Site | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Western corn rootworms | IL1 | – | – | – | 14.0 ± 4.04 | 16.3 ± 3.84 | 10.0 – 16.3 | 21.7 ± 5.24 | 19.7 ± 2.91 | 18.7 – 28.0 | – | – | – |
| | KS | – | – | – | 0.7 ± 0.33 | 0.7 ± 0.33 | 0.3 – 1.0 | – | – | – | – | – | – |
| | NE | – | – | – | 0.3 ± 0.33 | 0.7 ± 0.33 | 0.0 – 1.0 | 9.0 ± 1.00* | 3.0 ± 2.08 | 5.0 – 9.0 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not enumerated at this observation and site.

**Table H-7. Beneficial Arthropod Abundance on MON 87460 Compared to the Control and References at the IL1, KS, and NE Sites in a 2006 U.S. Field Study under Well-Watered Conditions**

| | | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| Arthropod | Site | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Brown lacewings | IL1 | – | – | – | – | – | – | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 1.0 |
| | KS | – | – | – | – | – | – | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 |
| Green lacewings | IL1 | – | – | – | 0.7 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.7 | 0.0 ± 0.00 | 1.0 ± 1.00 | 0.3 – 1.3 | – | – | – |
| | KS | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.7 | – | – | – | 0.7 ± 0.33 | 2.0 ± 0.58 | 0.0 – 1.7 | – | – | – |
| | NE | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 2.0 | 4.7 ± 0.88 | 2.3 ± 1.20 | 0.7 – 4.3 | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.0 – 1.3 |
| Ladybird beetles | IL1 | 2.3 ± 0.33 | 2.7 ± 0.67 | 3.7 – 5.7 | 4.7 ± 0.88 | 4.0 ± 1.73 | 2.3 – 3.7 | 3.0 ± 1.00 | 2.3 ± 1.20 | 2.3 – 3.0 | – | – | – |
| | KS | 4.7 ± 1.76 | 7.0 ± 0.58 | 4.0 – 7.0 | 0.7 ± 0.67 | 1.7 ± 0.33 | 1.7 – 2.7 | 0.3 ± 0.33 | 0.3 ± 0.33 | 0.3 – 1.0 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 1.0 |
| | NE | 0.7 ± 0.33 | 0.0 ± 0.00 | 0.7 – 2.7 | 2.7 ± 0.88 | 1.0 ± 0.58 | 1.7 – 3.3 | 4.3 ± 1.76 | 3.7 ± 1.86 | 1.0 – 9.3 | – | – | – |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.

**Table H-7 (Continued). Beneficial Arthropod Abundance on MON 87460 Compared to the Control and References at the IL1, KS, and NE Sites in a 2006 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Site | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Macro-parasitic hymenopterans[1] | IL1 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.3 – 0.7 | – | – | – | – | – | – | 2.7 ± 0.33 | 3.3 ± 0.33 | 1.7 – 4.0 |
| | KS | 0.0 ± 0.00[†] | 0.0 ± 0.00 | 0.0 – 0.0 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.0 ± 0.00 | 0.7 ± 0.67 | 0.0 – 0.7 | – | – | – |
| | NE | – | – | – | – | – | – | – | – | – | 0.3 ± 0.33 | 0.7 ± 0.33 | 0.7 – 2.0 |
| Micro-parasitic hymenopterans[2] | IL1 | 15.0 ± 2.52 | 12.3 ± 3.67 | 12.0 – 16.3 | 53.7 ± 9.94 | 42.0 ± 5.51 | 44.0 – 58.3 | 20.0 ± 4.73 | 17.3 ± 4.70 | 21.0 – 38.7 | 9.3 ± 2.19 | 9.0 ± 2.65 | 7.3 – 12.3 |
| | KS | 11.3 ± 2.96 | 13.3 ± 4.91 | 15.3 – 25.0 | 30.0* ± 3.51 | 42.3 ± 1.45 | 32.0 – 54.0 | 13.0* ± 0.58 | 23.3 ± 6.33 | 10.7 – 16.7 | 22.7 ± 4.63 | 22.0 ± 3.06 | 9.7 – 18.0 |
| | NE | 2.0 ± 0.58 | 3.0 ± 1.15 | 0.0 – 3.3 | 23.3 ± 4.67 | 19.7 ± 6.33 | 11.7 – 36.7 | 18.3 ± 5.55 | 17.0 ± 4.93 | 16.0 – 30.3 | 18.0 ± 7.51 | 21.0 ± 6.56 | 17.0 – 42.0 |
| Minute pirate bugs | IL1 | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.7 – 1.7 | 5.7 ± 0.88 | 4.7 ± 1.45 | 7.7 – 10.0 | 11.7 ± 3.71 | 5.3 ± 0.88 | 6.0 – 8.3 | 0.3 ± 0.33 | 0.3 ± 0.33 | 0.0 – 0.3 |
| | KS | 1.3 ± 0.67 | 0.3 ± 0.33 | 0.7 – 1.7 | 2.3 ± 1.20 | 5.0 ± 1.53 | 2.7 – 5.0 | 3.3 ± 1.20 | 4.3 ± 1.20 | 2.7 – 5.3 | 1.3 ± 0.67 | 1.3 ± 0.33 | 0.0 – 1.0 |
| | NE | – | – | – | 0.3 ± 0.33 | 0.3 ± 0.33 | 0.0 – 0.7 | 7.3 ± 0.88 | 8.7 ± 2.60 | 7.7 – 9.7 | 1.0 ± 0.58 | 0.7 ± 0.33 | 1.3 – 3.3 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.

[1] Greater than 5mm in length ; [2] Less than 5mm in length.

[†] p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-7 (Continued). Beneficial Arthropod Abundance on MON 87460 Compared to the Control and References at the IL1, KS, and NE Sites in a 2006 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Site | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Nabids | IL1 | – | – | – | 0.0 ± 0.00[†] | 0.0 ± 0.00 | 0.0 – 0.0 | – | – | – | – | – | – |
| Spiders | IL1 | 1.3 ± 0.88 | 2.7 ± 0.67 | 0.3 – 2.3 | – | – | – | 1.0 ± 0.00 | 2.0 ± 1.00 | 0.3 – 2.0 | 1.3 ± 0.67 | 1.0 ± 0.58 | 0.7 – 2.0 |
| | KS | – | – | – | 0.0 ± 0.00 | 0.7 ± 0.33 | 0.7 – 1.7 | – | – | – | 2.7 ± 1.20 | 1.0 ± 0.00 | 0.3 – 1.7 |
| | NE | 1.0 ± 0.58 | 1.0 ± 0.00 | 0.3 – 1.0 | 0.0 ± 0.00 | 1.0 ± 0.58 | 0.0 – 0.3 | 1.7 ± 0.88 | 0.7 ± 0.67 | 1.0 – 2.0 | 3.7 ± 0.67 | 4.0 ± 3.00 | 2.7 – 5.0 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.
[†] p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-8. Abiotic Stressor Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in a 2007 U.S. Field Study under Well-Watered Conditions**

| Abiotic stressor | Number of observations across all sites [∞] (IA1, IA2, IL1, IL2, IL3, IN, NE, OH, PA) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 90 | 90 |
| **Cold** | 3 | 3 |
| **Compaction (includes soil compaction)** | 7 | 7 |
| **Drought** | 2 | 2 |
| **Flood (includes excessive water)** | 5 | 5 |
| **Frost** | 2 | 2 |
| **Hail** | 14 | 14 |
| **Heat** | 18 | 18 |
| **Mineral toxicity** | 1 | 1 |
| **Nutrient deficiency** | 14 | 14 |
| **Wind** | 24 | 24 |

Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05). The experimental design was a randomized complete block with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn.

[∞] Site codes are as follows: IA1 = Jefferson County, IA; IA2 = Van Horne County, IA; IL1 = Stark County, IL; IL2 = Warren County, IL; IL3 = Clinton County, IL; IN = Boone County, IN; NE = York County, NE; OH = Fayette County, OH; PA = Berks County, PA

**Table H-9. Disease Stressor Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in a 2007 U.S. Field Study under Well-Watered Conditions**

| Disease | Number of observations across all sites [∞] (IA1, IA2, IL1, IL2, IL3, IN, NE, OH, PA) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 120 | 120 |
| **Anthracnose** | 7 | 7 |
| **Ear rot [1]** | 1 | 1 |
| **Ear rot [2]** | 8 | 8 |
| **Eyespot** | 8 | 8 |
| ***Fusarium*** | 3 | 3 |
| **Gray leaf spot** | 21 | 21 |
| **Leaf blight [3]** | 3 | 3 |
| **Northern corn leaf blight** | 14 | 14 |
| ***Pythium*** | 4 | 4 |
| **Seedling blight** | 6 | 6 |
| **Root rot** | 4 | 4 |
| **Rust [4]** | 21 | 21 |
| **Smut** | 6 | 6 |
| **Stalk rot [5]** | 1 | 1 |
| **Stalk rot [6]** | 8 | 8 |
| **Stewarts wilt** | 5 | 5 |

Note: No statistically significant differences between MON 87460 and the control ($\alpha$=0.05). The experimental design was a randomized complete block with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn.

[∞] Site codes are as follows: IA1 = Jefferson County, IA; IA2 = Van Horne County, IA; IL1 = Stark County, IL; IL2 = Warren County, IL; IL3 = Clinton County, IL; IN = Boone County, IN; NE = York County, NE; OH = Fayette County, OH; PA = Berks County, PA

[1] Ear rot assessed externally by observing the outside of the ear only; [2] Ear rot assessed by pulling husk back from ears of 5 non-systematically selected plants and evaluating the disease; [3] Includes southern leaf blight; [4] Includes leaf rust, common rust, and common/southern rust; [5] Stalk rot assessed externally by observing the outside of the stalk only; [6] Stalk rot assessed by splitting the stalks of 5 non-systematically selected plants and evaluating the disease

**Table H-10. Arthropod Stressor Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in a 2007 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Number of observations across all sites [∞] (IA1, IA2, IL1, IL2, IL3, IN, NE, OH, PA) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 99 | 98 |
| **Aphids (includes corn leaf aphids)** | 10 | 10 |
| **Leafhoppers** | 2 | 2 |
| **Thrips** | 2 | 2 |
| **Grasshoppers** | 6 | 6 |
| **Armyworms (includes fall armyworms)** | 10 | 10 |
| **Corn earworms [1]** | 6 | 6 |
| **Cutworms (includes black cutworms)** | 9 | 9 |
| **European corn borers [2, *]** | 22 | 21 |
| **Corn rootworms [3]** | 11 | 11 |
| **Flea beetles (includes corn flea beetles)** | 7 | 7 |
| **Grape colaspis** | 3 | 3 |
| **Japanese beetles** | 8 | 8 |
| **Billbugs** | 2 | 2 |
| **White grubs** | 1 | 1 |

[*] Differences were detected between MON 87460 and the control for European corn borer at the IA1 site during the fourth observation (moderate vs. slight). The difference detected was outside the range of the reference (no-sl)

Note: The experimental design was a randomized complete block with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn.

[∞] Site codes are as follows: IA1 = Jefferson County, IA; IA2 = Van Horne County, IA; IL1 = Stark County, IL; IL2 = Warren County, IL; IL3 = Clinton County, IL; IN = Boone County, IN; NE = York County, NE; OH = Fayette County, OH; PA = Berks County, PA

[1] External observation, ear husks were not pulled back for evaluation; [2] External observation; [3] Includes western corn rootworms and northern corn rootworms

**Table H-11. Quantitative Assessment for Corn Earworm and European Corn Borer Damage to MON 87460 Compared to the Control and References Combined across IA1, IL1, and IN Sites in a 2007 U.S. Field Study under Well-Watered Conditions**

| Pest Arthropod | Damage Assessment | MON 87460 | Control | Reference Range[1] |
|---|---|---|---|---|
| **Corn earworm** | Mean ± SE of 10 ears (0 – 9 rating scale) | 1.8 ± 0.95 | 2.6 ± 1.20 | 0.00 – 6.37 |
| **European corn borer** | Mean ± SE # of stalk entry/exit holes of 10 plants | 1.6 ± 0.35 | 1.4 ± 0.43 | 0.50 – 2.70 |
| **European corn borer** | Mean ± SE # of stalk galleries per plant of 10 plants | 1.4 ± 0.26 | 1.3 ± 0.35 | 0.48 – 2.28 |
| **European corn borer** | Mean ± SE stalk gallery length per plant of 10 plants (in.) | 2.1 ± 0.49 | 2.0 ± 0.80 | 0.62 – 4.50 |
| **European corn borer** | Mean ± SE live larvae per plant from 10 plants | 0.2 ± 0.09 | 0.2 ± 0.09 | 0.07 – 0.55 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).
[1] Reference range (the minimum and maximum values of the reference means).

**Table H-12. Quantitative Assessment for Corn Earworm and European Corn Borer Damage to MON 87460 Compared to the Control, and References at the IA1, IL1, and IN sites in a 2007 U.S. Field Study under Well-Watered Conditions**

| Pest Arthropod | Damage Assessment | Site | MON 87460 | Control |
|---|---|---|---|---|
| Corn earworm | Mean ± SE of 10 ears (0 – 9 rating scale) | IA1 | 0.2 ± 0.09 | 0.4 ± 0.09 |
| | Mean ± SE of 10 ears (0 – 9 rating scale) | IL1 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| | Mean ± SE of 10 ears (0 – 9 rating scale) | IN | 5.1 ± 1.57 | 7.2 ± 0.93 |
| European corn borer | Mean ± SE # of stalk entry/exit holes of 10 plants | IA1 | 0.9 ± 0.12 | 0.8 ± 0.19 |
| | Mean ± SE # of stalk entry/exit holes of 10 plants | IL1 | 1.2 ± 0.24 | 0.8 ± 0.20 |
| | Mean ± SE # of stalk entry/exit holes of 10 plants | IN | 2.7 ± 0.70 | 2.8 ± 0.85 |
| European corn borer | Mean ± SE # of stalk galleries per plant of 10 plants | IA1 | 0.8 ± 0.20 | 0.7 ± 0.15 |
| | Mean ± SE # of stalk galleries per plant of 10 plants | IL1 | 1.2 ± 0.24 | 0.7 ± 0.18 |
| | Mean ± SE # of stalk galleries per plant of 10 plants | IN | 2.2 ± 0.47 | 2.5 ± 0.61 |
| European corn borer | Mean ± SE stalk gallery length per plant from 10 plants (in.) | IA1 | 0.8 ± 0.25 | 0.8 ± 0.25 |
| | Mean ± SE stalk gallery length per plant from 10 plants (in.) | IL1 | 1.8* ± 0.39 | 0.7 ± 0.26 |
| | Mean ± SE stalk gallery length per plant from 10 plants (in.) | IN | 3.7 ± 0.71 | 4.6 ± 1.60 |
| European corn borer | Mean ± SE live larvae per plant from 10 plants | IA1 | 0.0 ± 0.03 | 0.0 ± 0.00 |
| | Mean ± SE live larvae per plant from 10 plants | IL1 | 0.4* ± 0.17 | 0.1 ± 0.03 |
| | Mean ± SE live larvae per plant from 10 plants | IN | 0.3 ± 0.18 | 0.5 ± 0.18 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

**Table H-13 Arthropod Pest Abundance on MON 87460 Compared to the Control and References at the IA1, IL1, and IN Sites in a 2007 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Site | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Aphids | IA1 | 5.7 ± 1.86 | 3.0 ± 1.73 | 3.7 – 6.7 | 4.7 ± 1.45 | 4.0 ± 1.73 | 1.7 – 6.3 | 32.0 ± 11.02 | 45.3 ± 9.96 | 18.0 – 44.3 | 25.3 ± 3.76 | 23.7 ± 14.66 | 10.3 – 51.0 |
| | IL1 | 45.7 ± 27.81 | 39.0 ± 23.25 | 7.0 – 94.7 | 2.0 ± 1.00 | 0.3 ± 0.33 | 1.0 – 2.7 | 49.0 ± 24.58 | 42.7 ± 9.35 | 15.7 – 126.7 | 4.0 ± 1.53 | 4.3 ± 1.45 | 3.3 – 6.3 |
| | IN | 8.3 ± 0.88 | 6.3 ± 1.33 | 8.7 – 18.3 | 11.3 ± 3.53 | 12.3 ± 5.84 | 4.7 – 27.0 | 7.7 ± 1.45 | 40.7 ± 29.34 | 3.0 – 118.3 | 6.7 ± 1.20 | 5.7 ± 0.88 | 1.0 – 3.3 |
| Delphacid planthoppers | IA1 | – | – | – | 0.3 ± 0.33 | 1.7 ± 1.67 | 0.0 – 0.7 | 18.3 ± 4.84 | 23.0 ± 8.33 | 9.3 – 21.0 | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.0 – 0.7 |
| | IL1 | 67.0 ± 15.50 | 80.0 ± 32.75 | 53.0 – 137.7 | 6.7 ± 3.18 | 13.7 ± 2.96 | 3.0 – 12.3 | 4.3 ± 1.86 | 4.7 ± 0.88 | 2.3 – 7.3 | 1.0 ± 0.58 | 1.3 ± 0.33 | 0.3 – 1.3 |
| | IN | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.3 – 1.7 | 100.0 ± 18.68 | 81.3 ± 17.70 | 47.7 – 92.0 | 0.3 ± 0.33 | 0.7 ± 0.33 | 0.3 – 1.7 |
| Grasshoppers | IN | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.0 ± 0.00[†] | 0.0 ± 0.00 | 0.0 – 0.0 | – | – | – | – | – | – |
| Leafhoppers | IA1 | 0.0[†] ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.00 | – | – | – | – | – | – | – | – | – |
| | IL1 | 12.7 ± 6.64 | 9.7 ± 3.84 | 7.7 – 15.7 | – | – | – | – | – | – | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.7 |
| | IN | 3.3 ± 1.45 | 2.7 ± 1.45 | 1.3 – 3.3 | 20.3 ± 13.45 | 15.0 ± 6.03 | 13.3 – 66.7 | 6.3 ± 4.37 | 12.3 ± 5.67 | 3.0 – 17.3 | 0.3 ± 0.33 | 0.7 ± 0.33 | 0.0 – 1.0 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant difference between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.
[†] p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-13 (Continued). Arthropod Pest Abundance on MON 87460 Compared to the Control and References at the IA1, IL1, and IN Sites in a 2007 U.S. Field Study under Well-Watered Conditions**

| Arthropod | Site | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Corn flea beetle | IA1 | 0.3 ± 0.33 | 0.3 ± 0.33 | 0.0 – 0.7 | 0.3 ± 0.33 | 0.7 ± 0.33 | 0.0 – 0.7 | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.3 – 1.0 | 0.0 ± 0.00 | 0.7 ± 0.67 | 0.0 – 0.0 |
| | IL1 | 23.0 ± 11.14 | 18.0 ± 6.51 | 10.3 – 26.7 | 7.7 ± 0.88 | 6.7 ± 0.88 | 3.7 – 10.0 | 1.0 ± 1.00 | 0.7 ± 0.33 | 0.0 – 2.0 | 0.3 ± 0.33 | 0.7 ± 0.67 | 0.3 – 0.7 |
| | IN | 0.7 ± 0.67 | 0.0 ± 0.00 | 0.0 – 0.7 | 15.0 ± 11.53 | 9.0 ± 3.06 | 8.7 – 19.3 | 9.7 ± 1.86 | 23.3 ± 8.82 | 4.3 – 23.7 | 7.7 ± 0.67 | 7.3 ± 2.85 | 1.7 – 5.7 |
| Northern corn rootworms | IA1 | – | – | – | – | – | – | 0.7 ± 0.33 | 1.7 ± 0.88 | 1.7 – 2.3 | 0.3 ± 0.33 | 0.7 ± 0.33 | 0.0 – 1.0 |
| | IL1 | – | – | – | 0.0 ± 0.00 | 0.7 ± 0.67 | 0.0 – 1.0 | 3.7 ± 0.33 | 3.0 ± 1.53 | 4.3 – 17.3 | – | – | – |
| Sap beetles | IA1 | 0.7 ± 0.67 | 0.3 ± 0.33 | 0.0 – 0.3 | 0.3 ± 0.33 | 0.7 ± 0.67 | 0.3 – 1.7 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 |
| | IL1 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | 1.0 ± 0.58 | 0.0 ± 0.00 | 0.0 – 0.7 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.7 |
| | IN | – | – | – | – | – | – | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.0 | 0.0 ± 0.00 | 0.7 ± 0.33 | 0.7 – 1.0 |
| Southern corn rootworms | IA1 | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.7 | – | – | – | – | – | – |
| | IL1 | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.

**Table H-13 (Continued). Arthropod Pest Abundance on MON 87460 Compared to the Control and References at the IA1, IL1, and IN sites in a 2007 U.S. Field Study under Well-Watered Conditions**

| | | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| **Arthropod** | **Site** | **MON 87460** | **Contr** | **Ref Range** | **MON 87460** | **Contr** | **Ref Range** | **MON 87460** | **Contr** | **Ref Range** | **MON 87460** | **Contr** | **Ref Range** |
| Western corn rootworms | IA1 | – | – | – | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.7 ± 0.67 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.0 ± 0.00[†] | 0.0 ± 0.00 | 0.0 – 0.0 |
| | IL1 | 26.0 ± 4.16 | 16.0 ± 5.20 | 10.0 – 44.7 | 6.3 ± 2.33 | 5.3 ± 1.86 | 2.3 – 6.0 | 16.7 ± 3.76 | 28.3 ± 9.40 | 24.3 – 37.0 | 0.3 ± 0.33 | 0.7 ± 0.67 | 0.3 – 1.3 |
| | IN | – | – | – | 32.0 ± 11.53 | 31.7 ± 5.78 | 33.0 – 39.3 | 25.0 ± 11.72 | 25.3 ± 7.62 | 15.3 – 41.3 | 0.0 ± 0.00[†] | 0.0 ± 0.00 | 0.0 – 0.0 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.
[†] p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-14. Beneficial Arthropod Abundance on MON 87460 Compared to the Control and References at the IA1, IL1, and IN Sites in a 2007 U.S. Field Study under Well-Watered Conditions**

| | | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| Arthropod | Site | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Araneae (spiders) | IA1 | 0.7 ± 0.33 | 0.7 ± 0.33 | 0.3 – 1.0 | 1.3 ± 0.88 | 0.3 ± 0.33 | 1.3 – 2.0 | 0.7 ± 0.67 | 2.3 ± 0.88 | 1.0 – 2.3 | 2.7 ± 0.88 | 1.0 ± 0.58 | 1.3 – 4.3 |
| | IL1 | 1.0 ± 1.00 | 0.3 ± 0.33 | 0.0 – 1.0 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 2.3 ± 0.67 | 0.7 ± 0.67 | 0.0 – 2.0 | 3.3 ± 0.88 | 3.0 ± 1.00 | 1.3 – 3.3 |
| | IN | 4.0 ± 1.73 | 3.7 ± 1.20 | 3.7 – 6.7 | 0.3 ± 0.33 | 0.3 ± 0.33 | 0.3 – 1.7 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 2.3 | 1.3 ± 0.88 | 0.7 ± 0.33 | 0.3 – 2.0 |
| Macro-parasitic hymenoptera[1] | IA1 | – | – | – | – | – | – | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.7 |
| | IN | – | – | – | – | – | – | 0.0 ± 0.00 | 0.7 ± 0.67 | 0.7 – 0.7 | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.0 – 0.3 |
| Micro-parasitic hymenoptera[2] | IA1 | 8.7 ± 1.20 | 6.7 ± 2.40 | 7.7 – 13.7 | 23.7 ± 4.48 | 27.3 ± 3.67 | 24.0 – 35.0 | 28.0 ± 10.21 | 21.0 ± 5.51 | 20.3 – 27.3 | 22.7 ± 2.40 | 36.7 ± 5.78 | 20.3 – 37.3 |
| | IL1 | 85.3 ± 22.70 | 63.0 ± 17.06 | 36.3 – 87.7 | 72.7 ± 13.42 | 77.7 ± 11.14 | 64.3 – 90.7 | 15.3 ± 1.76 | 11.7 ± 1.67 | 12.3 – 16.3 | 75.7 ± 5.46 | 92.7 ± 11.72 | 78.0 – 168.7 |
| | IN | 12.7 ± 3.38 | 9.3 ± 2.40 | 9.0 – 14.3 | 59.0 ± 10.44 | 72.0 ± 35.47 | 54.0 – 74.7 | 48.7* ± 19.70 | 118.3 ± 22.67 | 53.7 – 112.0 | 307.0 ± 9.71 | 256.0 ± 73.00 | 189.3 – 656.0 |
| Nabids | IN | 0.0 ± 0.00[†] | 0.0 ± 0.00 | 0.0 – 0.0 | 0.7 ± 0.67 | 0.3 ± 0.33 | 0.3 – 1.3 | – | – | – | – | – | – |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.

[1] Greater than 5mm in length.

[2] Less than 5mm in length.

[†] p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-14 (Continued). Beneficial Arthropod Abundance on MON 87460 Compared to the Control and References at the IA1, IL1, and IN Sites in a 2007 U.S. Field Study under Well-Watered Conditions**

| | | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| Arthropod | Site | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Orius | IA1 | 0.0† ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.0 | 3.7 ± 1.76 | 1.0 ± 0.58 | 3.7 – 7.0 | 15.3 ± 0.67 | 18.7 ± 3.76 | 5.7 – 12.7 | 2.3 ± 1.33 | 0.7 ± 0.33 | 0.0 – 2.3 |
| | IL1 | 12.7 ± 4.48 | 5.3 ± 2.67 | 3.7 – 17.3 | 0.7 ± 0.33 | 0.7 ± 0.33 | 0.3 – 1.0 | 9.3 ± 1.45 | 15.3 ± 0.67 | 6.0 – 15.3 | 7.0 ± 4.51 | 6.0 ± 1.15 | 7.3 – 21.3 |
| | IN | 0.0 ± 0.00 | 0.3 ± 0.33 | 0.0 – 0.0 | 4.3 ± 1.76 | 1.0 ± 0.00 | 1.3 – 3.7 | 16.0 ± 9.54 | 13.3 ± 4.26 | 5.0 – 16.0 | 0.7 ± 0.67 | 1.3 ± 0.67 | 0.0 – 2.3 |
| Brown lacewings | IA1 | – | – | – | – | – | – | 0.3 ± 0.33 | 0.7 ± 0.67 | 0.0 – 0.3 | – | – | – |
| | IL1 | – | – | – | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – |
| Green lacewings | IA1 | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – |
| | IL1 | – | – | – | – | – | – | 3.7 ± 0.33 | 5.7 ± 1.86 | 1.3 – 7.3 | – | – | – |
| | IN | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.
† p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-14 (Continued). Beneficial Arthropod Abundance on MON 87460 Compared to the Control and References at the IA1, IL1, and IN Sites in a 2007 U.S. Field Study under Well-Watered Conditions**

| | | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| Arthropod | Site | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Ladybird beetles | IA1 | 0.3 ± 0.33 | 1.3 ± 0.88 | 0.3 – 1.3 | 3.0 ± 0.58 | 3.0 ± 0.58 | 0.7 – 3.3 | 10.0 ± 2.31 | 15.3 ± 0.88 | 7.7 – 14.0 | 8.0 ± 2.65 | 10.3 ± 2.60 | 9.7 – 25.7 |
| | IL1 | 6.3 ± 2.03 | 8.7 ± 1.45 | 1.7 – 7.3 | 13.0 ± 1.53 | 6.3 ± 3.38 | 3.0 – 14.7 | 1.0 ± 0.00 | 1.3 ± 0.33 | 0.7 – 2.3 | 13.7 ± 1.86 | 16.7 ± 2.03 | 14.3 – 29.3 |
| | IN | 0.3 ± 0.33 | 0.0 ± 0.00 | 0.3 – 1.0 | 8.0 ± 0.58 | 6.3 ± 1.45 | 4.3 – 11.3 | 8.7 ± 2.73 | 4.3 ± 2.85 | 2.3 – 5.7 | 9.3 ± 1.20 | 7.3 ± 2.96 | 6.0 – 18.7 |

S.E. = standard error; the experimental design was a randomized complete block with three replicates.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not evaluated at this observation and site.

**Table H-15. Abiotic Stressor Incidence of MON 87460 Compared to the Control and References in a 2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices**

| Abiotic stressor | Number of observations across all sites [∞] (IAE, IAW, IL, IN, NE) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 47 | 47 |
| **Cold** | 2 | 2 |
| **Compaction** | 5 | 5 |
| **Flood** | 1 | 1 |
| **Frost** | 4 | 4 |
| **Hail** | 6 | 6 |
| **Heat** | 8 | 8 |
| **Nutrient deficiency** | 5 | 5 |
| **Wind** | 16 | 16 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a randomized complete block with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; NE = York County, NE.

**Table H-16. Disease Stressor Incidence of MON 87460 Compared to the Control and References in a 2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices**

| Disease | Number of observations across all sites [∞] (IAE, IAW, IL, IN, NE) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 67 | 67 |
| **Anthracnose** | 4 | 4 |
| **Ear rot** [1] | 5 | 5 |
| **Eyespot** | 3 | 3 |
| ***Fusarium*** | 1 | 1 |
| **Gray leaf spot** | 13 | 13 |
| **Leaf blight** | 1 | 1 |
| **Northern corn leaf blight** | 2 | 2 |
| **Northern leaf spot** | 5 | 5 |
| ***Pythium*** | 4 | 4 |
| **Seedling blight** | 5 | 5 |
| **Root rot** | 1 | 1 |
| **Rust** | 4 | 4 |
| **Southern leaf blight** | 2 | 2 |
| **Smut** | 4 | 4 |
| **Stalk rot** [2] | 6 | 6 |
| **Stewarts wilt** | 7 | 7 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a randomized complete block with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; NE = York County, NE.

[1] Ear rot assessed by pulling husk back from ears of 5 non-systematically selected plants and evaluating the disease;

[2] Stalk rot assessed by splitting the stalks of 5 non-systematically selected plants and evaluating the disease. At IL and IN, stalk rot data were collected both on a per plot basis and on 5 plants/plot

**Table H-17. Arthropod Stressor Incidence of MON 87460 Compared to the Control and References in a 2006 U.S. Field Study Established with Water Managed According to Local Agronomic Practices**

| Arthropod | Number of observations across all sites [∞] (IAE, IAW, IL, IN, NE) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 61 | 60 |
| **Aphids (includes corn leaf aphids)** | 5 | 5 |
| **Grasshoppers** | 12 | 11 |
| **Armyworms(includes fall armyworms)** | 2 | 2 |
| **Corn earworms** | 4 | 4 |
| **Cutworms** | 3 | 3 |
| **European corn borers [1]** | 13 | 13 |
| **Southwestern corn borers** | 1 | 1 |
| **Western bean cutworms** | 2 | 2 |
| **Corn rootworms [2]** | 7 | 7 |
| **Flea beetles (includes corn flea beetles)** | 2 | 2 |
| **Billbugs** | 1 | 1 |
| **Wireworms** | 3 | 3 |
| **White grubs** | 4 | 4 |
| **Seedcorn maggots** | 2 | 2 |

[*] Differences were detected between MON 87460 and the control for grasshopper at the IAE site during the third observation (none vs. slight). The difference detected was within the range of the reference (no-sl)

Note: The experimental design was a randomized complete block with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; NE = York County, NE.

[1] Includes corn borers; [2] Includes western corn rootworms and southern corn rootworms

**Table H-18. Chile 2006/2007: Abiotic Stressor Incidence of MON 87460 Compared to the Control and the References in a Field Study Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Abiotic stressor | Number of observations across all sites [∞] (CL, CT, LUM) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 29 | 29 |
| **Cold** | 1 | 1 |
| **Frost** | 3 | 3 |
| **Hail** | 1 | 1 |
| **Heat** | 9 | 9 |
| **Nitrogen deficiency** | 3 | 3 |
| **Wind** | 12 | 12 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: CL =Colina; CT Calera de Tango; LUM = Lumbreras

## Water-Limited Treatment

| Abiotic stressor | Number of observations across all sites [∞] (CL, CT, LUM) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 29 | 29 |
| **Cold** | 1 | 1 |
| **Frost** | 3 | 3 |
| **Hail** | 1 | 1 |
| **Heat** | 9 | 9 |
| **Nitrogen deficiency** | 3 | 3 |
| **Wind** | 12 | 12 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: CL =Colina; CT Calera de Tango; LUM = Lumbreras

**Table H-19. Chile 2006/2007: Disease Stressor Incidence of MON 87460 Compared to the Control and References in a Field Study Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Disease | Number of observations across all sites [∞] (CL, CT, LUM) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 35 | 35 |
| **Ear rot** | 2 | 2 |
| ***Fusarium*** | 3 | 3 |
| **Gray leaf spot** | 6 | 6 |
| **Leaf blight** | 5 | 5 |
| **Northern corn leaf blight** | 1 | 1 |
| **Root rot** | 3 | 3 |
| **Rust** | 9 | 9 |
| **Seedling blight** | 3 | 3 |
| **Smut** | 2 | 2 |
| **Stalk rot** | 1 | 1 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: CL =Colina; CT Calera de Tango; LUM = Lumbreras

## Water-Limited Treatment

| Disease | Number of observations across all sites [∞] (CL, CT, LUM) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 35 | 35 |
| **Ear rot** | 2 | 2 |
| ***Fusarium*** | 3 | 3 |
| **Gray leaf spot** | 6 | 6 |
| **Leaf blight** | 5 | 5 |
| **Northern corn leaf blight** | 1 | 1 |
| **Root rot** | 3 | 3 |
| **Rust** | 9 | 9 |
| **Seedling blight** | 3 | 3 |
| **Smut** | 2 | 2 |
| **Stalk rot** | 1 | 1 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: CL =Colina; CT Calera de Tango; LUM = Lumbreras

**Table H-20. Chile 2006/2007: Arthropod Stressor Incidence of MON 87460 Compared to the Control and References in a Field Study Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Arthropod | Number of observations across all sites [∞] (CL, CT, LUM) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 23 | 23 |
| **Aphids** | 9 | 9 |
| **Seedcorn maggots** | 3 | 3 |
| **Thrips** | 8 | 8 |
| **Wireworms** | 3 | 3 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: CL =Colina; CT Calera de Tango; LUM = Lumbreras

## Water-Limited Treatment

| Arthropod | Number of observations across all sites [∞] (CL, CT, LUM) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 23 | 23 |
| **Aphids** | 9 | 9 |
| **Seedcorn maggots** | 3 | 3 |
| **Thrips** | 8 | 8 |
| **Wireworms** | 3 | 3 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: CL =Colina; CT Calera de Tango; LUM = Lumbreras

**Table H-21. U.S. 2007 Study-1: Abiotic Stressor Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in Field Studies Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Abiotic stressor | Number of observations across all sites [∞] (CA, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 16 | 16 |
| **Drought** | 3 | 3 |
| **Hail** | 4 | 4 |
| **Heat** | 2 | 2 |
| **Heavy thunderstorm** | 1 | 1 |
| **Nitrogen deficiency** | 2 | 2 |
| **Wind** | 4 | 4 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: CA =Sutter County, CA; TX = Carson County, TX

## Water-Limited Treatment

| Abiotic stressor | Number of observations across all sites [@] (CA, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 16 | 16 |
| **Drought** | 3 | 3 |
| **Hail** | 4 | 4 |
| **Heat** | 2 | 2 |
| **Heavy thunderstorm** | 1 | 1 |
| **Nitrogen deficiency** | 2 | 2 |
| **Wind** | 4 | 4 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Observational data were collected at four crop developmental stages, Observation 1 = appx. V2-V4 growth stage; Observation 2 = appx. V10-V15 growth stage; Observation 3 = appx. VT-R3 growth stage; Observation 4 = appx. R6 growth stage.

[∞] Site codes are as follows: CA =Sutter County, CA; TX = Carson County, TX

**Table H-22. U.S. 2007 Study-1: Disease Damage Evaluations Using an Observational Severity Scale for MON 87460 Compared to the Control and References in Field Studies Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Disease | Number of observations across all sites [∞] (CA, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 18 | 18 |
| **Ear rot [1]** | 2 | 2 |
| ***Fusarium*** | 1 | 1 |
| **Gray mold** | 2 | 2 |
| **Maize dwarf mosaic virus** | 1 | 1 |
| **Rust** | 1 | 1 |
| **Seedling blight** | 1 | 1 |
| **Smut (head and ear)** | 4 | 4 |
| **Stalk rot [2]** | 2 | 2 |
| **Southern leaf blight** | 4 | 4 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with four replications; Observational data were collected at four crop developmental stages, Observation 1 = V2-V4, Observation 2 = V10-V15, Observation 3 = VT-R3, and Observation 4 = R6 growth stages of corn.

[∞] Site codes are as follows: CA =Sutter County, CA; TX = Carson County, TX

[1] ear rot assessed by pulling husk back from ears of 5 non-systematically selected plants and evaluating the disease

[2] stalk rot assessed by splitting the stalks of 5 non-systematically selected plants and evaluating the disease

## Water-Limited Treatment

| Disease | Number of observations across all sites [∞] (CA, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 18 | 18 |
| **Ear rot [1]** | 2 | 2 |
| ***Fusarium*** | 1 | 1 |
| **Gray mold** | 2 | 2 |
| **Maize dwarf mosaic virus** | 1 | 1 |
| **Rust** | 1 | 1 |
| **Seedling blight** | 1 | 1 |
| **Smut (head and ear)** | 4 | 4 |
| **Stalk rot [2]** | 2 | 2 |
| **Southern leaf blight** | 4 | 4 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with four replications; Observational data were collected at four crop developmental stages, Observation 1 = V2-V4, Observation 2 = V10-V15, Observation 3 = VT-R3, and Observation 4 = R6 growth stages of corn.

[∞] Site codes are as follows: CA =Sutter County, CA; TX = Carson County, TX

[1] ear rot assessed by pulling husk back from ears of 5 non-systematically selected plants and evaluating the disease

[2] stalk rot assessed by splitting the stalks of 5 non-systematically selected plants and evaluating the disease

**Table H-23. U.S. 2007 Study-1: Arthropod Damage Evaluated using an Observational Severity Scale for MON 87460 Compared to the Control and References in Field Studies Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Arthropod | Number of observations across all sites [∞] (CA, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 18 | 18 |
| **Aphids** | 4 | 4 |
| **Leafhoppers** | 2 | 2 |
| **Grasshoppers** | 1 | 1 |
| **Armyworms (includes fall armyworms)** | 2 | 2 |
| **Cutworms** | 1 | 1 |
| **European Corn Borers** | 2 | 2 |
| **Southwestern Corn Borers** | 2 | 2 |
| **Western Corn Rootworms** | 1 | 1 |
| **Mites (includes spider mites)** | 3 | 3 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with four replications; Observational data were collected at four crop developmental stages, Observation 1 = V2-V4, Observation 2 = V10-V15, Observation 3 = VT-R3, and Observation 4 = R6 growth stages of corn.

[∞] Site codes are as follows: CA =Sutter County, CA; TX = Carson County, TX

## Water-Limited Treatment

| Arthropod | Number of observations across all sites [∞] (CA, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 18 | 18 |
| **Aphids** | 4 | 4 |
| **Leafhoppers** | 2 | 2 |
| **Grasshoppers** | 1 | 1 |
| **Armyworms (includes fall armyworms)** | 2 | 2 |
| **Cutworms** | 1 | 1 |
| **European Corn Borers** | 2 | 2 |
| **Southwestern Corn Borers** | 2 | 2 |
| **Western Corn Rootworms** | 1 | 1 |
| **Mites (includes spider mites)** | 3 | 3 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with four replications; Observational data were collected at four crop developmental stages, Observation 1 = V2-V4, Observation 2 = V10-V15, Observation 3 = VT-R3, and Observation 4 = R6 growth stages of corn.

[∞] Site codes are as follows: CA =Sutter County, CA; TX = Carson County, TX

**Table H-24. U.S. 2007 Study-1: Quantitative Assessment for Corn Earworm and European Corn Borer Damage to MON 87460 Compared to the Control and References at TX in Field Studies Established with Well-Watered and Water-Limited Treatments**

**Well-Watered Treatment**

| Pest | Damage Assessment | MON 87460 | Control | Reference Range[1] |
|---|---|---|---|---|
| Corn earworm | Mean ± SE of 5 ears (0 – 9 rating scale) | 3.2 ± 0.32 | 3.4 ± 0.29 | 2.70 – 3.00 |
| European corn borer | Mean ± SE larvae/5 plants | 0.0[†] ± 0.00 | 0.0 ± 0.00 | 0.00 – 0.00 |
| European corn borer | Mean ± SE # of stalk entry/exit holes of 5 plants | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.00 – 0.05 |
| European corn borer | Mean ± SE # of stalk galleries per plant of 5 plants | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.00 – 0.03 |
| European corn borer | Mean ± SE stalk gallery length per plant of 5 plants (in.) | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.00 – 0.01 |

**Water-Limited Treatment**

| Pest | Damage Assessment | MON 87460 | Control | Reference Range[1] |
|---|---|---|---|---|
| Corn earworm | Mean ± SE of 5 ears (0 – 9 rating scale) | 2.5 ± 0.12 | 2.7 ± 0.09 | 2.60 – 2.80 |
| European corn borer | Mean ± SE larvae/5 plants | 0.0[†] ± 0.00 | 0.0 ± 0.00 | 0.00 – 0.00 |
| European corn borer | Mean ± SE # of stalk entry/exit holes of 5 plants | 0.0[†] ± 0.00 | 0.0 ± 0.00 | 0.00 – 0.00 |
| European corn borer | Mean ± SE # of stalk galleries per plant of 5 plants | 0.0[†] ± 0.00 | 0.0 ± 0.00 | 0.00 – 0.00 |
| European corn borer | Mean ± SE stalk gallery length per plant of 5 plants (in.) | 0.0[†] ± 0.00 | 0.0 ± 0.00 | 0.00 – 0.00 |

S.E. = standard error; the experimental design was a split plot design with four replications.
No statistically significant differences between MON 87460 and the control ($\alpha$=0.05).
[1] Reference range (the minimum and maximum values of the reference means).
[†] p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-25. U.S. 2007 Study-1: Arthropod Pest abundance on MON 87460 Compared to the Control and References at TX in Field Studies Established with Well-Watered and Water-Limited Treatments**

**Well-Watered Treatment**

| Arthropod | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Aphids | 5.5 ± 1.32 | 5.0 ± 1.08 | 7.5 – 10.0 | 67.0 ± 17.92 | 62.5 ± 13.86 | 50.3 – 79.3 | 6.0 ± 1.41 | 6.5 ± 2.22 | 4.3 – 6.0 | 17.8 ± 3.07 | 11.5 ± 3.52 | 9.8 – 17.5 |
| Delphacid planthoppers | 0.0 ± 0.00 | 0.3 ± 0.25 | 0.3 – 0.3 | 1.3 ± 0.48 | 1.3 ± 0.75 | 0.5 – 1.8 | 5.8 ± 2.46 | 5.3 ± 2.10 | 4.3 – 11.8 | 3.8 ± 0.85 | 4.5 ± 1.44 | 4.5 – 7.3 |
| Leafhoppers | 17.8 ± 2.69 | 11.8 ± 3.22 | 9.0 – 15.8 | 1.3 ± 0.75 | 1.3 ± 0.75 | 0.3 – 2.3 | 11.5 ± 2.99 | 13.8 ± 4.50 | 9.0 – 12.5 | 163.8 ± 45.48 | 159.3 ± 38.52 | 142.5 – 190.8 |
| Grasshoppers | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – | – | – | – |
| Corn flea beetles | 1.8 ± 0.48 | 1.3 ± 0.25 | 0.3 – 1.5 | 13.0 ± 4.02 | 11.0 ± 4.92 | 9.8 – 20.5 | 26.8 ± 6.69 | 23.8 ± 7.30 | 15.8 – 23.5 | 13.3 ± 1.31 | 15.0 ± 2.74 | 13.0 – 16.0 |
| Sap beetles | – | – | – | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – |
| Southern corn rootworms | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 1.0 ± 0.41 | 1.3 ± 1.25 | 0.8 – 1.8 | – | – | – | 0.3 ± 0.25 | 0.5 ± 0.29 | 0.3 – 1.3 |
| Western corn rootworms | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.5 | 0.0† ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.0 | 0.0† ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.0 |

S.E. = standard error; the experimental design was a split plot design with four replications.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not enumerated at this observation and site.
† p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-25 (Continued). U.S. 2007 Study-1: Arthropod Pest Abundance on MON 87460 Compared to the Control and References at TX in Field Studies Established with Well-Watered and Water-Limited Treatments**

**Water-Limited Treatment**

| Arthropod | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Aphids | 7.3 ± 3.28 | 5.5 ± 1.04 | 7.0 – 11.8 | 80.8 ± 23.26 | 80.0 ± 8.67 | 69.3 – 87.5 | 4.8 ± 1.11 | 8.3 ± 1.93 | 3.5 – 7.8 | 16.3 ± 2.02 | 16.8 ± 2.66 | 11.5 – 16.5 |
| Delphacid planthoppers | – | – | – | 1.8 ± 0.75 | 1.3 ± 0.48 | 0.8 – 2.0 | 4.8 ± 1.38 | 7.3 ± 2.93 | 2.8 – 10.0 | 5.3 ± 2.63 | 4.3 ± 1.93 | 5.0 – 8.8 |
| Leafhoppers | 9.3 ± 1.31 | 17.0 ± 6.82 | 8.5 – 20.5 | 1.8 ± 1.18 | 0.8 ± 0.25 | 0.5 – 1.8 | 10.8 ± 2.81 | 10.5 ± 4.29 | 8.8 – 12.5 | 137.5 ± 43.06 | 179.8 ± 32.53 | 96.8 – 171.0 |
| Grasshoppers | 0.3 ± 0.25 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – | – | – | – |
| Corn flea beetles | 1.3 ± 0.25 | 1.5 ± 0.50 | 0.3 – 1.3 | 21.5 ± 6.25 | 13.3 ± 2.21 | 9.3 – 18.3 | 18.0 ± 4.88 | 23.5 ± 9.21 | 12.5 – 23.8 | 16.5 ± 3.75 | 15.5 ± 2.53 | 7.8 – 15.8 |
| Sap beetles | – | – | – | – | – | – | – | – | – | – | – | – |
| Southern corn rootworms | – | – | – | 0.5 ± 0.29 | 0.8 ± 0.48 | 0.3 – 0.8 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | 2.5* ± 0.65 | 0.8 ± 0.48 | 0.8 – 1.5 |
| Western corn rootworms | – | – | – | 0.3 ± 0.25 | 0.0 ± 0.00 | 0.0 – 0.3 | 0.3 ± 0.25 | 0.8 ± 0.75 | 0.0 – 0.0 | 0.0 ± 0.00[†] | 0.0 ± 0.00 | 0.0 – 0.0 |

S.E. = standard error; the experimental design was a split plot design with four replications.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not enumerated at this observation and site.

[†] p-values could not be generated due to lack of variability in the data. MON 87460 was considered effectively not different from the control because the MON 87460 and control mean values were identical.

**Table H-26. U.S. 2007 Study-1: Arthropod Beneficial Abundance on MON 87460 Compared to the Control and References at TX in Field Studies Established with Well-Watered and Water-Limited Treatments**

**Well-Watered Treatment**

| Arthropod | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Araneae (spiders) | 17.5 ± 5.85 | 21.0 ± 3.85 | 15.0 – 24.5 | 4.0 ± 1.29 | 5.0 ± 1.00 | 2.5 – 2.8 | 2.8 ± 1.11 | 2.0 ± 1.08 | 1.8 – 2.8 | 10.8 ± 2.32 | 8.3 ± 2.32 | 7.8 – 9.0 |
| Brown lacewings | – | – | – | – | – | – | 0.5* ± 0.29 | 0.0 ± 0.00 | 0.0 – 0.5 | – | – | – |
| Green lacewings | – | – | – | 2.0 ± 0.41 | 0.5 ± 0.29 | 0.5 – 1.5 | 2.0 ± 0.91 | 3.5 ± 1.76 | 2.5 – 4.3 | 2.5 ± 1.55 | 2.5 ± 0.96 | 0.0 – 3.3 |
| Big-eyed bug | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – | – | – | – |
| *Orius* | 0.3 ± 0.25 | 0.8 ± 0.75 | 0.3 – 1.0 | 4.3 ± 0.63 | 4.8 ± 1.84 | 2.3 – 6.8 | 3.0 ± 0.71 | 3.0 ± 1.41 | 2.0 – 3.3 | 3.0 ± 1.08 | 2.8 ± 0.85 | 1.5 – 2.5 |
| Ladybird beetle | 1.3 ± 0.75 | 0.5 ± 0.29 | 0.5 – 0.8 | 5.0 ± 1.08 | 6.0 ± 1.35 | 5.0 – 6.8 | 8.8 ± 2.29 | 5.0 ± 1.73 | 5.3 – 12.0 | 3.0 ± 0.58 | 3.3 ± 0.48 | 1.3 – 3.3 |
| Macro-parasitic hymenoptera | – | – | – | 0.5 ± 0.29 | 1.0 ± 0.41 | 0.8 – 4.3 | – | – | – | 0.8 ± 0.48 | 1.5 ± 0.65 | 0.0 – 1.3 |
| Micro-parasitic hymenoptera | 35.0 ± 5.57 | 37.3 ± 7.42 | 21.3 – 32.8 | 118.5 ± 14.77 | 98.8 ± 18.43 | 101.5 – 128.5 | 59.3 ± 1.93 | 51.0 ± 5.43 | 39.3 – 49.5 | 217.3 ± 23.25 | 248.0 ± 13.35 | 189.0 – 222.8 |

S.E. = standard error; the experimental design was a split plot design with four replications.
*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not enumerated at this observation and site.

**Table H-26 (Continued). U.S. 2007 Study-1: Arthropod Beneficial Abundance on MON 87460 Compared to the Control and References at TX in Field Studies Established with Well-Watered and Water-Limited Treatments**

**Water-Limited Treatment**

| Arthropod | Mean Arthropod abundance ± S.E. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Collection 1 | | | Collection 2 | | | Collection 3 | | | Collection 4 | | |
| | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range | MON 87460 | Contr | Ref Range |
| Araneae (spiders) | 21.0 ± 3.08 | 19.3 ± 3.42 | 19.3 – 22.0 | 2.3 ± 0.48 | 2.5 ± 0.65 | 1.5 – 3.0 | 4.8 ± 2.17 | 1.8 ± 0.48 | 1.5 – 4.3 | 10.3 ± 1.93 | 6.0 ± 1.87 | 7.5 – 11.3 |
| Brown lacewings | – | – | – | – | – | – | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – |
| Green lacewings | – | – | – | 0.5 ± 0.50 | 0.5 ± 0.29 | 0.8 – 1.5 | 2.3 ± 0.63 | 2.0 ± 0.71 | 2.0 – 3.8 | 2.3 ± 0.85 | 1.5 ± 0.50 | 0.8 – 2.0 |
| Big-eyed bug | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.3 | – | – | – | – | – | – | – | – | – |
| *Orius* | 0.3 ± 0.25 | 0.0 ± 0.00 | 0.0 – 0.8 | 5.0 ± 1.22 | 7.0 ± 1.58 | 3.8 – 7.8 | 3.3 ± 1.97 | 2.8 ± 1.25 | 1.5 – 3.5 | 3.3 ± 1.49 | 1.3 ± 0.63 | 1.5 – 4.3 |
| Ladybird beetle | 0.3 ± 0.25 | 0.0 ± 0.00 | 0.3 – 1.8 | 6.5 ± 1.26 | 5.8 ± 0.25 | 4.8 – 7.5 | 2.8 ± 0.85 | 6.0 ± 0.91 | 6.0 – 9.0 | 2.8 ± 0.63 | 2.3 ± 1.11 | 1.3 – 2.3 |
| Macro-parasitic hymenoptera | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 – 0.8 | – | – | – | – | – | – | 1.3 ± 0.48 | 1.5 ± 0.65 | 0.5 – 2.3 |
| Micro-parasitic hymenoptera | 24.8 ± 2.90 | 27.3 ± 6.02 | 27.5 – 43.0 | 111.5 ± 7.80 | 101.8 ± 14.08 | 90.0 – 112.8 | 48.0 ± 4.02 | 51.8 ± 7.19 | 46.5 – 51.5 | 240.3 ± 53.00 | 199.8 ± 28.54 | 172.8 – 266.8 |

S.E. = standard error; the experimental design was a split plot design with four replications.
No statistically significant differences between MON 87460 and the control (α=0.05).
Contr = Control; Ref Range = minimum and maximum mean values of the references; dash (–) = arthropods not enumerated at this observation and site.

**Table H-27. U.S. 2007 Study-2: Abiotic Stressor Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in Field Studies Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Abiotic stressor | Number of observations across all sites [∞] (KS, NE, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 29 | 29 |
| **Drought** | 1 | 1 |
| **Hail** | 8 | 8 |
| **Heat** | 5 | 5 |
| **Water logging** | 3 | 3 |
| **Wind** | 12 | 12 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site codes are as follows: NE = York County, NE; KS = Pawnee County, KS; TX = Carson County, TX

## Water-Limited Treatment

| Abiotic stressor | Number of observations across all sites [∞] (TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 12 | 12 |
| **Drought** | 1 | 1 |
| **Hail** | 4 | 4 |
| **Heat** | 3 | 3 |
| **Wind** | 4 | 4 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site code is as follows: TX = Carson County, TX

**Table H-28. U.S. 2007 Study-2: Disease Damage Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in Field Studies Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Disease | Number of observations across all sites [∞] (KS, NE, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 42 | 42 |
| **Crazy top** | 1 | 1 |
| **Ear rot [1]** | 3 | 3 |
| **Gray leaf spot** | 7 | 7 |
| **Gray mold** | 3 | 3 |
| **Maize dwarf mosaic** | 1 | 1 |
| **Northern corn leaf blight** | 7 | 7 |
| **Rust [2]** | 8 | 8 |
| **Seedling blight** | 2 | 2 |
| **Smut** | 3 | 3 |
| **Stalk rot [3]** | 3 | 3 |
| **Southern corn leaf blight** | 4 | 4 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site codes are as follows: NE = York County, NE; KS = Pawnee County, KS; TX = Carson County, TX

[1] Ear rot assessed externally by observing the outside of the ear only; [2] Includes common rust and leaf rust; [3] Stalk rot assessed by splitting the stalks of 5 non-systematically selected plants and evaluating the disease

## Water-Limited Treatment

| Disease | Number of observations across all sites [∞] (TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 14 | 14 |
| **Ear rot [1]** | 1 | 1 |
| **Gray mold** | 3 | 3 |
| **Maize dwarf mosaic** | 1 | 1 |
| **Northern corn leaf blight** | 3 | 3 |
| **Seedling blight** | 1 | 1 |
| **Stalk rot [2]** | 1 | 1 |
| **Southern corn leaf blight** | 4 | 4 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site code is TX = Carson County, TX

[1] Ear rot assessed externally by observing the outside of the ear only; [2] Stalk rot assessed by splitting the stalks of 5 non-systematically selected plants and evaluating the disease

**Table H-29. U.S. 2007 Study-2: Arthropod Damage Evaluations using an Observational Severity Scale for MON 87460 Compared to the Control and References in Field Studies Established with Well-Watered and Water-Limited Treatments**

## Well-Watered Treatment

| Arthropod | Number of observations across all sites [∞] (KS, NE, TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 36 | 36 |
| **Aphids** | 1 | 1 |
| **Leafhoppers** | 1 | 1 |
| **Grasshoppers** | 4 | 4 |
| **Armyworms** | 5 | 5 |
| **Corn earworms** | 4 | 4 |
| **Cutworms (includes black cutworms)** | 2 | 2 |
| **European corn borers** | 8 | 8 |
| **Southwestern corn borers** | 3 | 3 |
| **Corn rootworm [1]** | 6 | 6 |
| **Wireworms** | 1 | 1 |
| **Leafrollers** | 1 | 1 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site codes are as follows: NE = York County, NE; KS = Pawnee County, KS; TX = Carson County, TX

[1] Includes western corn rootworm and southern corn rootworm

## Water-Limited Treatment

| Arthropod | Number of observations across all sites [∞] (TX) | Number of observations where no differences were detected across all sites |
|---|---|---|
| **Total** | 12 | 12 |
| **Aphids** | 1 | 1 |
| **Grasshoppers** | 1 | 1 |
| **Armyworms** | 1 | 1 |
| **Corn earworms** | 3 | 3 |
| **Cutworms (includes black cutworms)** | 1 | 1 |
| **European corn borers** | 2 | 2 |
| **Southwestern corn borers** | 2 | 2 |
| **Corn rootworm [1]** | 1 | 1 |

Note: No differences were detected between MON 87460 and the control. The experimental design was a split plot design with three replications; Data were collected at four crop developmental stages, observation 1 = V2-V4, observation 2 = V10-V15, observation 3 = VT-R3, and observation 4 = R6 growth stages of corn

[∞] Site code is as follows: TX = Carson County, TX

[1] Includes western corn rootworm and southern corn rootworm

## Appendix I. Water Management and Environmental Data for U.S. and Chile Field Studies during 2006 and 2007

This section contains water management and temperature data for the phenotypic characterization field sites presented in Appendices F, G, and H.

**Contents**

## I-1. Calculated and Accumulated Water Data for U.S. and Chile Field Studies during 2006 and 2007

### I.1.1. U.S. 2006 and 2007 Well-Watered Sites

*Site Code, Test Location, and Year*

Site Code IA1, Jefferson County, Iowa, 2006.

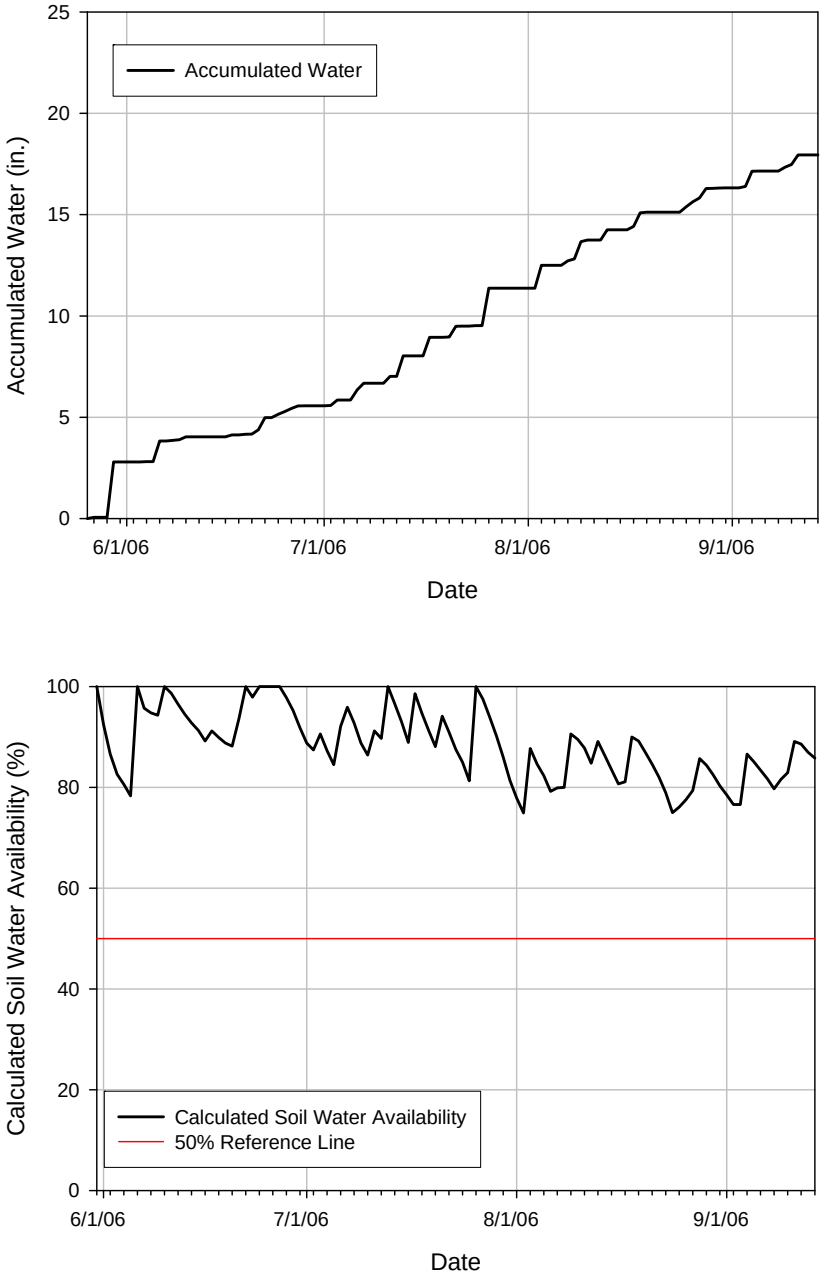



**Figure I-1. Calculated and Accumulated Soil Water Values at IA1 in 2006**

(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IA2, Benton County, Iowa, 2006.

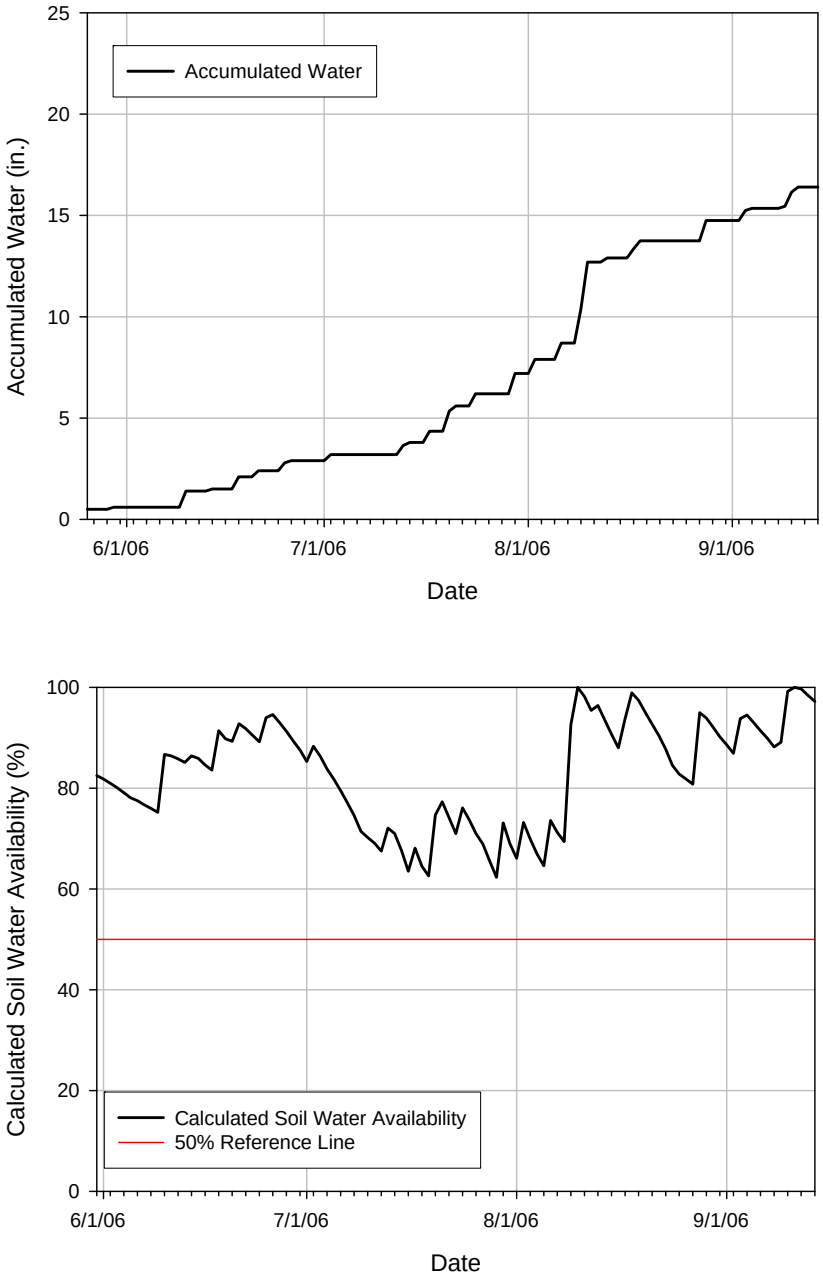



**Figure I-2. Calculated and Accumulated Soil Water Values at IA2 in 2006**

(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IL1, Stark County, Illinois, 2006.

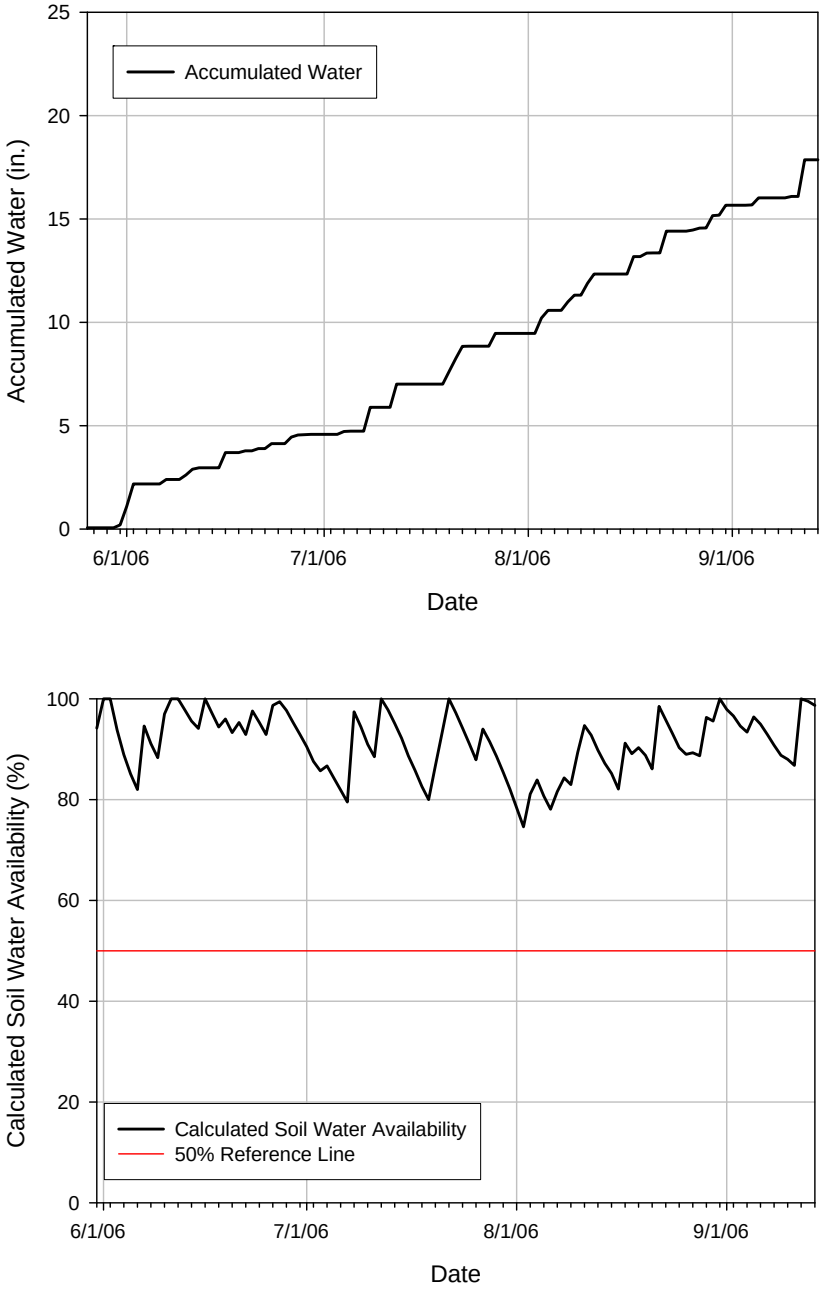
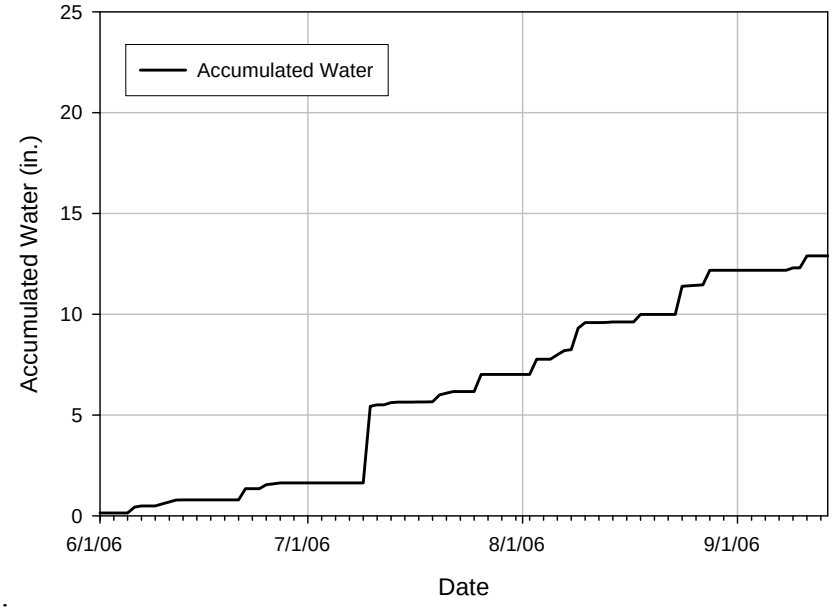



**Figure I-3. Calculated and Accumulated Soil Water Values at IL1 in 2006**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IL2, Warren County, Illinois, 2006.




.

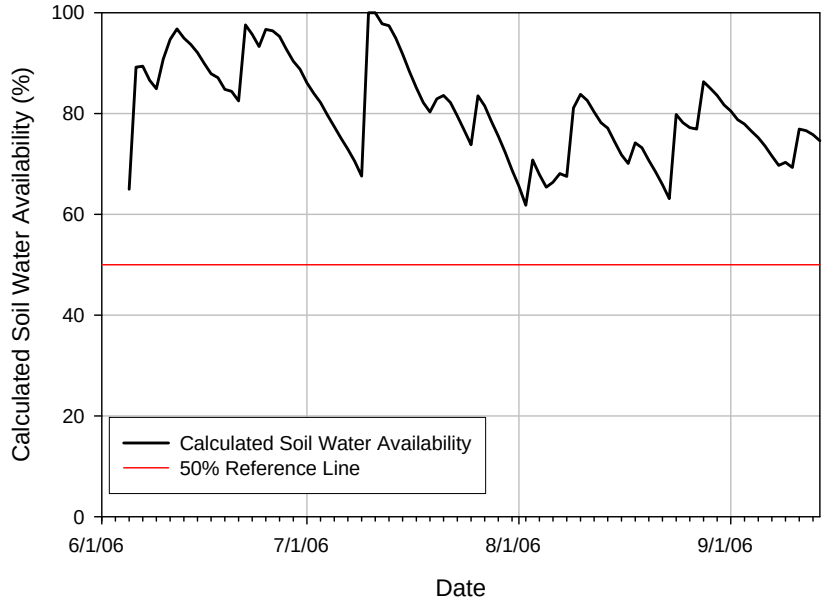



**Figure I-4. Calculated and Accumulated Soil Water Values at IL2 in 2006**

(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IN1, Boone County, Indiana, 2006.

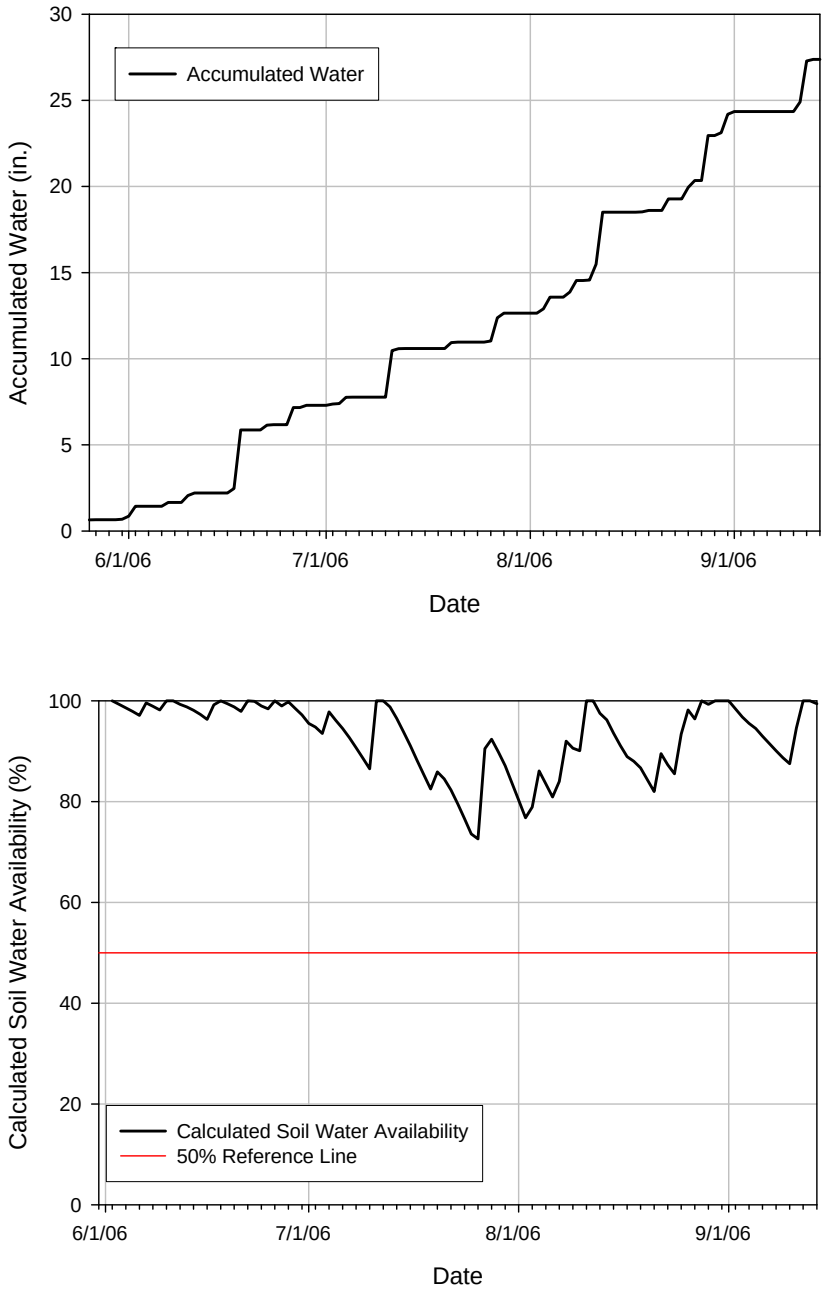



**Figure I-5. Calculated and Accumulated Soil Water Values at IN1 in 2006**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IN2, Parke County, Indiana, 2006.

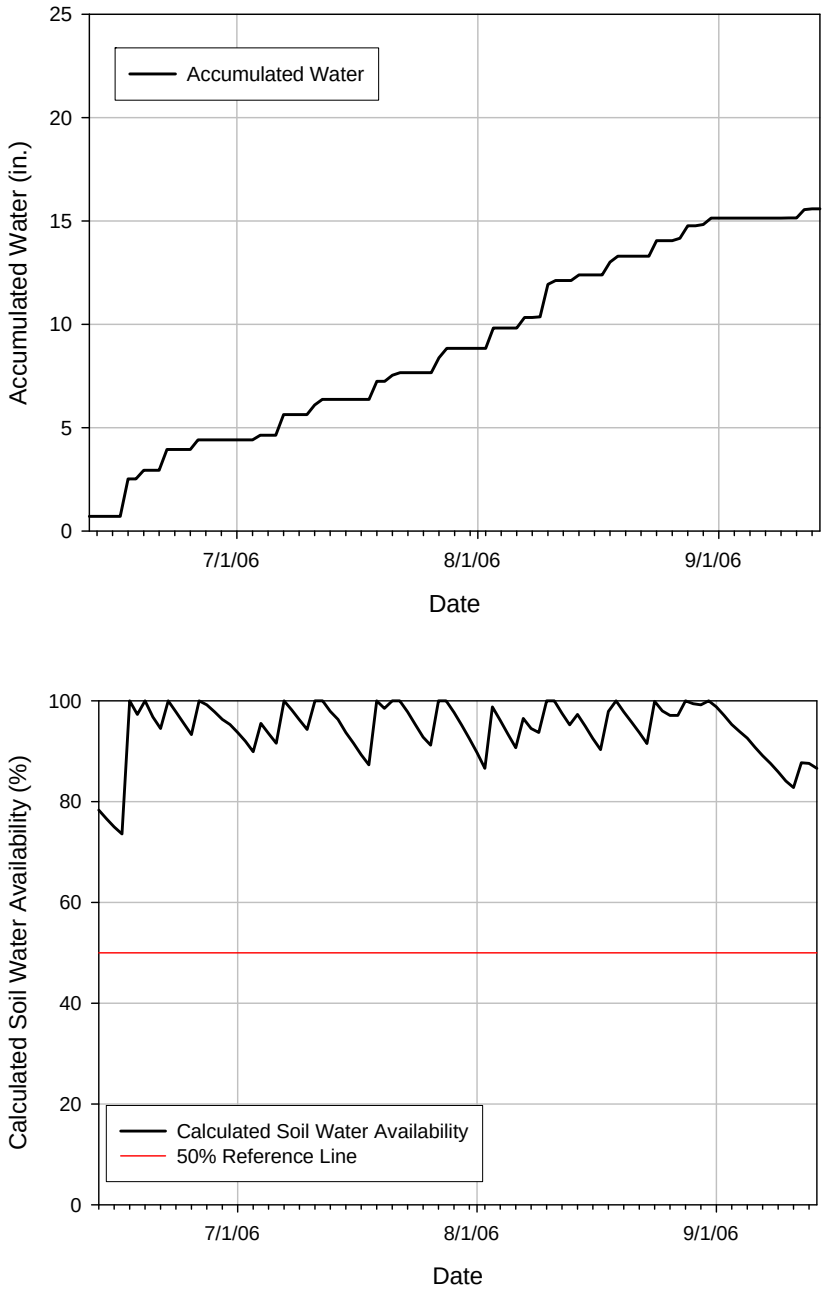



**Figure I-6. Calculated and Accumulated Soil Water Values at IN2 in 2006**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code KS, Pawnee County, Kansas, 2006.

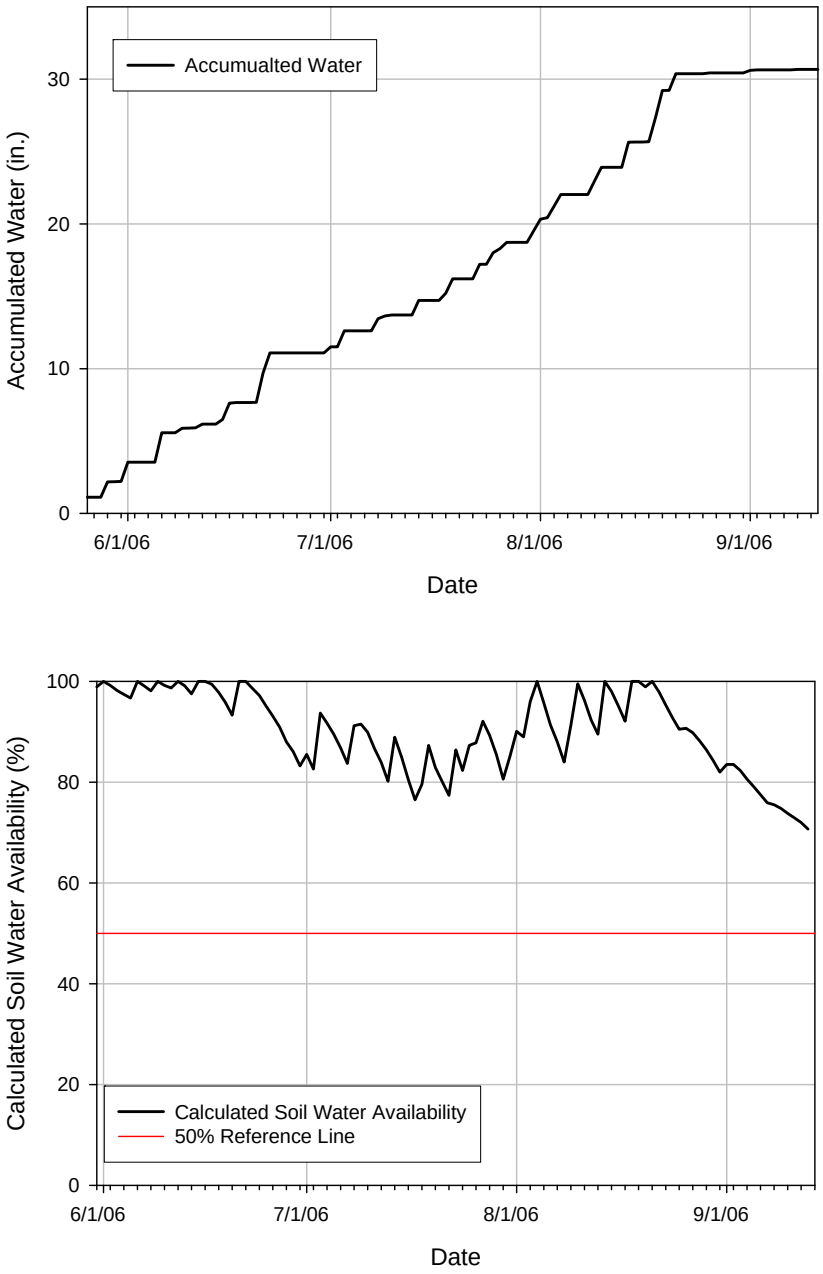



**Figure I-7. Calculated and Accumulated Soil Water Values at KS in 2006**

(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code NE, York County, Nebraska, 2006.

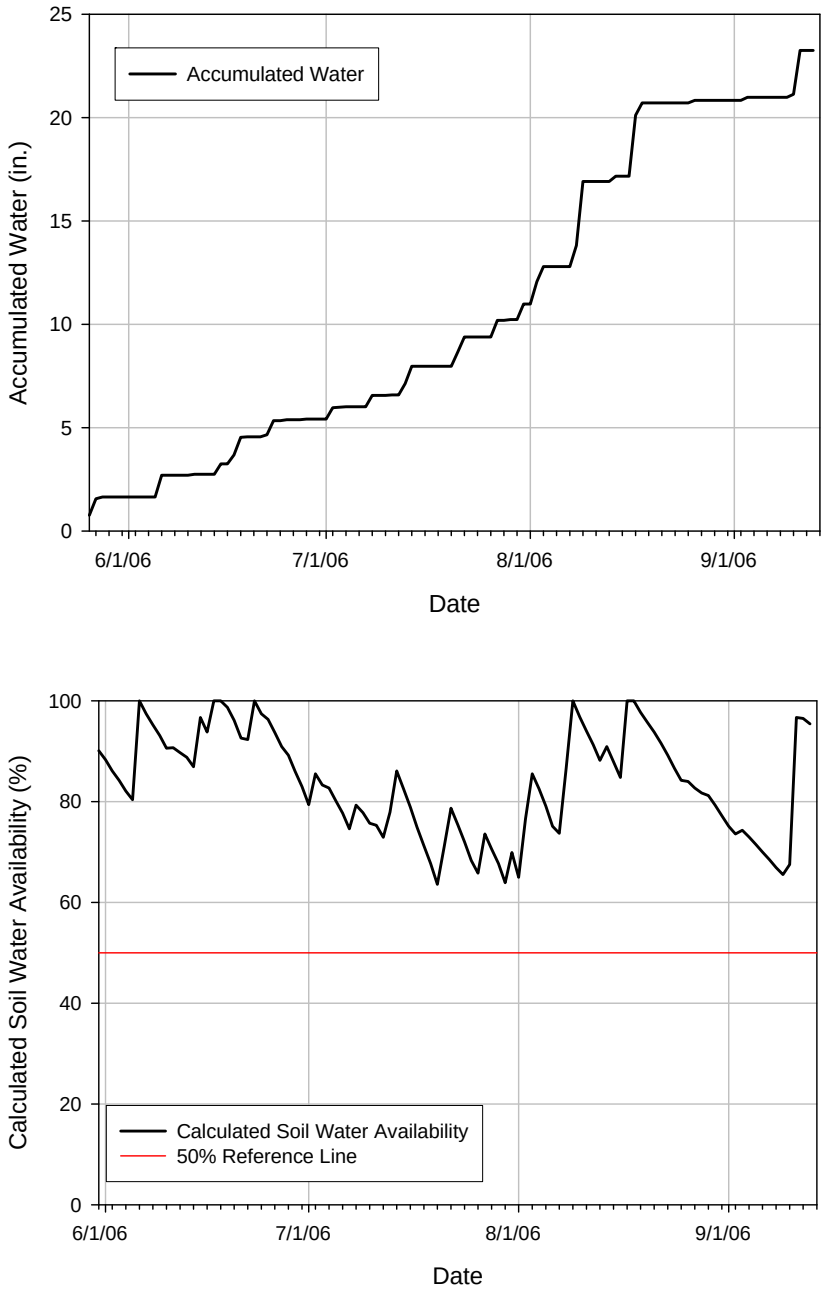
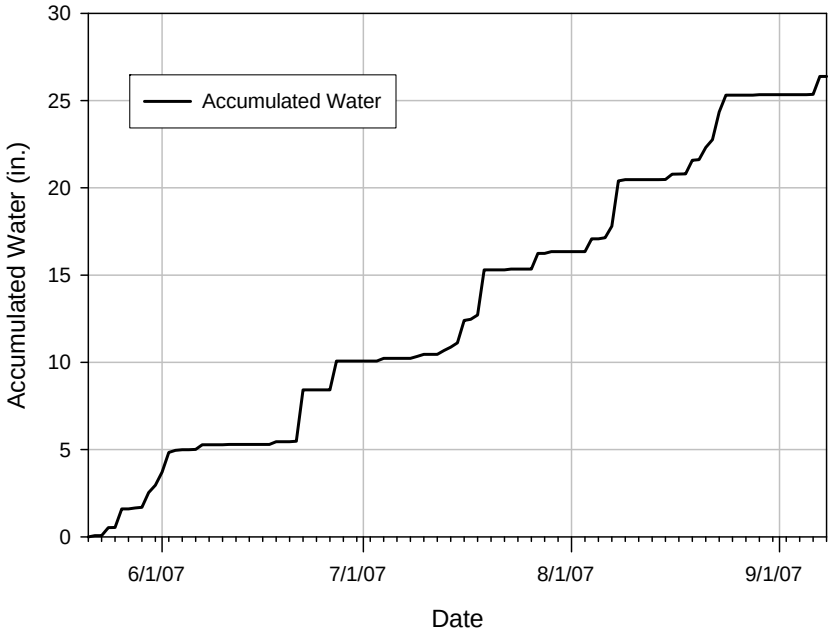



**Figure I-8. Calculated and Accumulated Soil Water Values at NE in 2006**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IA1, Jefferson County, Iowa, 2007.



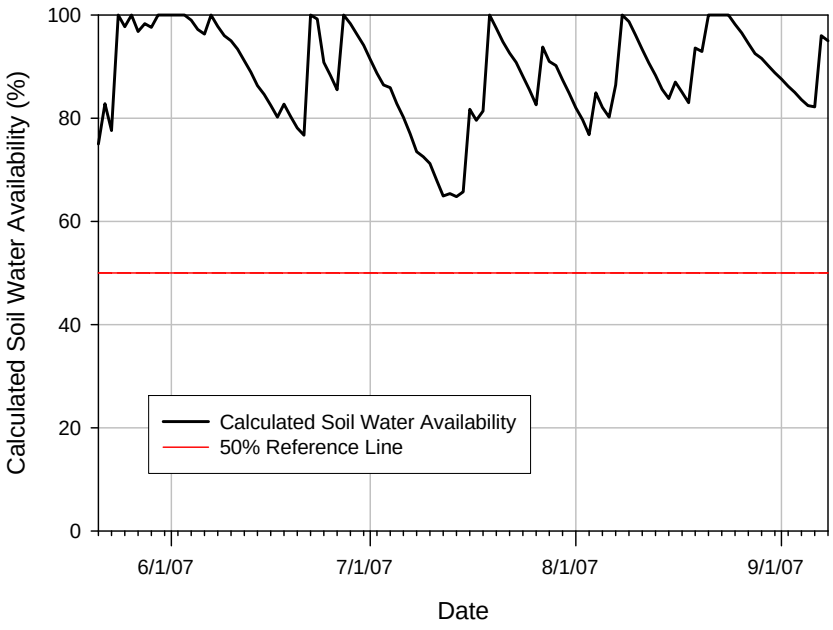




**Figure I-9. Calculated and Accumulated Soil Water Values at IA1 in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IA2, Van Horne County, Iowa, 2007.

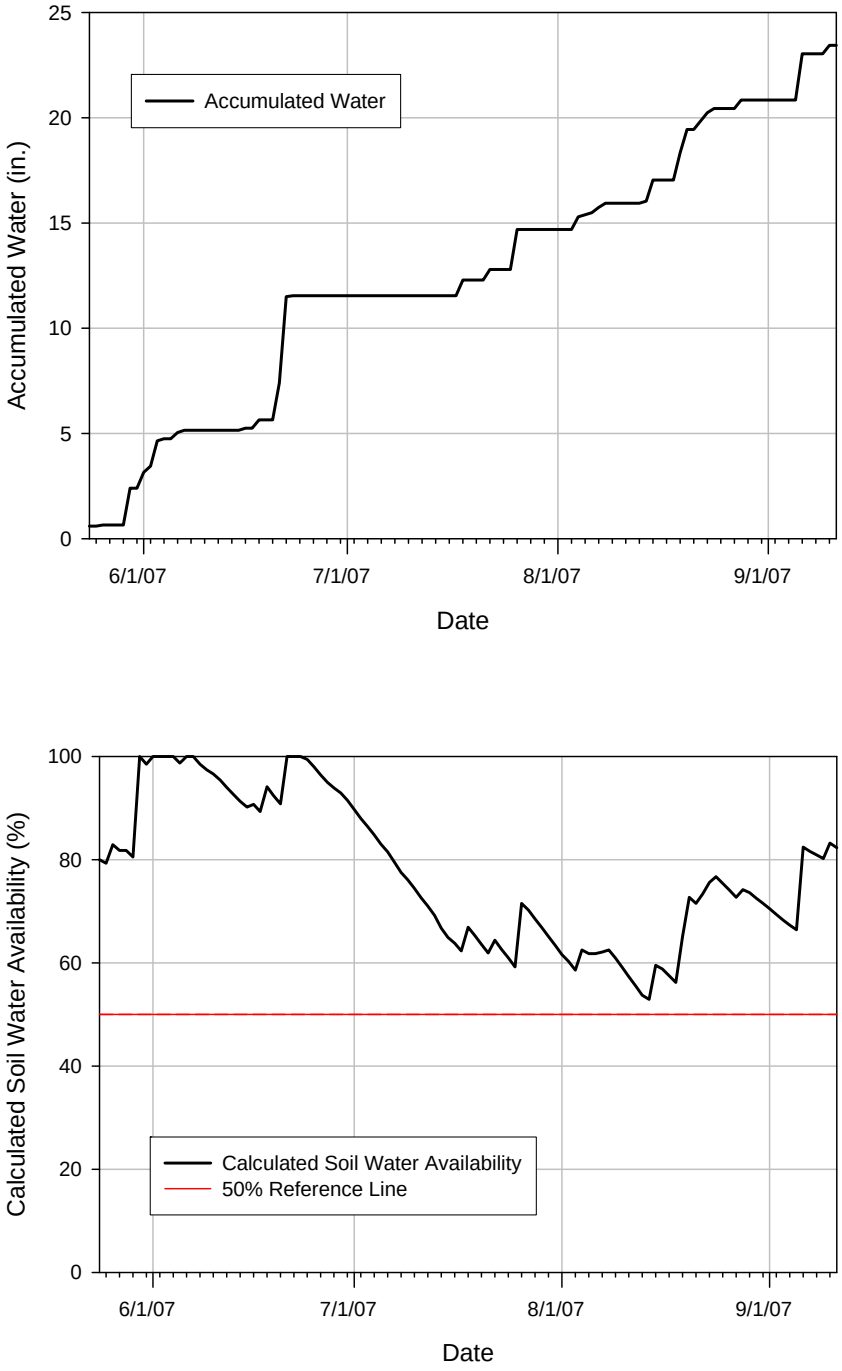



**Figure I-10. Calculated and Accumulated Soil Water Values at IA2 in 2007**

(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IL1, Stark County, Illinois, 2007.

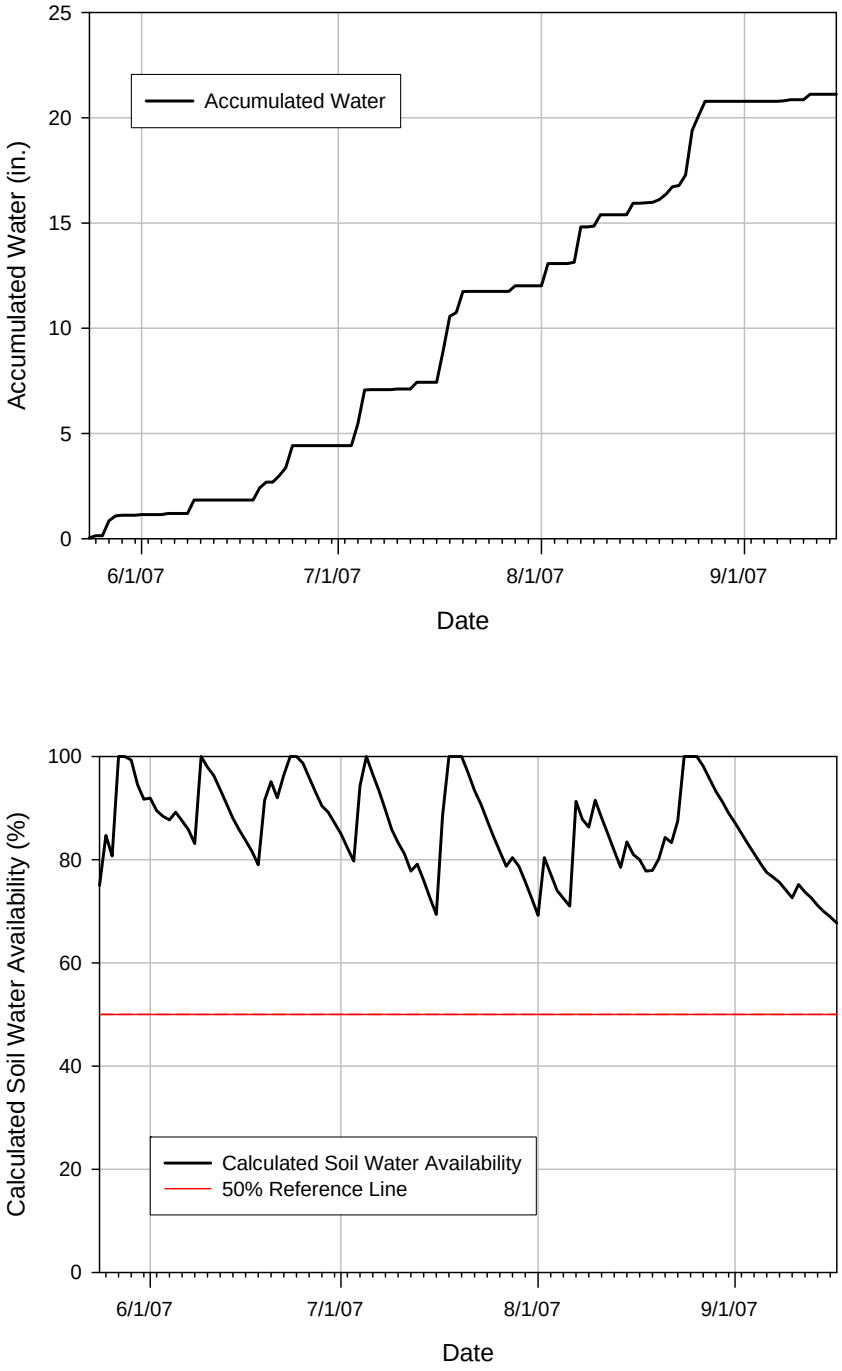



**Figure I-11. Calculated and Accumulated Soil Water Values at IL1 in 2007**

(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IL2, Warren County, Illinois, 2007.

Water data not available for this site.

**Figure I-12. Calculated and Accumulated Soil Water Values at IL2 in 2007**

*Site Code, Test Location, and Year*
Site Code IL3, Clinton County, Illinois, 2007.

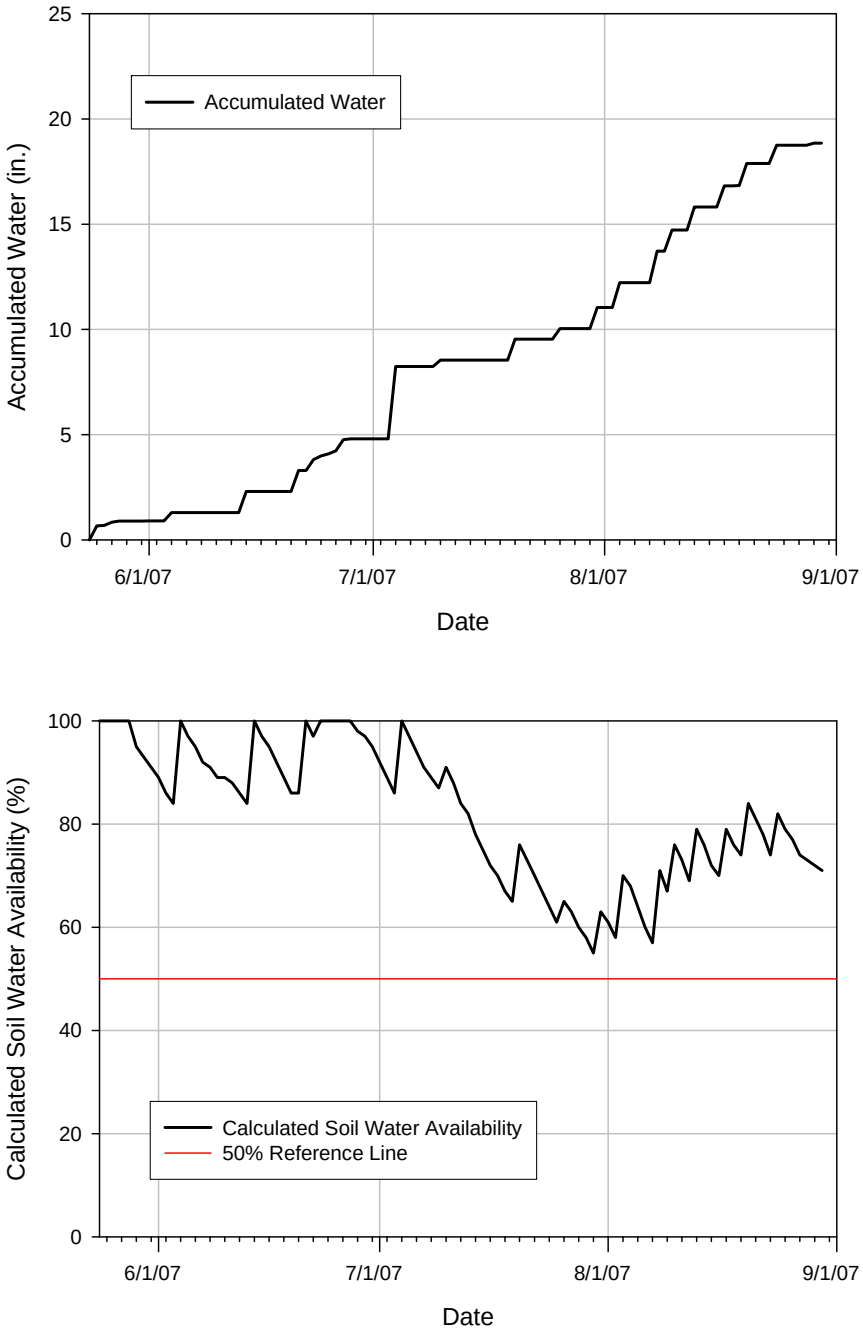



**Figure I-13. Calculated and Accumulated Soil Water Values at IL3 in 2007**

(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code IN, Boone County, Indiana, 2007.

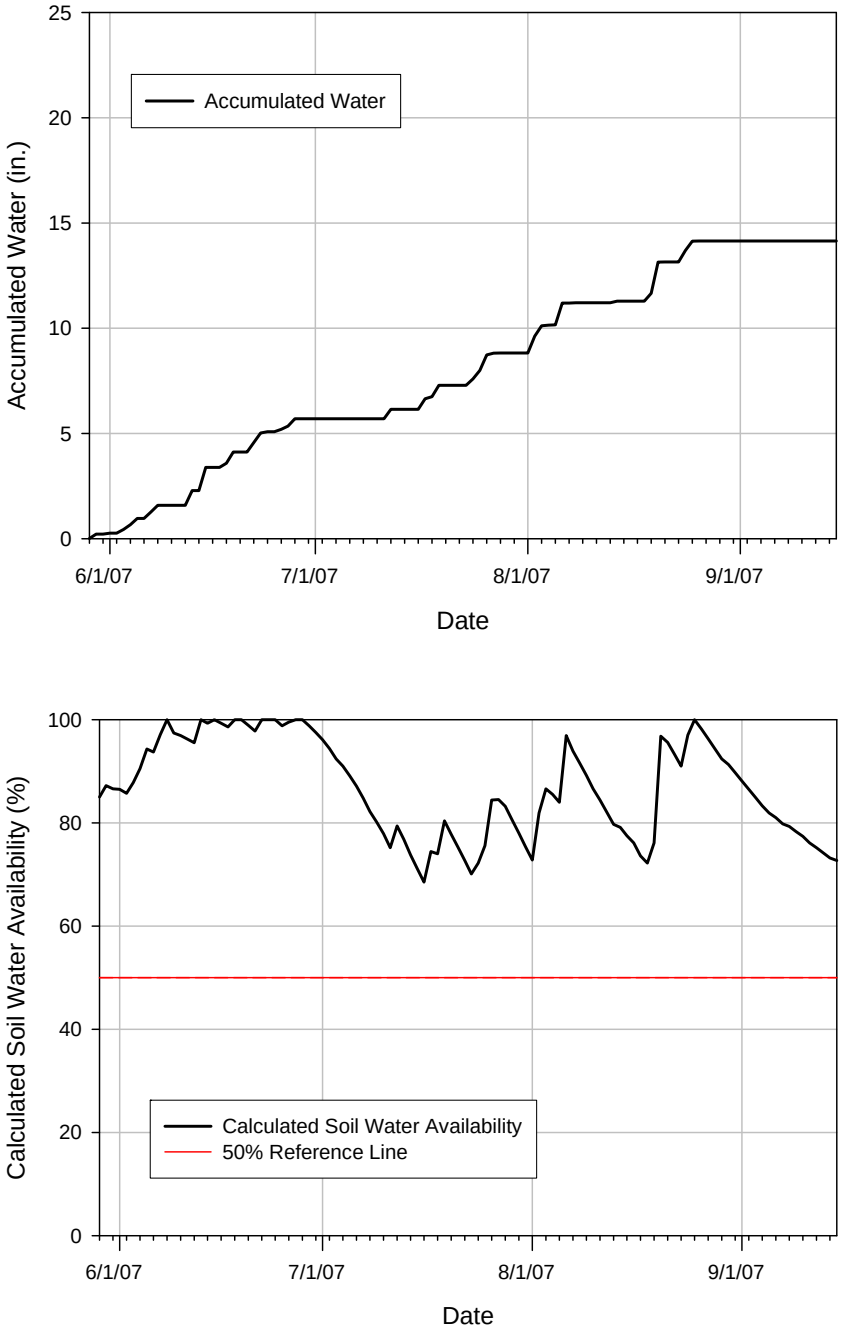



**Figure I-14. Calculated and Accumulated Soil Water Values at IN in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code NE, York County, Nebraska, 2007.

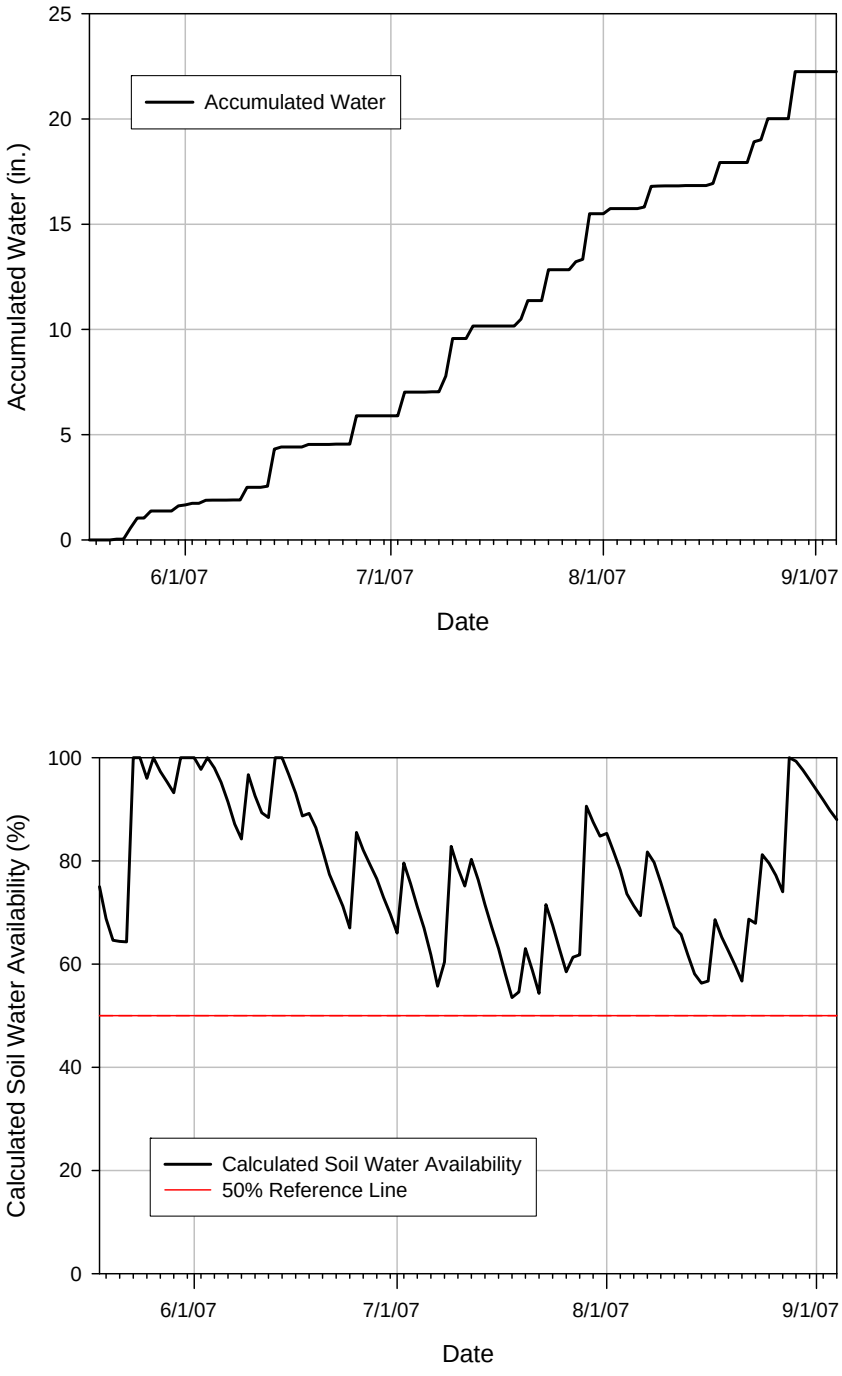



**Figure I-15. Calculated and Accumulated Soil Water Values at NE in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code OH, Fayette County, Ohio, 2007.

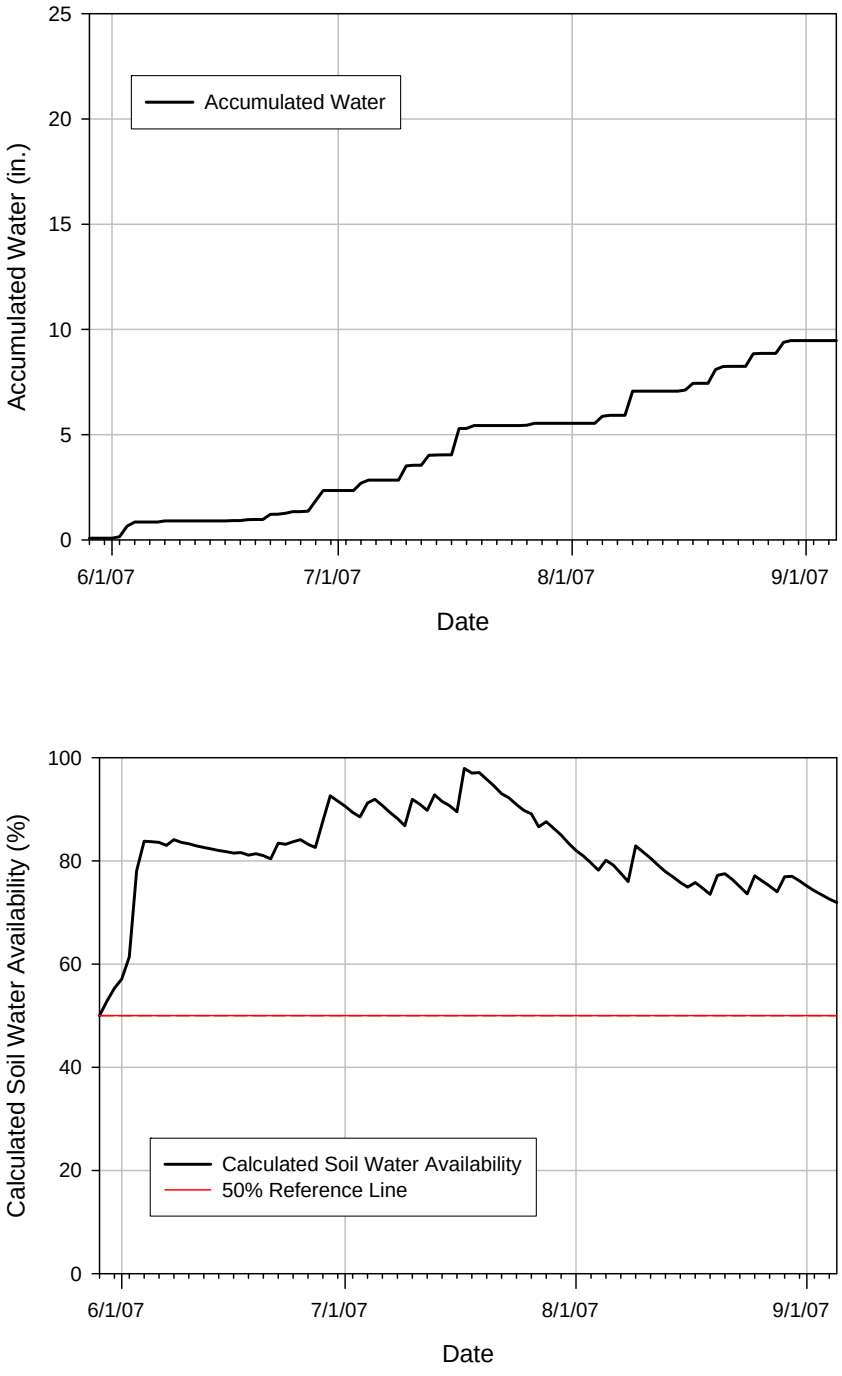



**Figure I-16. Calculated and Accumulated Soil Water Values at OH in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code PA, Berks County, Pennsylvania, 2007.

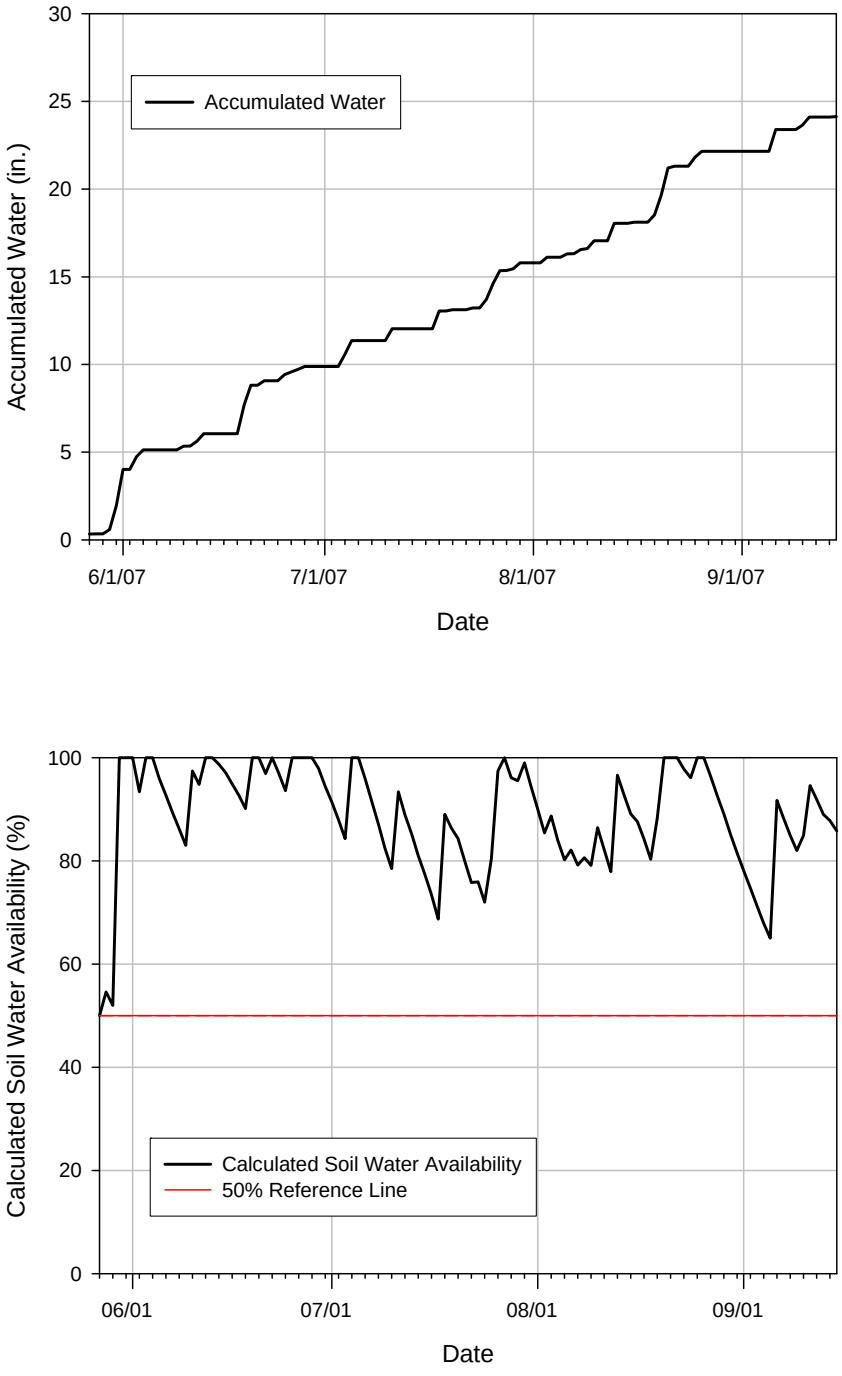



**Figure I-17. Calculated and Accumulated Soil Water Values at PA in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

### I.1.2. U.S. 2006 Typical Local Practice Sites, Accumulated Water Data Only

*Site Code, Test Location, and Year*
Site Code IAE, Benton County, Iowa, 2006.

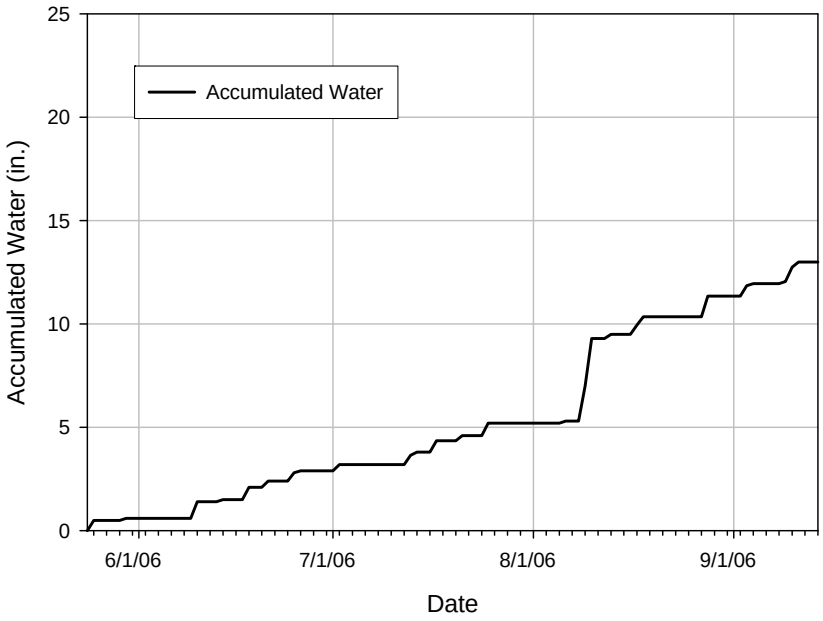



**Figure I-18. Accumulated Water at IAE in 2006**

*Site Code, Test Location, and Year*
Site Code IAW, Greene County, Iowa, 2006.

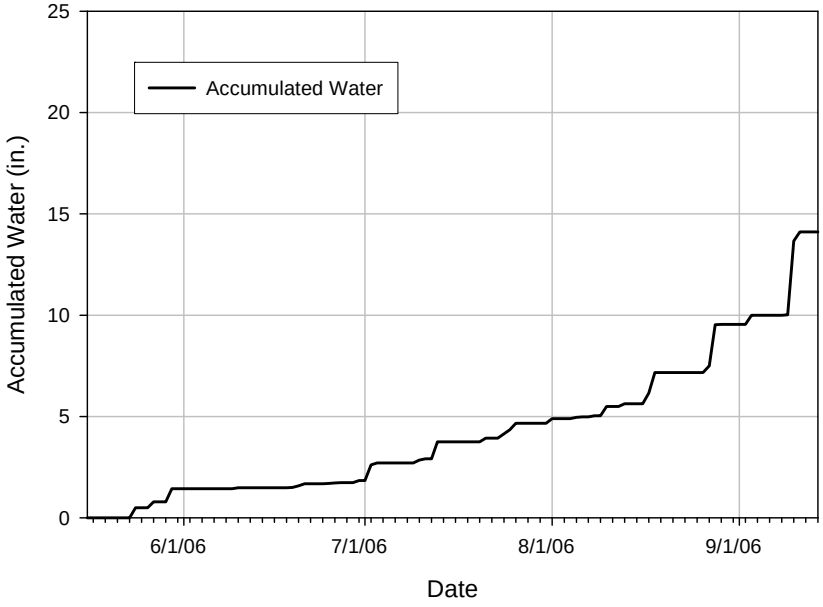



**Figure I-19. Accumulated Water at IAW in 2006**

*Site Code, Test Location, and Year*
Site Code IL, Stark County, Illinois, 2006.

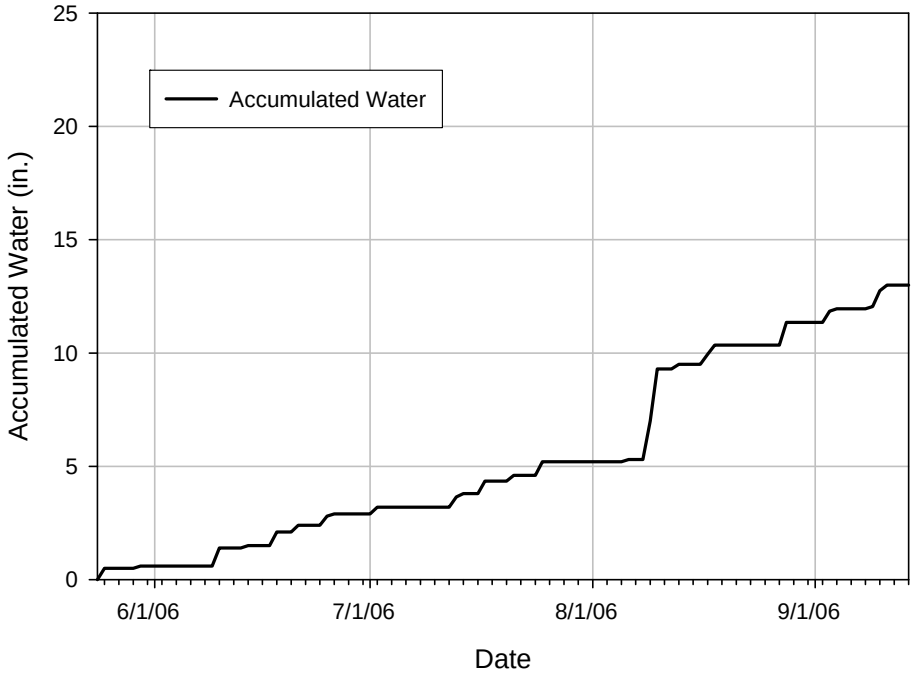



**Figure I-20. Accumulated Water at IL in 2006**

*Site Code, Test Location, and Year*
Site Code IN, Parke County, Indiana, 2006.

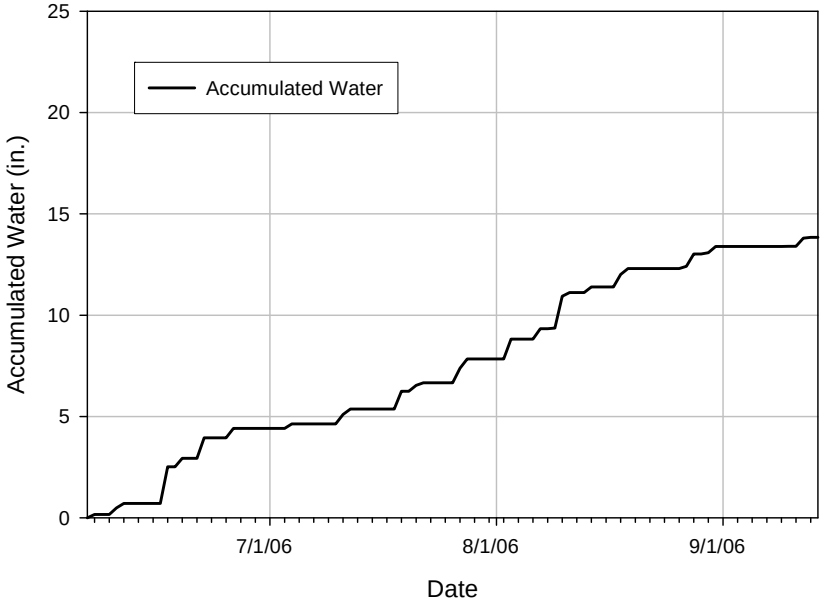



**Figure I-21. Accumulated Water at IN in 2006**

*Site Code, Test Location, and Year*
Site Code NE, York County, Nebraska, 2006.

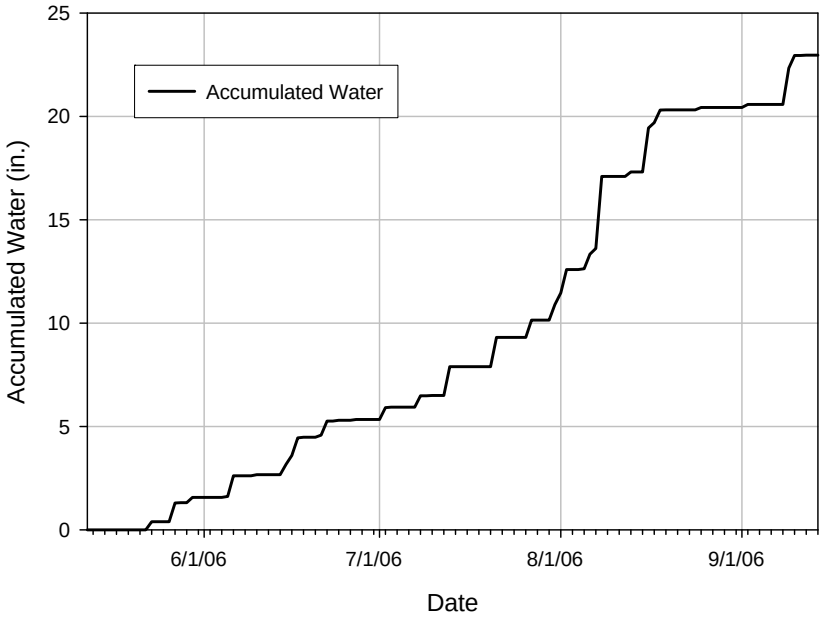



**Figure I-22. Accumulated Water at NE in 2006**

### I.1.3. Chile 2006/2007 Well-Watered and Water-Limited Sites, Accumulated Water Data Only

*Site Code, Test Location, and Year*
Site Code CL, Colina, Chile, 2006/2007.

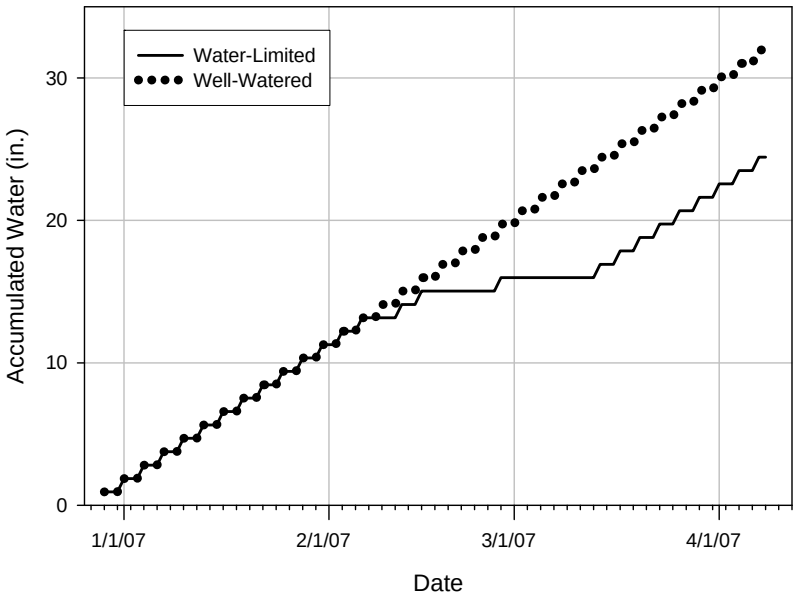



**Figure I-23. Accumulated Water at CL in 2006/2007**

*Site Code, Test Location, and Year*
Site Code CT, Calera de Tango, Chile, 2006/2007.

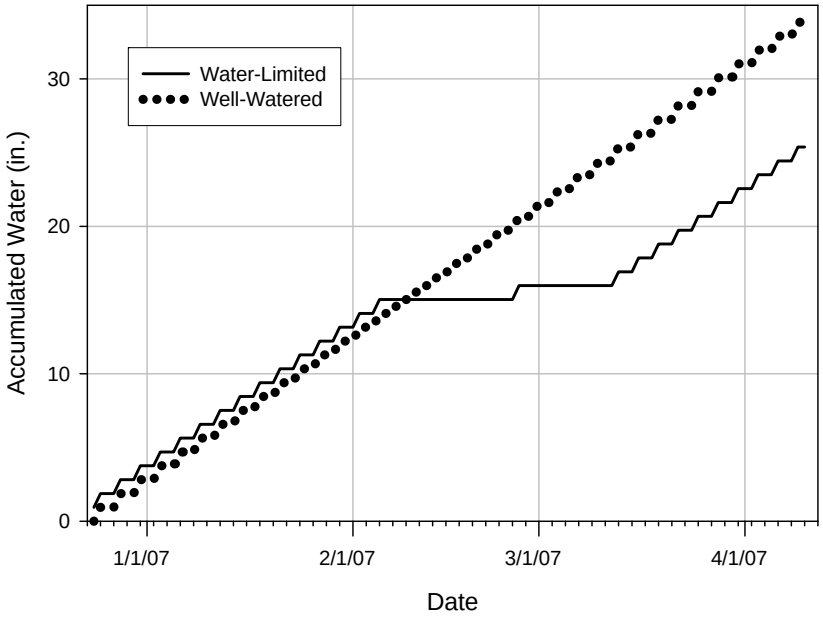



**Figure I-24. Accumulated Water at CT in 2006/2007**

*Site Code, Test Location, and Year*
Site Code LUM, Lumbreras, Chile, 2006/2007.

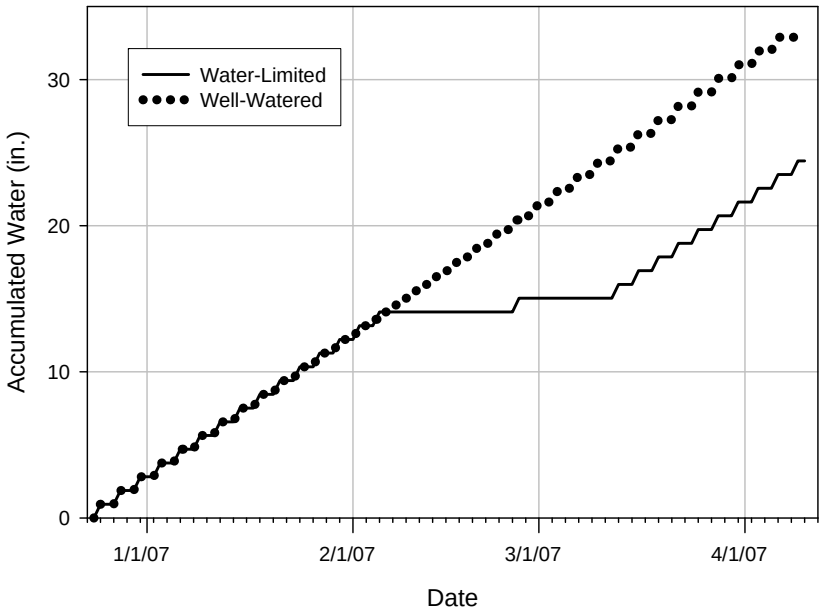


**Figure I-25. Accumulated Water at LUM in 2006/2007**

*Site Code, Test Location, and Year*
Site Code QUI, Quillota, Chile, 2006/2007.

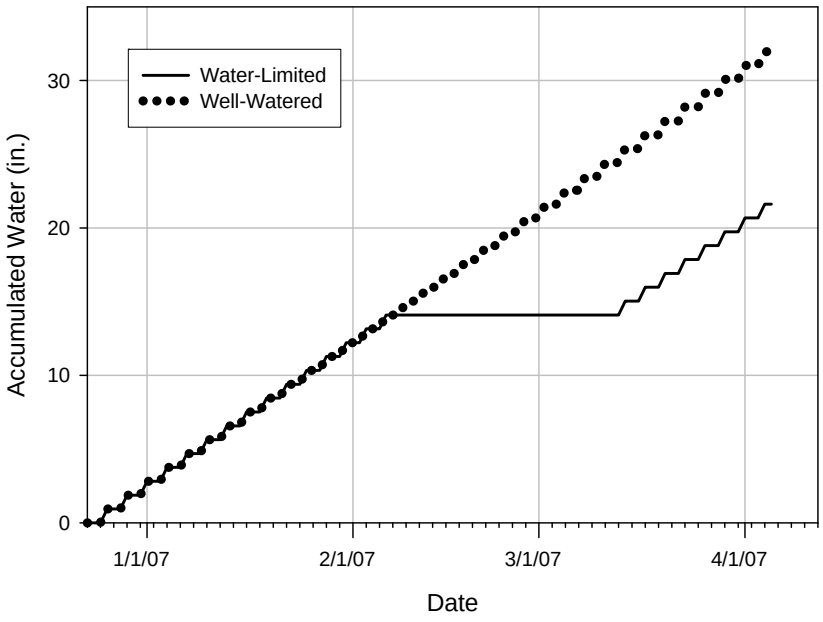
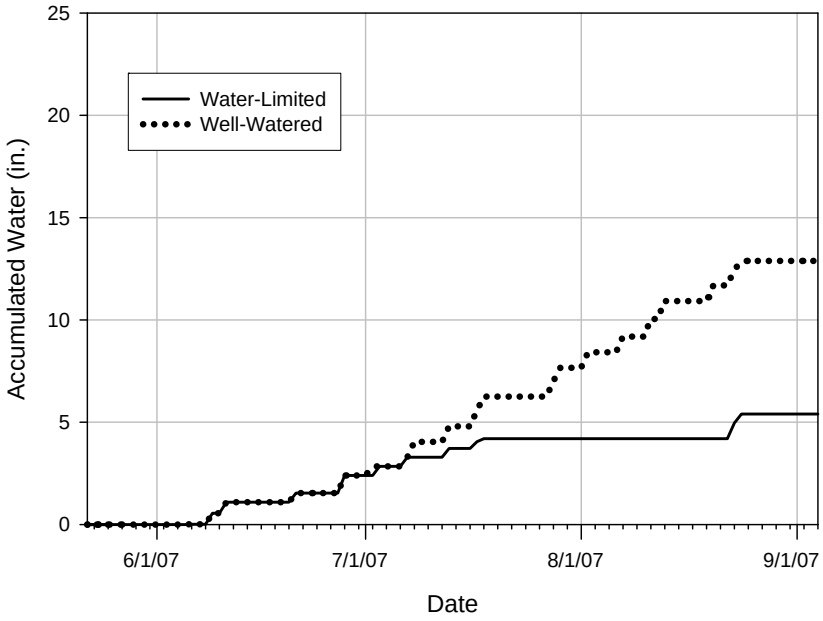



**Figure I-26. Accumulated water at QUI in 2006/2007**

### I.1.4. U.S. 2007 Study-1 Well-Watered and Water-Limited Sites

*Site Code, Test Location, and Year*
Site Code CA, Sutter County, California, Study-1 in 2007.



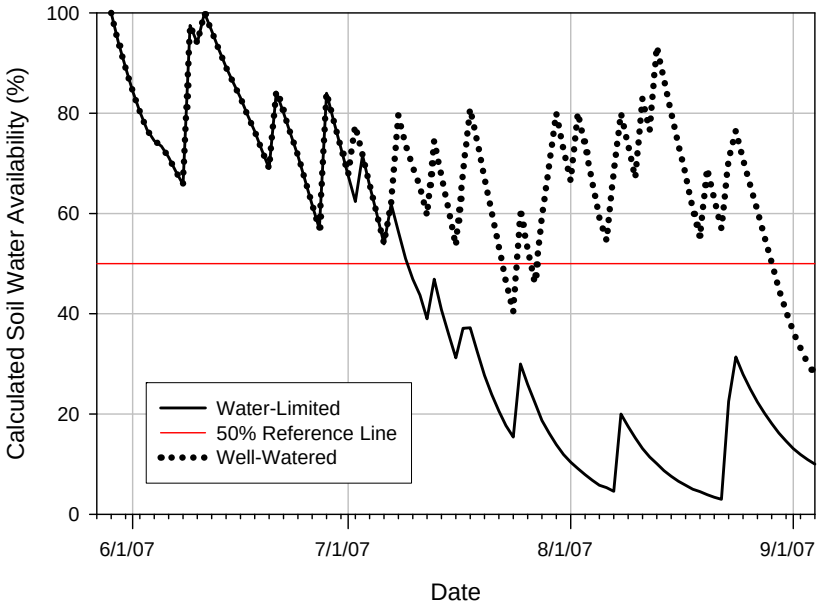
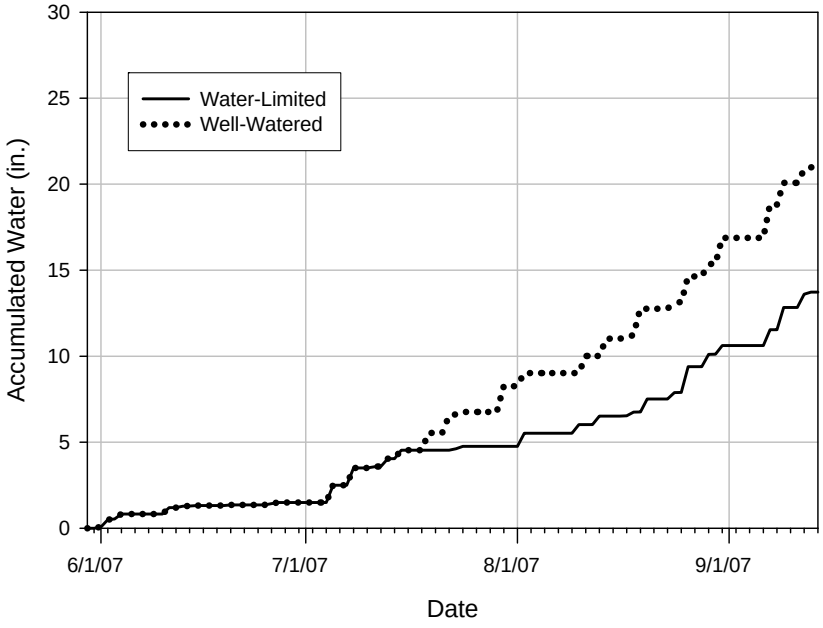




**Figure I-27. Calculated and Accumulated Soil Water Values at CA Study-1 in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code TX, Carson County, Texas, Study-1 in 2007.



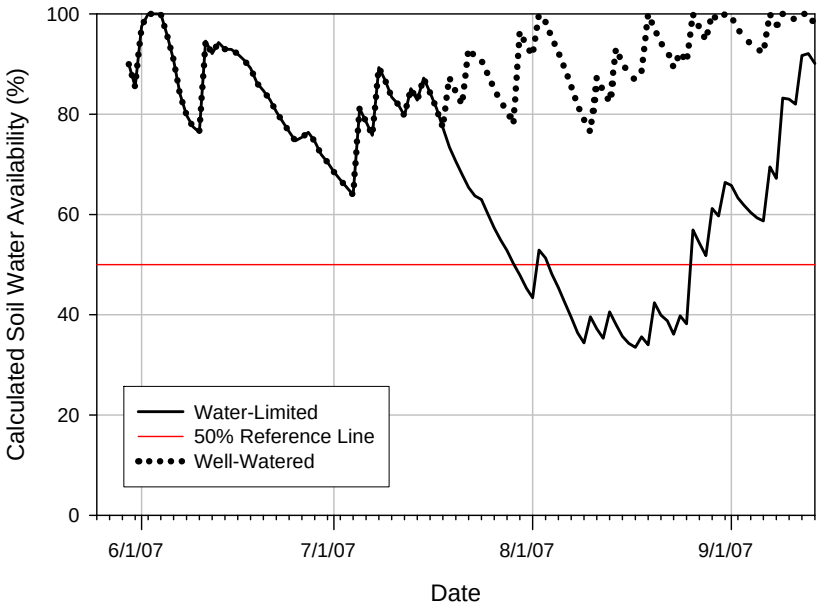
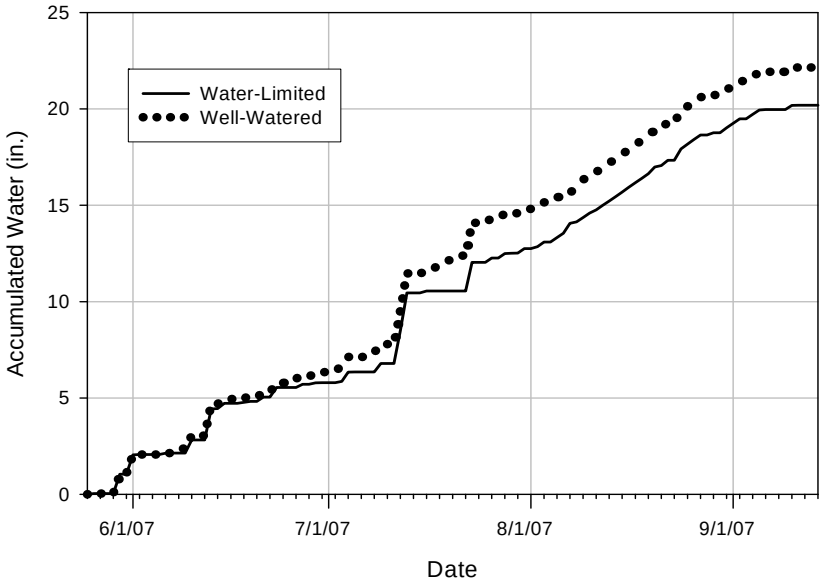




**Figure I-28. Calculated and Accumulated Soil Water Values at TX Study-1 in 2007**

(Note: moisture levels below 50% of field capacity represent stress conditions)

### I.1.5. U.S. 2007 Study-2 Well-Watered and Water-Limited Sites

*Site Code, Test Location, and Year*
Site Code KS, Pawnee County, Kansas, Study-2 in 2007.



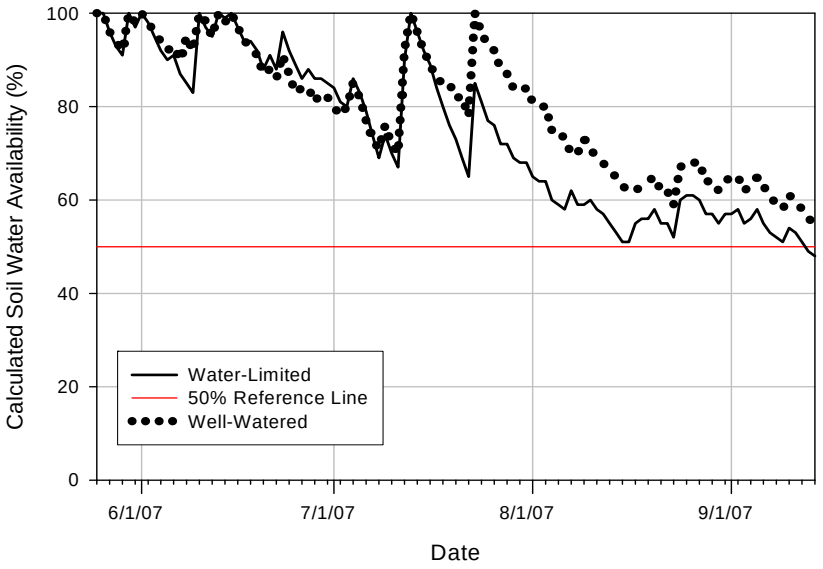




**Figure I-29. Calculated and Accumulated Soil Water Values at KS Study-2 in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code NE, York County, Nebraska, Study-2 in 2007.

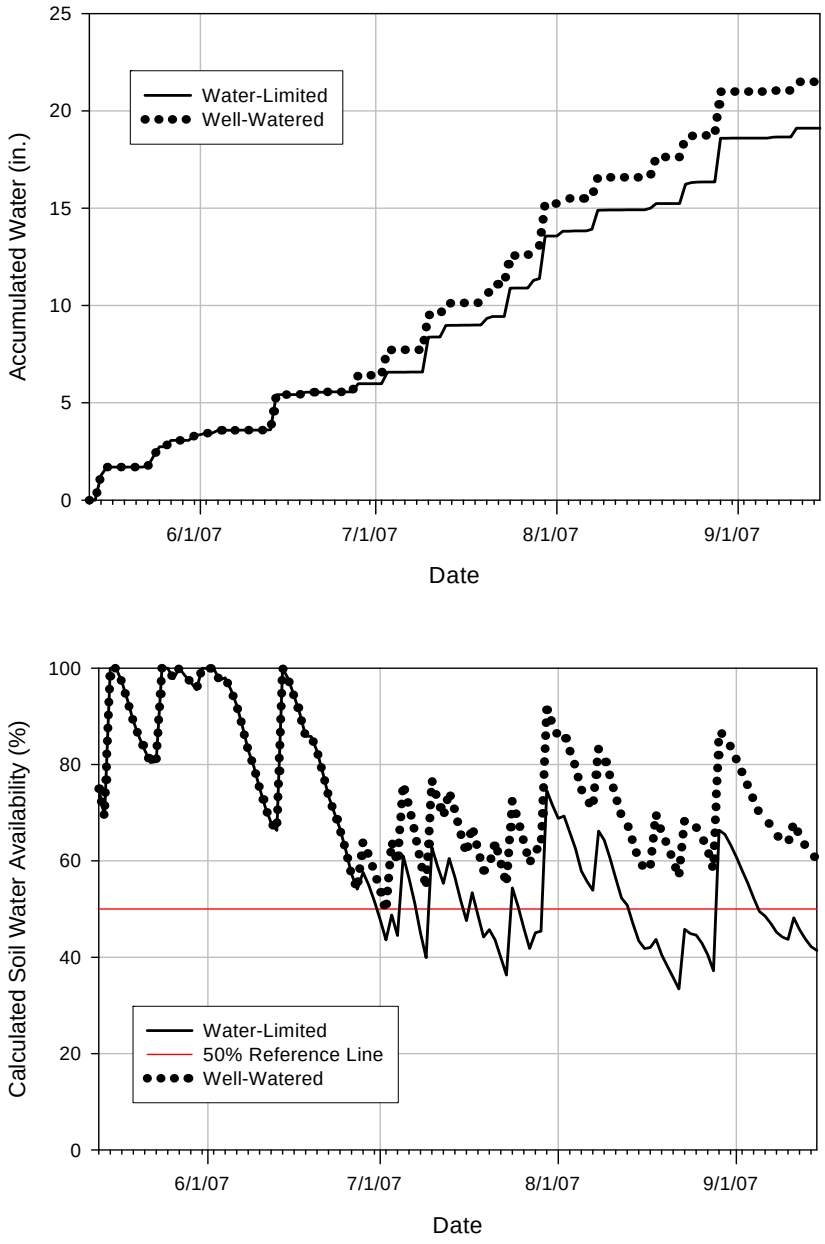



**Figure I-30. Calculated and Accumulated Soil Water Values at NE Study-2 in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

*Site Code, Test Location, and Year*
Site Code TX, Carson County, Texas, Study-2 in 2007.

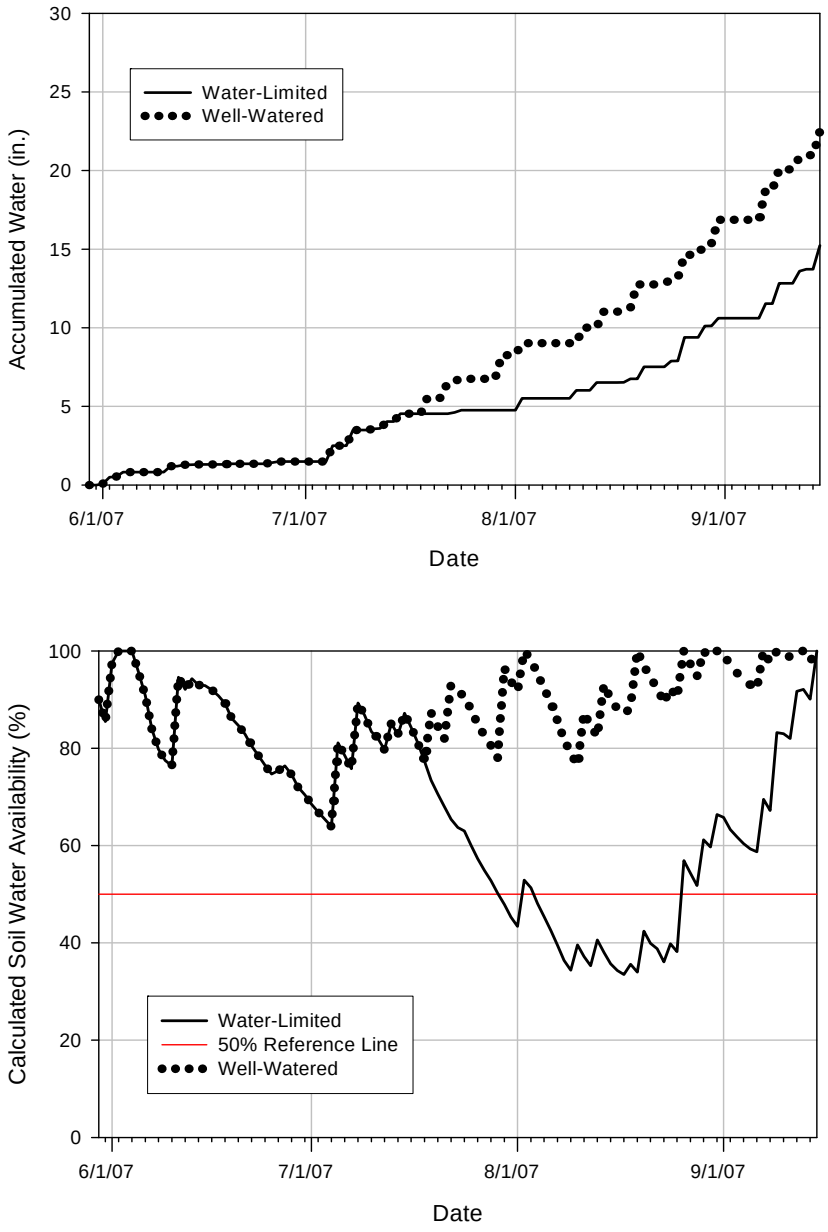



**Figure I-31. Calculated and Accumulated Soil Water Values at TX Study-2 in 2007**
(Note: moisture levels below 50% of field capacity represent stress conditions)

## I.2. Temperature Data for U.S. and Chile Field Studies during 2006 and 2007

### I.2.1. U.S. 2006 and 2007 Well-Watered Sites Temperature Data

**Table I-1. U.S. 2006 Well-Watered Sites - Monthly Average Minimum and Maximum Temperatures and Temperature Range**

| Study | May | | | June | | | July | | |
|---|---|---|---|---|---|---|---|---|---|
| Site[1] | Min[2] | Max | Range[3] | Min | Max | Range | Min | Max | Range |
| IA 1 | 52.2 | 71.9 | 33-91 | 60.9 | 81.1 | 51-90 | 66.8 | 88.1 | 53-96 |
| IA 2 | 51.1 | 72.5 | 30-98 | 59.5 | 81.1 | 45-97 | 64.9 | 85.6 | 54-95 |
| IL 1 | 50.5 | 71.7 | 36-94 | 57.1 | 80.9 | 46-92 | 64.2 | 87.6 | 46-97 |
| IL 2 | 50.5 | 71.1 | 33-94 | 59.5 | 80.4 | 48-90 | 65.1 | 87.2 | 54-96 |
| IN 1 | 51.2 | 70.4 | 37-92 | 58.8 | 79.7 | 49-92 | 65.5 | 85.0 | 50-96 |
| IN 2 | 51.1 | 70.6 | 40-92 | 58.1 | 80.1 | 52-89 | 64.8 | 86.3 | 50-96 |
| KS | 53.3 | 78.8 | 39-99 | 61.7 | 88.6 | 53-99 | 66.5 | 92.8 | 55-104 |
| NE | 51.4 | 79.1 | 37-96 | 61.3 | 88.1 | 52-101 | 65.5 | 91.3 | 55-103 |

| Study | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|
| Site | Min | Max | Range | Min | Max | Range | Min | Max | Range |
| IA 1 | 64.6 | 82.1 | 55-92 | 51.0 | 72.9 | 33-85 | 39.2 | 59.2 | 21-92 |
| IA 2 | 62.0 | 86.5 | 55-96 | 48.9 | 75.6 | 32-94 | 36.8 | 58.8 | 19-94 |
| IL 1 | 61.8 | 82.2 | 51-96 | 48.2 | 74.4 | 34-88 | 35.8 | 60.0 | 19-92 |
| IL 2 | 64.2 | 82.5 | 55-97 | 51.1 | 72.9 | 35-84 | 38.0 | 60.9 | 22-94 |
| IN 1 | 62.4 | 81.8 | 52-92 | 52.0 | 72.6 | 37-85 | 41.9 | 61.4 | 27-85 |
| IN 2 | 63.2 | 82.7 | 55-93 | 52.8 | 74.5 | 37-87 | 40.6 | 63.1 | 25-90 |
| KS | 65.8 | 88.8 | 52-104 | 51.2 | 77.7 | 37-93 | 41.9 | 67.7 | 22-96 |
| NE | 63.0 | 86.1 | 52-100 | 50.0 | 75.8 | 36-93 | 37.2 | 63.0 | 18-96 |

[1]Study sites: IA1 = Jefferson Co., IA; IA2 = Benton, Co., IA; IL1 = Stark Co., IL; IL2 = Warren Co., IL; IN1 = Boone Co., IN; IN2 = Parke Co., IN; KS = Pawnee Co., KS, NE = York Co., NE

[2]Average daily minimum and maximum temperature (°F)

[3]Range of daily minimum and maximum temperatures (°F)

**Table I-2. U.S. 2007 Well-Watered Sites - Monthly Average Minimum and Maximum Temperatures and Temperature Range**

| Study | May | | | June | | | July | | |
|---|---|---|---|---|---|---|---|---|---|
| Site[1] | Min[2] | Max[2] | Range[3] | Min | Max | Range | Min | Max | Range |
| IA1 | 56.1 | 76.7 | 43-87 | 62.3 | 81.5 | 52-93 | 63.5 | 84.9 | 54-91 |
| IA2 | 54.0 | 76.6 | 39-89 | 61.6 | 80.4 | 48-92 | 61.5 | 84.2 | 52-91 |
| IL1 | 53.8 | 79.4 | 32-91 | 58.3 | 82.4 | 42-94 | 58.1 | 83.2 | 49-91 |
| IL2 | 57.3 | 78.1 | 44-87 | 61.6 | 82.2 | 50-91 | 60.7 | 81.9 | 53-90 |
| IL3 | 59.7 | 79.8 | 40-88 | 64.2 | 85.4 | 53-92 | 64.7 | 85.7 | 55-91 |
| IN | 53.7 | 81.0 | 30-90 | 58.0 | 84.5 | 44-95 | 56.8 | 84.3 | 45-93 |
| MO | 57.1 | 78.3 | 42-89 | 62.8 | 82.6 | 51-92 | 65.0 | 86.4 | 54-92 |
| NE | 55.5 | 76.0 | 45-91 | 60.2 | 82.4 | 48-90 | 65.9 | 86.7 | 56-96 |
| OH | 51.4 | 80.1 | 38-92 | 59.7 | 84.6 | 48-96 | 59.5 | 83.1 | 48-93 |
| PA | 52.1 | 86.0 | 38-93 | 58.4 | 82.2 | 47-93 | 59.7 | 84.1 | 46-94 |

| Study | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|
| Site1 | Min | Max | Range | Min | Max | Range | Min | Max | Range |
| IA1 | 67.2 | 85.9 | 53-93 | 54.2 | 79.4 | 34-90 | 47.4 | 67.4 | 31-87 |
| IA2 | 65.6 | 84.0 | 49-90 | 50.3 | 78.1 | 30-89 | 44.6 | 65.8 | 25-88 |
| IL1 | 64.1 | 85.5 | 48-93 | 50.9 | 82.7 | 29-93 | 43.4 | 70.1 | 25-90 |
| IL2[4] | 67.3 | 86.0 | 58-93 | - | - | - | - | - | - |
| IL3 | 69.6 | 95.0 | 55-104 | 60.0 | 84.4 | 40-96 | 50.6 | 72.0 | 31-92 |
| IN | 63.5 | 87.0 | 49-95 | 53.7 | 82.0 | 36-93 | 49.4 | 70.7 | 32-91 |
| MO | 69.6 | 90.8 | 60-99 | 57.8 | 83.3 | 45-93 | 47.9 | 69.9 | 32-87 |
| NE | 66.7 | 86.3 | 56-95 | 53.5 | 79.0 | 40-90 | 44.9 | 67.1 | 29-87 |
| OH | 64.3 | 88.8 | 51-98 | 53.2 | 83.9 | 35-95 | 48.3 | 71.7 | 28-93 |
| PA | 62.1 | 84.8 | 52-96 | 52.6 | 80.9 | 39-91 | 47.7 | 72.3 | 29-90 |

[1] Study sites: IA1 = Jefferson Co., IA; IA2 = Van Horne Co., IA; IL1 = Wyoming Co., IL; IL2 = Monmouth Co., IL; IL3 = Carlyle Co., IL; IN = Sheridan Co., IN; MO = Bethel Co., MO; NE = York Co., NE; OH = New Holland Co., OH; PA = Hamburg Co., PA

[2] Average daily minimum and maximum temperature (°F)

[3] Range of daily minimum and maximum temperatures (°F)

[4] The IL2 site was destroyed by a storm on August 28, 2007 and subsequent weather data was not collected

## I.2.2. U.S. 2006 Typical Local Practice Sites Temperature Data

**Table I-3. U.S. 2006 Typical Local Practice Sites - Monthly Average Minimum and Maximum Temperatures and Temperature Range**

| Study Site [1] | May | | | June | | | July | | |
|---|---|---|---|---|---|---|---|---|---|
| | Min[2] | Max[2] | Range[3] | Min | Max | Range | Min | Max | Range |
| IAE | 51 | 72 | 30 - 98 | 60 | 81 | 45 - 97 | 65 | 86 | 54 - 95 |
| IAW | 50 | 72 | 34 - 91 | 59 | 84 | 49 - 93 | 63 | 88 | 52 - 99 |
| IL | 51 | 72 | 36 - 94 | 57 | 81 | 46 - 92 | 64 | 88 | 46 - 97 |
| IN | 51 | 71 | 40 - 92 | 58 | 80 | 52 - 89 | 65 | 86 | 50 - 96 |
| NE | 51 | 79 | 37 - 96 | 61 | 88 | 52 - 101 | 65 | 91 | 55 - 103 |

| Study Site | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Range | Min | Max | Range | Min | Max | Range |
| IAE | 62 | 86 | 55 - 96 | 49 | 76 | 32 - 94 | 37 | 59 | 19 - 94 |
| IAW | 66 | 83 | 58 - 93 | 48 | 71 | 34 - 86 | 38 | 60 | 23 - 90 |
| IL | 62 | 82 | 51 - 96 | 48 | 75 | 34 - 88 | 36 | 60 | 19 - 92 |
| IN | 63 | 83 | 55 - 93 | 53 | 75 | 37 - 87 | 41 | 63 | 25 - 90 |
| NE | 63 | 86 | 52 - 100 | 50 | 76 | 36 - 93 | 37 | 63 | 18 - 96 |

[1]Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; NE = York County, NE.
[2]Average daily minimum and maximum temperature (°F)
[3]The range is the absolute maximum and minimum temperature in each month.

### I.2.3. Chile 2006/2007 Well-Watered and Water-Limited Sites Temperature Data

**Table I-4. Chile 2006/2007 Well-Watered and Water-Limited Sites - Monthly Average Minimum and Maximum Temperatures and Temperature Range**

| Study | January | | | February | | | March | | |
|---|---|---|---|---|---|---|---|---|---|
| Site[1] | Min[2] | Max[2] | Range[3] | Min | Max | Range | Min | Max | Range |
| CL | 52.9 | 87.6 | 45.9-97.2 | 49.3 | 84.6 | 41.5-94.1 | 47.3 | 82.4 | 40.8-94.1 |
| CT | 51.6 | 83.7 | 45.9-91.0 | 49.5 | 79.3 | 42.0-89.6 | 49.5 | 78.8 | 41.5-88.2 |
| LUM | 52.0 | 81.1 | 46.8-88.9 | 50.4 | 77.5 | 41.5-88.9 | 48.9 | 78.8 | 41.5-94.1 |
| QUI | 51.4 | 83.3 | 47.5-91.8 | 50.9 | 78.6 | 43.7-89.6 | 48.6 | 77.4 | 40.8-93.4 |

| Study | April | | | May | | |
|---|---|---|---|---|---|---|
| Site[1] | Min | Max | Range | Min | Max | Range |
| CL | 40.8 | 74.3 | 33.1 - 88.9 | 33.1 | 66.6 | 25.5 - 78.1 |
| CT | 41.0 | 71.4 | 30.6 - 86.7 | 36.7 | 65.7 | 28.9 - 77.4 |
| LUM | 41.9 | 72.5 | 32.2 - 87.4 | 36.5 | 67.1 | 28.0 - 76.6 |
| QUI | 43.9 | 73.6 | 33.8 - 91.8 | 37.4 | 68.0 | 28.9 - 76.6 |

[1] Study sites: CL = Colina; CT = Calera de Tango; LUM = Lumbreras; QUI = Quillota.
[2] Average daily minimum and maximum temperature (°F)
3The range is the absolute maximum and minimum temperature in each month.

### I.2.4. U.S. Study-1 Well-Watered and Water-Limited Sites Temperature Data

**Table I-5. U.S. 2007 Study-1 Well-Watered and Water-Limited Sites - Monthly Average Minimum and Maximum Temperatures and Temperature Range**

| Study | May | | | June | | | July | | |
|---|---|---|---|---|---|---|---|---|---|
| Site[1] | Min[2] | Max[2] | Range[3] | Min | Max | Range | Min | Max | Range |
| CA | 50.7 | 84.5 | 39.0-94.0 | 55.1 | 91.5 | 47.0-107.0 | 58.2 | 95.8 | 52.0-109.0 |
| TX | 50.8 | 75.9 | 38.0-86.0 | 57.3 | 86.0 | 43.0-96.0[2] | 61.0 | 94.8 | 57.0-100.0 |

| Study | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|
| Site[1] | Min | Max | Range | Min | Max | Range | Min | Max | Range |
| CA | 57.4 | 95.4 | 49.0-107.0 | 52.0 | 86.0 | 41.0-105.0 | 45.7 | 73.3 | 40.0-87.0 |
| TX | 63.7 | 95.9 | 59.0-105.0 | 56.2 | 86.9 | 46.0-105.0 | 43.9 | 80.0 | 32.0-98.0[2] |

[1]Source for weather data: University of California, Agriculture and Natural Resources. IPM Online for Yuba City, CA, and Record of Climatological Observations, Panhandle Station for Carson Count, TX.
[2]Data missing or not reported for June 10, June 26-30, and for October 30-31
[3]The range is the absolute maximum and minimum temperature in each month.

### I.2.5. U.S. Study-2 Well-Watered and Water-Limited Sites Temperature Data

**Table I-6. U.S. 2007 Study-2 Well-Watered and Water-Limited Sites - Monthly Average Minimum and Maximum Temperatures and Temperature Range**

| Study | May | | | June | | | July | | |
|---|---|---|---|---|---|---|---|---|---|
| Site[1] | Min[2] | Max[2] | Range[3] | Min | Max | Range | Min | Max | Range |
| KS | 55.3 | 78.3 | 42-84 | 61.3 | 85.4 | 48-91 | 66.0 | 91.2 | 60-95 |
| NE | 55.0 | 76.0 | 42-91 | 60.0 | 82.0 | 46-91 | 66.0 | 87.0 | 55-98 |
| TX | 53.0 | 76.0 | 44-86 | 60.0 | 84.0 | 44-89 | 64.0 | 90.0 | 59-96 |

| Study | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|
| Site[1] | Min | Max | Range | Min | Max | Range | Min | Max | Range |
| KS | 69.5 | 94.6 | 60-102 | 58.1 | 85.4 | 44-96 | 44.5 | 75.8 | 28-91 |
| NE | 67.0 | 86.0 | 55-96 | 53.0 | 79.0 | 41-91 | 45.0 | 67.0 | 30-89 |
| TX | 66.0 | 93.0 | 62-98 | 60.0 | 85.0 | 46-98 | 47.0 | 76.0 | 32-91 |

[1]Note: Average Daily Minimum and Average Daily Maximum values were provided by the principle investigator at each site. The Range values were downloaded from the internet source: http://www.wunderground.com using the nearest airport weather data for each site.

[2] Average daily minimum and maximum temperature (°F)

3The range is the absolute maximum and minimum temperature in each month.

## Appendix J. Materials, Methods and Individual Site Results for Seed Dormancy and Germination

### J.1. Materials

Seed of MON 87460, the conventional control corn, and reference corn were produced in Greene County, IA, Stark County, IL, and Pawnee County, KS in 2006.

| Material | Material Type | Phenotype | Production[1] Site |
|---|---|---|---|
| MON 87460 | Test | Drought tolerant | IA |
| H1548126 | Control | Conventional | IA |
| H8991 | Reference | Conventional | IA |
| DKC 61-50 | Reference | Conventional | IA |
| 33N29 | Reference | Conventional | IA |
| MON 87460 | Test | Drought tolerant | IL |
| H1548126 | Control | Conventional | IL |
| 33K39 | Reference | Conventional | IL |
| M-3744 | Reference | Conventional | IL |
| M-3765 | Reference | Conventional | IL |
| MON 87460 | Test | Drought tolerant | KS |
| H1548126 | Control | Conventional | KS |
| S-2721 | Reference | Conventional | KS |
| 32B33 | Reference | Conventional | KS |
| 33H25 | Reference | Conventional | KS |

### J.2. Characterization of the Materials

The presence or absence of MON 87460 insert was verified by event-specific PCR analysis for MON 87460, control, and reference corn. The results of these verifications were as expected with the following exceptions: analysis of the H1548126 seed from the IA production site revealed the presence of MON 87460 at ≤1.84%. In addition, the analysis of seed of three reference substances from the IA production site and two reference substances from the KS production site revealed the presence of MON 87460 at ≤1.84%. This result was not unexpected as the seed of the test, control and reference substances were produced in a common nursery. The level of MON 87460 was low and was deemed to have no negative effect on the quality of the study or interpretation of the results.

### J.3. Performing Facility and Experimental Methods

Dormancy and germination evaluations were conducted at BioDiagnostics, Inc. in River Falls, WI. The principal investigator was certified to conduct seed dormancy and germination testing consistent with the standards established by the Association of Official Seed Analysts (AOSA), a seed trade association (AOSA, 2000; AOSA, 2006).

Seven germination chambers were used in the study and each chamber was maintained dark under one of the following seven temperature regimes: constant temperature of approximately 5, 10, 20, or 30°C or alternating temperatures of approximately 10/20, 10/30, or 20/30°C. The alternating temperature regimes were maintained at the lower temperature for 16 h and the higher temperature for 8 h. The temperature inside each germination chamber was monitored and recorded throughout the duration of the study.

Germination towels for MON 87460, control, and reference materials were prepared per the facility SOPs. Each germination towel represented one replication. The types of data collected depended on the temperature regime. Each rolled germination towel in the AOSA-recommended temperature regime (i.e., 20/30°C) was assessed periodically during the study for normally germinated, abnormally germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed as defined by AOSA guidelines (AOSA, 2006). Each rolled germination towel in the additional temperature regimes (i.e., 5, 10, 20, 30, 10/20 and 10/30°C) was assessed periodically during the study for germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed.

### J.4. Statistical Analysis

Statistical analyses were performed by Monsanto Statistics Technology Center. The data were analyzed according to a split-plot design, with production site as the whole plot and seed substance type as the sub-plot. SAS® (SAS Version 9.1.3, 2002-2003) was used to compare MON 87460 and the control material for each characteristic across all sites with a level of statistical significance of 5% ($\alpha = 0.05$). A combined-site analysis was conducted when no significant production site-by-seed substance type interactions were detected. An individual-site analysis was conducted when production site-by-seed substance type interactions were detected. Summary statistics were provided for each production site and temperature regime combination. The test substance was not statistically compared to the reference substances, and no comparisons were made across temperature regimes. The reference range was calculated from the minimum and maximum mean values observed in the references.

For the AOSA recommended temperature range, the data reported for each seed substance included the percentage of normal germinated, abnormal germinated, dead, viable firm swollen, and viable hard seed. For non-AOSA temperatures, the data reported for each seed substance included the percentage of germinated, dead, viable firm swollen, and viable hard seed.

### J.5. Individual Site Seed Dormancy and Germination Results

MON 87460, the control, and reference seed materials were produced at three sites to assess germination characteristics of seed grown under various environmental conditions.

® SAS is a registered trademark of SAS Institute, Inc.

No production site × seed substance interactions were detected for any combination except percent germinated and percent viable firm swollen at 10°C. These two characteristics were analyzed separately by site to account for the interaction. In percent germinated and percent viable firm swollen seed at 10°C, significant production site × seed substance type interactions were detected. Analyses were therefore conducted for these two variables at this temperature on an individual site basis. No differences were detected between MON 87460 and the control at the IL or KS production sites. For the IA site, MON 87460 had greater percent germination and lower percent viable firm swollen seed than the control (91.5 vs. 87.0; 6.5 vs. 11.2, respectively). However, no differences were detected at other sites, and the values for MON 87460 and the control at 10°C were within the reference range for both percent germination and percent viable firm swollen seed (Table J-1). Furthermore, of the 15 comparisons over the germination characteristics from the across-site analysis, no statistically significant differences were detected between MON 87460 and the control (Table VIII-3).

The biological characteristics evaluated in this study are used to characterize MON 87460 in the context of plant pest risk assessment. The results of this study, in particular the absence of hard seed, support a conclusion that seed of MON 87460 does not exhibit altered dormancy and germination characteristics compared to the conventional control. These results support a conclusion of no increased pest potential of MON 87460 compared to conventional corn.

## J.6. References

AOSA. 2000. Tetrazolium Testing Handbook. Association of Official Seed Analysts. Lincoln, NE.

AOSA. 2006. Rules for Testing Seeds. Association of Official Seed Analysts. Lincoln, NE.

**Table J-1. Individual Site Analysis for Germination of MON 87460 and the Control in 2006**

| | | IA[2,3] Mean ± S.E. | | IL[2,3] Mean ± S.E. | | KS[2,3] Mean ± S.E. | | Reference Range | |
|---|---|---|---|---|---|---|---|---|---|
| Temperature | Characteristic | MON 87460 | Control | MON 87460 | Control | MON 87460 | Control | Min | Max |
| 10°C | Germinated | 91.5 ± 1.52 | 87.0* ± 1.74 | 86.0 ± 1.58 | 89.0 ± 0.41 | 92.0 ± 1.78 | 94.0 ± 0.41 | 87.7 | 99.0 |
| | Viable firm swollen | 6.5 ± 1.86 | 11.2* ± 1.40 | 13.3 ± 1.18 | 10.5 ± 0.65 | 7.8 ± 1.65 | 5.3 ± 0.48 | 0.0 | 8.3 |

S.E. = standard error.

*Indicates significant differences detected between MON 87460 and the control ($p \leq 0.05$).

[1] Analysis run only on variables where production site × seed substance interaction prevented combined-site analysis.

[2] IA = Greene County, IA; IL = Stark County, IL; KS = Pawnee County, KS.

[3] Totals are a mean percent.

## Appendix K. Materials and Methods for Pollen Morphology and Viability Analysis

### K.1. Test, Control, and Reference Substances

Assessment for pollen morphology and viability was conducted in a U.S. field study in CA during 2007 on pollen collected from plants that received either the well-watered or water-limited treatment (Tables F-1 and F-6).

#### K.1.1. Test Substance

The test substance was MON 87460.

#### K.1.2. Control Substance

The control substance was conventional corn DM1718, which has a genetic background similar to the test substance but did not possess the drought tolerance trait.

#### K.1.3. Reference Substances

The reference substances were commercially available corn hybrids: DKC63-78, RX715, RX754RR2, and DKC61-50.

### K.2. Experimental Methods

*Plant Production*

MON 87460, a conventional control, and four references were grown in California under similar agronomic conditions, with the exception of irrigation treatment. The study was arranged in a split-plot design with four replications, where irrigation treatment was the whole-plot and plant substance type was the sub-plot. A pollen sample was collected from three plants per plot and was fixed and stained with 1:5 diluted Alexander stain. Pollen viability and pollen diameter were evaluated and general pollen morphology was observed. The test substance was compared to the control substance for mean pollen diameter and percentage viable pollen.

*Pollen Collection*

Tassel bags were placed on three to five, non-systematically selected plants per plot during pollen shed. The following morning, pollen was collected from each of the bagged plants and transferred to a uniquely labeled tube. Within approximately 30 minutes of collection, Alexander's stain solution (Alexander, 1980) in a 1:5 dilution was added to each tube (at least 2:1 (v/v) stain to pollen) to fix and stain the pollen, rendering the pollen non-viable; the tubes were closed and the contents shaken until thoroughly mixed. Subsamples were placed on wet ice within 30 minutes of pollen collection and maintained under those conditions until receipt at the performing laboratory. Pollen collected from each plant in a plot represented a subsample, and three to five subsamples made up one pollen sample.

*Data collection*

Pollen samples were assessed for viability, diameter and general morphology. Slides were prepared by aliquoting 15 µl of suspended pollen / stain solution onto a slide. Pollen samples were viewed under an Olympus Provis AX70 light/fluorescence microscope with an Olympus DP70 digital color camera. The associated PC computer [run on Microsoft Windows 2000 Professional (© 1981-1999, Microsoft Corp.)] had microscope and camera software (DP Controller v1.2.1.108 and DP Manager v1.2.1.107, respectively) (© 2001-2003, Olympus Optical Co., Ltd.).

To assess pollen viability, one hundred pollen grains were evaluated under the 10X ocular lens (100X magnification) for each of the three to five subsamples per plot, and the mean of each whole sample (calculated from the subsamples) was analyzed. When exposed to the staining solution, viable pollen grains stained purple due to the presence of vital cytoplasmic content, while dead pollen grains stained clear to light blue-green. In addition, viable pollen grains appeared round and turgid, whereas non-viable pollen grains may have appeared flaccid, depending on the degree of hydration.

Pollen diameter was measured using Image-Pro Plus v4.5.1.27 (© 1993-2002, Media Cybernetics, Inc.) software to view digital images. For a single subsample per pollen sample, pollen diameter was measured along two perpendicular axes for ten viable pollen grains. The mean of the 20 diameter measurements per sample was analyzed.

Pollen morphology was observed from digital images for one of the three to five samples for each test, control, or reference substance.

## K.3. Statistical Analysis

Monsanto Statistics Technology Center performed the statistical analysis. The design was a split plot with four replications, where treatment was the whole plot and substance type was the sub-plot. SAS® was used to compare the test substance to the control substance within each treatment for pollen viability and diameter, with a significance level of 5% ($\alpha = 0.05$). A reference range consisting of minimum and maximum mean values of the reference substances in each treatment was reported for each characteristic.

## K.4. Reference

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology. 55:13-18.

® SAS is a registered trademark of SAS Institute, Inc.

## Appendix L. Materials and Methods for Volunteer Potential Assessment

In some crops, seed remaining in the field after harvest have the potential to over-winter and volunteer in the subsequent cropping season. The purpose of this study was to evaluate volunteer potential of MON 87460 compared to conventional corn. These data are used to assess pest potential, and ultimately, the environmental risk of the biotechnology-derived crop as compared to the conventional crop.

### L.1. Study Sites

The experiment was established at Jefferson County, Iowa (RL); Guthrie County, Iowa (BG), and Parke County, Indiana (RV). These three locations provided a range of environmental and agronomic conditions representative of major corn growing regions where commercial production of MON 87460 is expected. The Principal Investigator at each site was familiar with corn production and the occurrence and identification of corn volunteers.

### L.2. Test, Control, and Reference Substances

Starting seed for the test, control and reference substances were produced during 2006 in Jefferson County, IA (Tables F-1 and F-2).

The viability of the starting seed was determined in the laboratory (BioDiagnostics Inc.) by conducting warm germination testing of each test, control, and reference starting seed lot. A sub-report that includes details of the germination testing is included in the study file. The seed germination was ≥ 90% for all test, control, and reference lots.

In this study, emerged plants of MON 87460, the control, or references were evaluated.

#### L.2.1. Test Substance

The test substance was MON 87460.

#### L.2.2. Control Substance

The control substance was conventional corn H1548126, which has a genetic background similar to the test substance but did not possess the drought tolerance trait.

#### L.2.3. Reference Substances

The reference substances were conventional, commercially available corn hybrids: C63-78, 33H25, H8991, C60-15, H8920, and 33N29.

## L.3. Experimental Methods

At each site, the study areas used for volunteer potential assessment had not been used for corn production during the 2006 growing season nor were corn seed observed to be inadvertently present.

Planting, soil, and cropping history information for each site are presented in Table K-1. In November 2006, plots were established at each of the three study sites in a randomized complete block design with three replications. Each plot was 20 ft long by 5 ft wide. Alleyways between blocks were 3 to 10 ft in length. To avoid mixing of substances between adjacent plots during seed incorporation, a confined seeding area of 18 ft by 3 ft (1 ft from each edge of the plot) was established. Each plot was hand-planted by uniformly scattering approximately 200 seed on the soil surface within the confined plot area. Seed were then incorporated with a disk or field cultivator.

Agronomic practices used to prepare and maintain each study site were characteristic of each respective region. No irrigation was applied to the study areas and no plot management was required after the seed were scattered in the plots.

Volunteer plant counts were taken after planting in Fall 2006 until soil temperatures dropped below 50°F and re-commenced in Spring 2007 approximately a week prior to the average local planting date for each field site. Volunteer plant counts were taken approximately every two weeks thereafter until mid-June, for a total of six to seven observations at each site.

## L.4. Data Assessment

Experienced scientists familiar with the experimental design and evaluation criteria were involved in all components of data collection and summarization. Study personnel ensured that assessments were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Any unexpected observations or issues during the study that would impact the study objectives were noted by the Study Director.

## L.5. Statistical Analysis

Statistical analysis of cumulative plant count data was to be conducted according to a randomized complete block design with three replications. However, because no volunteer plants emerged at any sites, no analysis was warranted.

**Table L-1. Planting, Soil type, and Cropping History Information for Volunteer Potential Study**

| Site[1] | Planting Date | Planting Depth (in) | Soil Series Description, Organic Matter (%), and pH | 2006 Crop | 2005 Crop |
|---|---|---|---|---|---|
| BG | 11/16/06 | 1 - 2 | Loam, 3-4%, 6.5 | soybean | soybean |
| RL | 11/22/06 | 1.5 | Taintor silty clay loam, 2.4%, 7.3 | none | soybean |
| RV | 11/25/06 | 1 - 2.5 | Reesville silt loam, 2.0%, 7.5 | soybean | corn |

[1] BG = Guthrie County, IA; RL =Jefferson County, IA; RV = Parke County, IN.

## Appendix M. Materials and Methods for Survival Outside Cultivation Assessment

Monsanto Company has developed drought tolerant corn MON 87460 that provides a yield benefit when yield is limited by water availability. The purpose of this study was to assess the ability of plants of MON 87460 to survive in unmanaged, competitive environments compared to a conventional corn control.

### M.1. Study Site Descriptions

Field trials were established in 2007 at four locations to evaluate whether MON 87460 could establish and persist in unmanaged environments. The study sites listed below were located within major corn growing regions of the U.S. where seed or grain may be incidentally returned to the environment during harvest, handling, or transport.

- Effingham County, Illinois (site code: IL)
- Shelby County, Missouri (site code: MO)
- York County, Nebraska (site code: NE)
- Carson County, Texas (site code: TX)

### M.2. Test, Control, and Reference Substances

The starting seed of the test, control, and reference substances are summarized in Table M-1. Since seed loss is more likely during harvest operations, starting seed of the F2 generation, produced by self-pollination, were used. The test and control starting seed were produced in Stark County, IL, in 2006. The reference starting seed were produced in Greene County, IA, Stark County, IL, or Pawnee County, KS. Within the production site, the study substances were grown under similar agronomic conditions. Plant phenotypic and agronomic characteristics of the test, control, and reference substances were evaluated in this study.

#### M.2.1. Test Substance

The test substance was MON 87460.

#### M.2.2. Control Substance

The control substance was H1548126, which has background genetics similar to the test substance with the exception of the drought tolerance trait.

#### M.2.3. Reference Substances

The reference substances were commercially-available corn hybrids that did not express the drought tolerance trait.

**Table M-1. Test, Control, and Reference Starting Seed for Survival Outside Cultivation Study**

| Site Code[1] | Substance Name | Substance Type | Phenotype/ Genotype |
|---|---|---|---|
| All | MON 87460 | Test | Drought-tolerant |
| All | H1548126 | Control | Conventional |
| IL, TX | DKC 61-50 | Reference | Conventional |
| NE, MO | 33N29 | Reference | Conventional |
| NE | 33K39 | Reference | Conventional |
| MO | M-3744 | Reference | Conventional |
| NE | M-3765 | Reference | Conventional |
| MO, IL, TX | 33H25 | Reference | Conventional |
| IL, TX | 32B33 | Reference | Conventional |

[1] Study sites: IL = Effingham County., IL; MO = Shelby County., MO; NE = York County., NE; and TX = Carson County., TX.

## M.3. Experimental Methods

### M.3.1. Plot Design

The experiment was established at each of the four sites in a randomized complete block design with three replications. Each plot was approximately 100 $ft^2$ in size, consisting of four rows, approximately 10 ft. in length, with an inter-row spacing of approximately 30 inches. A target of 100 seeds per plot were to be planted; however, due to a seed packaging error, several plots were planted with less than 100 seeds. The minimum number of seed planted in any plot was 53 at the IL site and 76 at the TX site. Planting occurred at the MO and NE sites before a recount could be requested. The Principal Investigator at the MO site, however, confirmed that he planted a minimum of 50 seeds. Therefore, the minimum number of seed planted in any plot was conservatively estimated to be 50 based on the information received.

### M.3.2. Planting and Study Area Description

Within each study area, the study substances were grown under similar conditions. The study areas consisted of unmanaged areas and no plot preparation was performed. No fertilizers, tillage, or herbicides were applied to the study area prior to or after planting. Seed in each plot was incorporated into the soil using methods appropriate to the site (e.g., no-till or drill planting equipment or by hand). Planting information and soil description of each study site are provided in Table M-2.

The IL study area was typical of Conservation Reserve Program (CRP) acres, with a mix of native grasses, forbs, and weeds. The ground cover was estimated to be approximately 60%. The area had last been used for corn and soybean rotation in 2003. The MO study area was in an area adjacent to a lake and consisted of annual grasses, broadleaf weeds, and volunteer wheat. The ground cover was estimated to be approximately 98%. The area had last been used for soybean in 2006 and winter wheat in 2005-2006. The NE study area was adjacent to a field watered by a center pivot irrigation system and contained a mixture of weeds. The ground cover was estimated to be approximately 25%. The area was usually not used for crop production. The TX study area was a pasture of native grasses. The ground cover was estimated to be approximately 85%. The area had last been cultivated in 1983.

Weather data collected at each site throughout the growing season is presented in Table M-3. Weather conditions at the sites during 2007 were typical for their respective regions and there were no unusual weather events.

### M.3.3. Data Collection and Assessment

The characteristics evaluated and evaluation timing are presented in Table M-4. Experienced scientists familiar with the experimental design and evaluation criteria were involved in all components of data collection, summarization, and analysis. Study personnel assessed that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated by the Study Director for evidence of biologically relevant changes and for possible evidence of an unexpected plant response. Any unexpected observations or issues during the study that would impact the study objectives were noted by the Study Director. Data were then subjected to statistical analysis as indicated below.

### M.3.4. Statistical Analysis

Analysis of variance was conducted according to a randomized complete block design using SAS® (Version 9.2). The level of significance was $\alpha$=0.05. For each statistically analyzed characteristic, MON 87460 was compared to the control at each site. There was no intention to analyze the data across all sites since the sites were selected for their varying attributes. The reference range was determined for each characteristic from the minimum and maximum mean values of the references.

® SAS is a registered trademark of the SAS Institute.

**Table M-2. Study Area Planting Information and Soil Description for Survival Outside Cultivation study**

| Study Site[1] | Planting Date (mm/dd/yy) | Planting Rate (seeds/plot)[2] | Planting Depth (in) | Plot Size (ft) | Reps | Soil Series; Organic Matter; pH |
|---|---|---|---|---|---|---|
| IL | 06/11/07 | 53-100 | 1.2-1.5 | 10 x 10 | 3 | Bluford silt loam; 1.7%; 6.7 |
| MO | 05/29/07 | 53-100 | 1.3 | 10 x 10 | 3 | Putnam loam; 2.0%; 5.7 |
| NE | 06/01/07 | 53-100 | 0.5 | 10 x 10 | 3 | Hastings silt loam; 3.0%; 6.6 |
| TX | 06/09/07 | 76-100 | 0.5-0.8 | 10 x 10 | 3 | Pullman silty clay loam; 2.9%; 6.9 |

[1] Study sites: IL = Effingham County., IL; MO = Shelby County., MO; NE = York County., NE; and TX = Carson County., TX.
[2] Planting rate was documented for each plot at the IL and TX sites. Planting rate at the MO and NE sites were estimated based on counts from the IL and TX sites.

**Table M-3. Monthly Temperature and Rainfall Amounts for Survival Outside Cultivation Study**

| Study | May | | | | June | | | | July | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site[1] | Low[2] | High[3] | Range[4] | Rain[5] | Low | High | Range | Rain | Low | High | Range | Rain |
| IL | 55.8 | 80.3 | 38-89 | 3.0 | 61.8 | 85.9 | 53-97 | 3.4 | 61.6 | 85.1 | 54-94 | 3.2 |
| MO | 57.1 | 78.3 | 42-89 | 4.3 | 62.8 | 82.6 | 51-92 | 3.6 | 65.0 | 86.4 | 54-92 | 0.8 |
| NE | 55.5 | 76.0 | 45-91 | 5.8 | 60.2 | 82.4 | 48-90 | 2.2 | 65.9 | 86.7 | 56-98 | 6.9 |
| TX | 50.8 | 75.9 | 38-86 | 2.4 | 57.3 | 86.0 | 43-96 | 0.5 | 61.0 | 94.8 | 57-100 | 0.5 |

| Study | August | | | | September | | | | October | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Low | High | Range | Rain | Low | High | Range | Rain | Low | High | Range | Rain |
| IL | 66.6 | 93.5 | 57-100 | 1.0 | 56.5 | 84.7 | 40-98 | 2.4 | 47.2 | 72.6 | 29-93 | 3.7 |
| MO | 69.6 | 91.1 | 60-99 | 6.3 | 57.8 | 83.3 | 45-93 | 1.6 | 47.9 | 69.2 | 32-87 | 4.4 |
| NE | 66.7 | 86.3 | 56-95 | 4.8 | 53.5 | 79.0 | 40-90 | 2.9 | 44.9 | 67.2 | 29-87 | 5.2 |
| TX | 63.7 | 95.9 | 59-105 | 0.9 | 56.2 | 86.9 | 46-105 | 2.3 | 43.9 | 80.0 | 32-98 | 0.7 |

[1] Study sites: IL = Effingham County., IL; MO = Shelby County., MO; NE = York County., NE; and TX = Carson County., TX.
[2] Average daily low temperature (°F)
[3] Average daily high temperature (°F)
[4] Range of daily high and low temperatures (°F)
[5] Total rainfall (inches)

**Table M-4. Characteristics Evaluated at Each Site for Survival Outside Cultivation Study**

| Characteristic | Timing of Evaluation | Evaluation Description |
|---|---|---|
| Early stand count | V2-V4 growth stage (at least one plant) | Number of emerged plants per plot |
| Growth stage monitoring | V2-V4 growth stage (at least one plant) and every two to four weeks thereafter | Average growth stage of emerged corn plants using Iowa State University guidance (Herman, 1997) |
| Vigor monitoring | V2-V4 growth stage (at least one plant) and every two to four weeks thereafter | Vigor rating on a 1-9 scale, where 1-3 = above average, 4-6 = average, 7-9 = below average, based on PI experience with corn |
| Late vegetative plant height | V10 growth stage (approximately 50% of plants) | The average height of up to five plants |
| Pollen shed interval | Pollen shed | Date when the earliest plant begins shedding pollen and the date when the latest plant stops shedding pollen |
| Silking interval | Silking | Date when the earliest plant begins silking and the date when the latest plant stops silking |
| Final stand count | Within one week of typical harvest time | Number of standing plants per plot |
| Plant height at maturity | R6 growth stage (approximately 50% of plants) | The average height of up to five plants |
| Number of ears produced | Harvest | Number of ears produced per plot |
| Number of seed produced | Harvest | Number of seed produced per plot |
| Average number of ears per plant | Derived from other data | Number of ears produced divided by the final stand count per plot |
| Replacement value | Derived from other data | Ratio of the number of seed produced to the number of seed planted |

## Appendix N. USDA Notifications Approved for MON 87460

Field trials of MON 87460 were conducted in the U.S. since 2002. The protocols for these trials include field performance, agronomics, and generation of field materials and data necessary for this petition. In addition to the phenotypic assessment data provided for MON 87460, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2007 and 2008 seasons, are still in preparation. A list of trials conducted under USDA notification and the status of the final reports for these trials are provided in Table N-1.

**Table N-1. USDA Notifications Approved for MON 87460 and Status of Trials Conducted Under these Notifications.**

| USDA No. | Effective Date (m/d/y) | Release State (sites) | Trial Status |
|---|---|---|---|
| **2002 Field Trials** | | | |
| 02-256-06n | 11/19/02 | HI (3) | Submitted to USDA |
| 02-256-07n | 10/13/02 | PR (2) | Submitted to USDA |
| | | | |
| **2003 Field Trials** | | | |
| 03-059-04n | 03/30/03 | CA, KS | Submitted to USDA |
| 03-218-02n | 10/08/03 | HI (5) | Submitted to USDA |
| 03-276-09n | 11/02/03 | PR (2) | Submitted to USDA |
| 03-276-10n | 11/02/03 | PR (2) | Submitted to USDA |
| 03-317-01r | 04/07/04 | CA (5),CT (2), IA (10), IL (11), KS (7), MO (1) | Submitted to USDA |
| 03-317-01r * | 03/24/05 | CA(7), IA (8), IL (20), KS (8) | Submitted to USDA |
| *Amendment 1 | For Permit 03-317-01r | | |
| | | | |
| **2004 Field Trials** | | | |
| 04-022-03n | 03/25/04 | CT (2), IA (2), IL (2), KS, MI (2), MN | Submitted to USDA |
| 04-036-04n | 03/22/04 | CT (2), IA (2), IL (2), KS, MI (2), MN, WI (2) | Submitted to USDA |
| 04-042-12n | 03/12/04 | CA (3), KS (7), NE | Submitted to USDA |
| 04-042-13n | 03/12/04 | IA (5), IL (10) | Submitted to USDA |
| 04-058-06n | 03/28/04 | CT | Submitted to USDA |
| 04-237-03n | 09/30/04 | PR (2) | Submitted to USDA |
| 04-238-01n | 09/30/04 | HI (4), PR (2) | Submitted to USDA |
| 04-238-03n | 09/27/04 | HI (3) | Submitted to USDA |
| | | | |
| **2005 Field Trials** | | | |
| 05-045-07n | 03/22/05 | IA (6), IL (5) | Submitted to USDA |
| 05-045-08n | 03/22/05 | CA (6), KS (5), NE, TX | Submitted to USDA |
| 05-053-25n | 03/17/05 | CT (2) | Submitted to USDA |
| 05-088-01r | 08/12/05 | HI (3), PR | Submitted to USDA |
| 05-171-02n | 08/15/05 | HI (6), PR (2) | Submitted to USDA |
| 05-213-06n | 09/06/05 | HI (5), PR (2) | Submitted to USDA |
| 05-230-01n | 10/03/05 | HI (5), PR (2) | Submitted to USDA |
| 05-290-01r | 02/23/06 | CA(8), CT (2), IA (15), IL (27), IN (7), KS (9) | Submitted to USDA |
| | | | |
| **2006 Field Trials** | | | |
| 06-039-06n | 05/18/06 | PR (3) | Submitted to USDA |
| 06-039-07n | 05/18/06 | HI (12) | Submitted to USDA |
| 06-045-23n | 04/17/06 | IA (7), IL (2), IN (4), KS, NE | Submitted to USDA |
| 06-052-08n | 04/24/06 | IL (2), KS(8), NE (3), TX | Submitted to USDA |
| 06-052-10n | 03/23/06 | CA (6), KS (5), NE | Submitted to USDA |

Table N-1 continues on next page.

**Table N-1 (continued). USDA Notifications Approved for MON 87460 and Status of Trials Conducted under these Notifications.**

| USDA No. | Effective Date (m/d/y) | Release State (sites) | Trial Status |
|---|---|---|---|
| 06-054-04n | 03/20/06 | IL (7) | Submitted to USDA |
| 06-054-05n | 04/24/06 | IA (7), KS | Submitted to USDA |
| 06-054-06n | 03/23/06 | CA (6) | Submitted to USDA |
| 06-054-07n | 03/23/06 | IL (7), IN (4), NE | Submitted to USDA |
| 06/089-01r | 07/01/06 | HI (3), PR | Submitted to USDA |
| 06-089-05n | 05/18/06 | HI (5), PR (2) | Submitted to USDA |
| 06-118-01n | 05/09/06 | IA | Submitted to USDA |
| 06-142-103n | 08/15/06 | HI (14) | Submitted to USDA |
| 06-142-106n | 08/15/06 | PR (3) | Submitted to USDA |
| 06-153-102n | 08/13/06 | HI (2) | Submitted to USDA |
| 06-233-101n | 09/20/06 | IA (2), IN | Submitted to USDA |
| 06-272-105n | 11/14/06 | HI (7) | Submitted to USDA |
| 06-275-105n | 11/08/06 | HI (10) | Submitted to USDA |
| 06-347-107n | 01/12/07 | KS | Cancelled |
| 06-347-109n | 01/12/07 | NE (2) | Cancelled |
| 06-348-103n | 01/13/07 | OK | Submitted to USDA |
| 06-353-101n | 01/29/07 | HI (7) | Submitted to USDA |
| 06-353-102n | 01/29/07 | HI (10) | Submitted to USDA |
| 06-353-103n | 01/18/07 | IN (4) | Submitted to USDA |
| 06-354-104n | 01/19/07 | KS (5) | Submitted to USDA |
| 06-355-102n | 01/20/07 | SD (15) | Submitted to USDA |
| 06-355-103n | 01/20/07 | SD (10) | Submitted to USDA |
| 06-355-105n | 01/20/07 | NE (13) | Submitted to USDA |
| 06-355-106n | 01/20/07 | NE (15) | Submitted to USDA |
| 06-355-107n | 01/20/07 | KS (5), NE (10) | Submitted to USDA |
| 06-355-108n | 01/20/07 | OK | Submitted to USDA |
| | | | |
| **2007 Field Trials** | | | |
| 07-084-102r | 04/12/07 | CA (8), CT (2), IA (19), IL (31), IN (6), KS (18) | Submitted to USDA |
| 07-010-106n | 02/09/07 | OK | Cancelled |
| 07-012-102n | 03/18/07 | NM | Submitted to USDA |
| 07-024-103n | 02/23/07 | TX | Submitted to USDA |
| 07-024-107n | 02/23/07 | KS | Submitted to USDA |
| 07-024-109n | 02/23/07 | NE (2) | Submitted to USDA |
| 07-024-111n | 02/23/07 | CO | Submitted to USDA |
| 07-028-120n | 03/17/07 | CO | Submitted to USDA |
| 07-033-107n | 03/21/07 | IL(3), KS (3), MS | Submitted to USDA |
| 07-033-108n | 03/19/07 | TX (2) | Submitted to USDA |
| 07-036-109n | 03/21/07 | KS (7), TX | Submitted to USDA |
| 07-036-110n | 03/19/07 | CA (8), CT (2) | Submitted to USDA |
| 07-037-120n | 03/22/07 | IL | Submitted to USDA |
| 07-037-121n | 03/18/07 | IA, KS | Submitted to USDA |

Table N-1 continues on next page.

**Table N-1 (continued). USDA Notifications Approved for MON 87460 and Status of Trials Conducted under these Notifications.**

| USDA No. | Effective Date (m/d/y) | Release State (sites) | Trial Status |
|---|---|---|---|
| 07-040-104n | 03/26/07 | IA (2), IL (3), KS, NE | Submitted to USDA |
| 07-044-108n | 03/22/07 | CA, TX, KS (2) | Submitted to USDA |
| 07-045-108n | 04/13/07 | IL (2) | Submitted to USDA |
| 07-052-106n | 03/23/07 | IN, PA | Submitted to USDA |
| 07-052-113n | 03/23/07 | IL (2) | Submitted to USDA |
| 07-053-103n | 03/24/07 | IA, MO, OH | Submitted to USDA |
| 07-053-105n | 04/03/07 | IA, IL | Submitted to USDA |
| 07-054-104n | 03/25/07 | OK | Submitted to USDA |
| 07-054-110n | 03/25/07 | NE, TX | Submitted to USDA |
| 07-058-107n | 03/29/07 | HI (5), PR (2) | Submitted to USDA |
| 07-061-102rm | 06/08/07 | HI (5), PR | Submitted to USDA |
| 07-065-114n | 04/06/07 | HI (4) | Submitted to USDA |
| 07-065-115n | 04/09/07 | PR (3) | Submitted to USDA |
| 07-065-116n | 04/05/07 | MO | Submitted to USDA |
| 07-066-111n | 04/06/07 | IL (3), NE (2) | Submitted to USDA |
| 07-084-102r | 04/12/07 | CA (6), CT (2), IA (8), IL (21), IN (5), KS (9) | Submitted to USDA |
| 07-215-101n | 09/02/07 | PR (4) | Submitted to USDA |
| 07-215-102n | 09/01/07 | HI (6) | In Process |
| 07-243-104n | 09/30/07 | HI (5), PR (2) | In Process |
| 07-257-108rm | 02/14/08 | HI (5), PR | In Process |
| 07-292-104n | 11/20/07 | HI | In Process |
| 07-292-105n | 11/20/07 | PR | In Process |
| 07-295-101n | 12/06/07 | HI | In Process |
| 07-295-105n | 12/07/07 | PR | In Process |
| 07-295-109n | 11/21/07 | HI | In Process |
| 07-295-110n | 12/06/07 | PR | In Process |
| 07-295-113n | 12/06/07 | HI, PR | In Process |
| 07-295-114n | 12/06/07 | HI, PR | In Process |
| 07-324-101rm | 04/11/08 | CA (5), CT (2), IA (10), IL (13), IN (3), KS (11), MD, OH | In Process |
| 07-351-101n | 01/18/08 | HI (5), PR (2) | In Process |
| | | | |
| **2008 Field Trials** | | | |
| 08-016-102n | 02/15/08 | IL (2) | In Process |
| 08-016-104n | 02/15/08 | KS (11) | In Process |
| 08-016-106n | 02/15/08 | KS (15) | In Process |
| 08-016-113n | 02/15/08 | MO | In Process |
| 08-016-114n | 02/15/08 | NE (12) | In Process |

Table N-1 continues on next page.

**Table N-1 (continued). USDA Notifications Approved for MON 87460 and Status of Trials Conducted under these Notifications.**

| USDA No. | Effective Date (m/d/y) | Release State (sites) | Trial Status |
|---|---|---|---|
| 08-016-115n | 02/15/08 | NE (9) | In Process |
| 08-016-116n | 02/23/08 | IN (2) | In Process |
| 08-016-117n | 02/23/08 | MI (2) | In Process |
| 08-016-118n | 02/15/08 | OH | In Process |
| 08-016-121n | 02/22/08 | OK | In Process |
| 08-017-103n | 02/25/08 | SD (12) | In Process |
| 08-017-104n | 02/16/08 | SD (14) | In Process |
| 08-017-105n | 02/25/08 | TN (2) | In Process |
| 08-017-106n | 02/16/08 | WI (2) | In Process |
| 08-017-107n | 02/16/08 | IA (15) | In Process |
| 08-017-108n | 02/16/08 | IL (14) | In Process |
| 08-017-110n | 02/20/08 | MN (8) | In Process |
| 08-017-111n | 02/16/08 | NE (15) | In Process |
| 08-043-127n | 03/13/08 | TX (2) | In Process |
| 08-056-110n | 03/26/08 | TX | In Process |
| 08-056-111n | 03/26/08 | CA (4) | In Process |
| 08-056-113n | 03/26/08 | KS (5) | In Process |
| 08-058-108n | 03/28/08 | NE | In Process |
| 08-058-110n | 03/28/08 | IL, IN, LA, NE | In Process |
| 08-058-112n | 03/28/08 | CO | In Process |
| 08-058-116n | 03/28/08 | KS (3), SD (3) | In Process |
| 08-058-117n | 03/28/08 | TX | In Process |
| 08-058-118n | 03/28/08 | KS | In Process |
| 08-063-105n | 04/02/08 | AR, CA (2) | In Process |
| 08-063-109n | 04/02/08 | KS | In Process |
| 08-072-102n | 04/11/08 | CA | In Process |
| 08-079-104n | 04/18/08 | TX | In Process |
| 08-080-105n | 04/19/08 | OK | In Process |
| 08-080-107n | 04/24/08 | CA | In Process |
| 08-080-110n | 04/19/08 | TX (2) | In Process |
| 08-084-105n | 04/25/08 | IA, NE | In Process |
| 08-091-108n | 04/30/08 | TX | In Process |
| 08-106-111rm | 06/30/08 | HI (5), PR | In Process |
| 08-108-103n | 05/17/08 | IL, MS | In Process |
| 08-113-101n | 05/22/08 | TX | In Process |
| 08-185-103n | 08/02/08 | HI (2) | In Process |
| 08-200-102n | 08/17/08 | HI (4), PR (2) | In Process |
| 08-242-103n | 09/26/08 | HI (2) | In Process |
| 08-297-105n | 11/22/08 | HI, PR (2) | In Process |

## Appendix O. Petitioner's Environmental Assessment

This section provides a brief summary of three key areas to be covered in an environmental assessment prepared by APHIS for MON 87460 under NEPA: (1) Alternatives, (2) the Affected Environment, and (3) Potential Environmental Consequences. The significance of the potential environmental impact of MON 87460 takes into consideration both the context and intensity of the proposed action of deregulation in whole for MON 87460. This assessment provides data and analysis that appropriately addresses and evaluates potential elements of each as described in the implementing NEPA regulations (40 CFR Part 1508.27). MON 87460 has been the subject of numerous field trials conducted in the U.S. under APHIS notification since 2002. Information has been developed from these field trials, other tests and the literature to specifically assess whether the drought tolerance trait or the plant transformation process altered MON 87460 corn in any way that would impart plant pest characteristics or cause significant environmental impacts, including cumulative impacts.

### O.A. Alternatives to Consider

The action of deregulation is governed by 7 CFR 340.6 (d)(3)(i) which states that APHIS may approve the petition in whole or in part, resulting in one of three possible outcomes from Monsanto's petition:

- **No action**
  - MON 87460 would remain a regulated article
- **Approval in part**
  - MON 87460 would be granted deregulated status with some restrictions (e.g., geographic)
- **Approval in whole**
  - MON 87460 would be granted full deregulated status

Rejection of the "no action" and "approval in part" options is dependent upon a finding of no plant pest risk for MON 87460. MON 87460 has been thoroughly characterized and extensive information presented in Sections I through IX of this petition demonstrates that MON 87460 does not present a plant pest risk. On the basis of this analysis, Monsanto is requesting an "approval in whole" or full deregulated status for MON 87460. Information and arguments presented in this section will further demonstrate that MON 87460 does not present a significant environmental impact; thus, the requirements of NEPA are satisfied by an Environmental Assessment and Finding of No Significant Impact (FONSI). A comparison of likely environmental impacts between the "no action" and "approval in whole" alternatives to be considered under 7 CFR 340.6 (d)(3)(i) is presented in Table O-1.

**Table O-1. Comparison of Likely Environmental Impacts Between "No Action" and "Approval in Whole" Alternatives**

| Attribute/Measure | No Action Alternative | Approval in Whole Alternative |
|---|---|---|
| **Meets Purpose and Need Objectives** | No | Yes |
| Unlikely to Pose Plant Pest Risk | Satisfied through use of regulated field trials | Satisfied through use of regulated field trials and safety assessment |
| Commercial Use | Unchanged | Unchanged due to well established Stewardship policies and lack of plant pest potential |
| **Management Practices** | | |
| Corn Seed Production | Unchanged | Unchanged; seed production will be in accordance with OECD and AOSCA standards |
| Cropping Practices | Unchanged | Unchanged due to agronomic and phenotypic equivalency to conventional corn |
| Specialty Corn Production | Unchanged | Unchanged due to well established processes developed to protect seed purity |
| Pesticide Use | Unchanged | Unchanged due to agronomic and phenotypic equivalency to conventional corn |
| **Human and Animal Health** | | |
| Risk to Human and Animal Health | Unchanged | Unchanged; supported by low oral toxicity, protein safety, and compositional equivalence |
| Risk to Worker Safety | Unchanged | Unchanged since pesticide use practices will not change |
| **Environment** | | |
| Risk to Plants | Unchanged | Unchanged due to no increase in plant pest potential, including weediness, and low potential for gene introgression |
| Risk to Animals | Unchanged | Unchanged; supported by low oral toxicity, protein safety, and compositional equivalence |
| Land Use Including CRP | Unchanged | Unchanged; no more likely to survive on marginal farmland or displace other crops in Western Dryland than conventionally bred drought tolerant corn |
| Water Use | Unchanged | Unchanged or may result in some decrease in irrigation |
| Cumulative Impacts | Unchanged | Unchanged for land use, agronomic practices, specialty corn production, biodiversity, risk to threatened or endangered species and non-target organisms due to agronomic and phenotypic equivalence to conventional corn, well-established seed production practices, lack of weediness, food and feed safety; possible positive economic and environmental benefits due to yield stability under water-limiting conditions and potential for decrease in irrigation |

## O.B. Affected Environment

Corn is an intensively cultivated row crop that is planted in almost every state in the U.S. The two largest corn producing regions are the Midwest contributing 65% of U.S. corn production and the Great Plains contributing 25% of U.S. production. Over the last several decades breeding programs have been successful at increasing the ability of conventional corn to withstand drought, resulting in hybrids that are characterized as having very good to excellent drought tolerance. These hybrids are currently planted widely throughout the Great Plains and the Midwest. MON 87460 would provide an additional tool for growers faced with uncertain weather conditions throughout the Corn Belt, particularly in the Western Dryland region of the Great Plains.

The affected environment is described in detail in Section IX of this document. The environment includes corn, its uses as human food and animal feed, areas where corn is currently grown commercially, land where corn may be grown given the characteristics of the trait, and animal and plant communities. The affected environment also includes related agricultural practices such as tillage, crop rotation, pesticide use, weed management, irrigation practices, and non-agricultural lands. Finally, the affected environment includes related corn production and marketing practices such as specialty corn production, including organic corn production, seed production, and related human activities associated with marketing of corn grain.

## O.C. Potential Environmental Consequences

### O.C.1. Corn Production

#### O.C.1.1. Acreage and Areas of Corn Production

MON 87460, with the exception of reduced yield loss under water limited conditions, is agronomically and phenotypically equivalent to conventional corn. Therefore it is not anticipated that the introduction of MON 87460 will have any significant impact on total current U.S. corn acreage or the areas in which corn is produced. Between 1996 and 2006, a period during which improved conventionally bred drought tolerant corn hybrids (Campos, 2006) and new biotechnology-derived corn products were introduced, total corn acreage remained relatively steady, averaging 78 M acres per year (http://www.nass.usda.gov). In 2007, the total corn acreage increased by 15% from 78 M to 92.9 M acres. The increase was attributed to increased demand from ethanol producers (http://www.ncga.com/node/83) and strong exports sales. The increased corn acreage was obtained primarily by planting fewer acres of soybean in the Corn Belt and Great Plains (USDA-NASS, 2007).

A small portion of the increase in 2007 corn acreage came from land that had previously been enrolled in the Conservation Reserve Program (CRP). The CRP is a voluntary federal program offering annual rental payments over a 10-year contract period, as well as cost-share assistance, to producers establishing specific types of plant cover on marginal farmland. According to the Farm Service Agency: “CRP plantings and practices offer our nation vast environmental benefits, including reducing soil erosion, improving surface and ground water quality, creating wildlife habitat, restoring wetlands, sequestering carbon, preserving soil productivity and reducing offsite wind erosion

damage." Of the 36 M acres enrolled in the CRP in 2006, 21.2 M acres or 59% were from Great Plains states with 7.9 M acres or 22% from Midwest "corn-belt" states (USDA-FSA-CRP, 2007).

Corn prices are a significant driver of decisions to withdraw land from the CRP. In 2007, re-enrollment for 15.7 M eligible acres with CRP contracts due to expire dropped by 2.6 M acres to 13.1 M acres compared with 2006 (USDA-FSA-CRP, 2007) Corn commodity prices were at an all-time high at $4/bushel in 2007 (http://www.nass.usda.gov). Consequently, some growers did not renew their enrollment in the CRP and instead planted their land to crops, a decision driven by market demands and corn prices (Hart, 2006). It is highly likely much of the corn planted on these marginal farmland acres were conventional hybrids with good drought tolerance characteristics. In this scenario, it is important to recall that MON 87460 requires the same agronomic management practices as conventionally bred drought tolerant corn in order to produce acceptable yields. Under severe water deficit, corn grain yield for MON 87460, as well as for conventionally bred drought tolerant corn, can be reduced to zero. Therefore MON 87460 is no more likely to impact changes in corn acreage and land use including marginal land use, than a conventional drought tolerant hybrid.

### O.C.1.2. Cropping Practices

Given that, with the exception of enhanced yield under water-limited conditions, MON 87460 is agronomically and phenotypically equivalent to conventional corn and that numerous drought tolerant conventional corn hybrids are already planted in the U.S., it is not anticipated that the introduction of MON 87460 will have any impact on current cropping practices including seed production, crop rotation, tillage, or disease, insect, and weed management (Section IX).

In the event this product is commercialized, it is likely that the drought tolerance trait expressed in MON 87460 will become part of breeding programs. However it is not anticipated that the availability of the MON 87460 drought tolerance trait will result in a reduction in the selection of hybrids currently available to growers or, based on the mechanism of action and field observations, introduction of an enhanced susceptibility to insects or diseases.

Corn is extremely sensitive to soil moisture deficits. Due to the arid climate there is a significant amount of acreage under irrigation in the Great Plains. The growing market for biofuels and worldwide concern over food shortages that have caused commodity prices to increase significantly may result in changes to cropping and irrigation patterns in the Great Plains as growers plant more of their acres to corn. Given the above, it is foreseeable that growers in the future may evaluate whether MON 87460 and other drought tolerant hybrids can be used in dryland production without benefit of irrigation. While the introduction of MON 87460 may facilitate an increase in dryland productivity, it will be the result of the market forces noted above, not to the availability of a new drought tolerant hybrid per se.

### O.C.1.3. Specialty Corn Production

When considering impacts to the human environment, specialty and organic grower concerns with regard to the possibility of a decrease in the value of their crops due to potential gene flow should be addressed. For several decades the corn industry has created and adopted systems to maintain and preserve the purity of corn germplasm developed for various uses, including specialty and organic uses. To maintain the genetic purity of inbred and hybrid corn populations, production activities for each type are isolated from one another and from commercial grain production (Westgate et al., 2004; Wych, 1988). Isolation is achieved through various means but may include physical separation to prevent cross pollination, temporal isolation by planting at different times to stagger pollination times of different materials, detasseling, and the use of cytoplasmic male sterility. Specialty corn producers use isolation measures to attain commercially acceptable purity standards for their crops. Organic corn producers use process-based standards outlined in the USDA's National Organic Program to ensure that there is no opportunity for gene flow from biotechnology-derived corn to non-biotechnology derived corn. Consequently, the advent of biotechnology-derived crops does not appear to have had a negative impact on specialty or organic crop production. Indeed, organic production has seen its fastest growth in the decade since biotechnology-derived crops came on the market. From 1995 to 2005, organic corn acreage increased by 300% in the U.S. During the same timeframe, the acreage of biotechnology-derived corn increased from none to 43% of total U.S. acreage for all crops in 2006 (USDA-NASS 2006).

The U.S. acreage devoted to corn hybrids with biotechnology traits has grown dramatically since the first such hybrid was planted in 1996. Those traits include insect resistance and herbicide tolerance. In 2006, approximately 61% of U.S. corn acres were planted with a hybrid that contains a biotechnology trait (James, 2006; http://www.ers.usda.gov/Data/BiotechCrops/ExtentofAdoptionTable1.htm). Despite this large acreage of biotechnology-derived corn, about 5% of the total corn production acres are devoted to production of non-biotechnology, specialty corn hybrids, including popcorn, waxy corn, high oil corn, white corn, blue corn, Indian corn, and high- and modified-protein corn hybrids (USGC, 2007).

Organic farming is a small but rapidly growing sector of the U.S. agriculture industry with about 0.5% of all U.S. cropland and 0.5% of all U.S. pasture being certified organic in 2005 (USDA-ERS, 2007). Only a small percentage of the most widely planted U.S. field crops (0.16% of corn, 0.17% of soybean, and 0.48% wheat) were grown under certified organic farming systems (Table O-2). Driven by a desire to have a single, consistent national standard that could result in greater public confidence in foods that were labeled as "organic," Congress passed The Organic Foods Production Act of 1990 (OFPA). The OFPA required USDA to establish a National Organic Program (NOP) to develop uniform standards and a certification process for those producing and handling food products offered for sale as organically produced (USDA-ARS, 2000). The OFPA requires the NOP to be process-based, specifically describing the NOP as "an organic certification program for producers and handlers of agricultural products that have been

produced using organic methods."[1]. No claims or assumptions regarding the quality or content of organic products may be made beyond the fact that the product was produced under these standards. Importantly, NOP prohibits the *use* of biotechnology: growers selling crops as organic may not *intentionally* plant seed produced through biotechnology. USDA repeatedly stated in the finalized NOP that the mere presence of plant materials produced through biotechnology in a crop will not jeopardize the integrity of products labeled as organic:

> "We have not accepted the comments that requested adding the products of excluded methods to the definition [of excluded methods]. The emphasis and basis of these standards is on process, not product. We have specifically structured the provisions relating to excluded methods to refer to the use of methods. Including the products of excluded methods in the definition would not be consistent with this approach to organic standards as a process-based system" (USDA-ARS, 2000).

Although small, organic production has been one of the fastest growing segments of U.S. agriculture for the past decade. The U.S. had less than one million acres of certified organic farmland when Congress passed the OFPA. By the time USDA implemented national organic standards in 2002, certified organic farmland had doubled, and doubled again between 2002 and 2005 (USDA-ERS, 2007). For example, from 1995 to 2005 the organic corn acreage increased by 300% in the U.S. (Table O-2), while during the same time frame the biotechnology-derived corn acreage increased from none to 52% of total U.S. acreage for corn in 2005 (USDA-NASS, 2006).

These national statistics do not suggest that the increase in organic corn production and the increase in biotechnology-derived corn production are correlated, but they do indicate that the adoption of biotechnology-derived corn did not adversely affect organic corn production. Accordingly, there is no reason to expect that Monsanto's drought tolerant corn product, MON 87460, will have any negative impact on the production of organic corn or other specialty corn.

[1] 7 U.S.C. § 6503(a).

**Table O-2. Certified Organic and Total U.S. Acreage of Selected Crops (USDA-ERS, 2007)**

| Item | Total certified organic (acres) | | | | | | | | U.S. Cropland 2005 (acres) | Certified organic to total (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1995 | 1997 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | | |
| **Total cropland:** | 638,500 | 850,173 | 1,218,905 | 1,302,392 | 1,299,632 | 1,451,601 | 1,452,353 | 1,723,271 | 340,650,083 | 0.51% |
| ***Grains--*** | | | | | | | | | | |
| Corn | 32,650 | 42,703 | 77,912 | 93,551 | 96,270 | 105,574 | 99,111 | 130,672 | 81,759,000 | 0.16% |
| Wheat | 96,100 | 125,687 | 181,262 | 194,640 | 217,611 | 234,221 | 214,244 | 277,487 | 57,229,000 | 0.48% |
| Oats | 13,250 | 29,748 | 29,771 | 33,254 | 53,459 | 46,074 | 42,616 | 46,465 | 4,246,000 | 1.09% |
| Barley | 17,150 | 29,829 | 41,904 | 31,478 | 34,031 | 30,265 | 26,629 | 39,271 | 3,875,000 | 1.01% |
| Sorghum | -- | 3,075 | 1,602 | 938 | 3,043 | 4,152 | 4,453 | 6,042 | 6,454,000 | 0.09% |
| Rice | 8,400 | 11,043 | 26,870 | 29,022 | 22,381 | 20,152 | 22,173 | 26,428 | 3,384,000 | 0.78% |
| Spelt | 12,350 | 1,704 | 12,606 | 7,639 | 6,939 | 9,719 | 6,203 | 8,169 | -- | -- |
| Millet | 18,550 | 12,285 | 15,103 | 23,366 | 17,575 | 26,935 | 21,036 | 14,175 | 565,000 | 2.51% |
| Buckwheat | 13,250 | 7,616 | 10,599 | 14,311 | 8,388 | 8,086 | 7,960 | 6,364 | -- | -- |
| Rye | 2,900 | 4,365 | 7,488 | 7,056 | 9,644 | 11,616 | 10,289 | 8,597 | 1,433,000 | 0.60% |
| ***Beans--*** | | | | | | | | | | |
| Soybean | 47,200 | 82,143 | 136,071 | 174,467 | 126,540 | 122,403 | 114,239 | 122,217 | 72,142,000 | 0.17% |
| Dry beans | -- | 4,641 | 14,010 | 15,080 | 2,430 | 9,836 | 7,642 | 10,561 | 1,659,300 | 0.64% |
| Dry peas & lentils | 5,900 | 5,187 | 10,144 | 9,362 | 7,476 | 16,188 | 15,893 | 17,757 | 571,000 | 3.11% |
| ***Oilseeds--*** | | | | | | | | | | |
| Flax | 5,850 | 8,053 | 25,076 | 20,672 | 20,484 | 14,940 | 35,104 | 30,843 | 983,000 | 3.14% |
| Sunflowers | 14,200 | 10,894 | 19,342 | 15,295 | 7,624 | 7,121 | 9,742 | 6,087 | 2,709,000 | 0.22% |

### O.C.1.4. Commercial Use

Corn is a globally traded commodity and the U.S. is the single largest exporter. The commercial use of corn in the U.S. would not be feasible without approvals from key trading partners. While there have been occasional market disruptions in the past involving agricultural biotechnology products, these have not occurred when the product has successfully completed the determination process in key import markets such as Canada, Mexico, Japan, (Section I.F.2) and in no instance was any market disruption due to plant death, damage, or disease from the biotechnology-derived product. Monsanto does not intend to commercially release MON 87460 until all key corn import markets with functioning regulatory systems have also granted approval of MON 87460. The lack of significant adverse effects on non-target organisms, a lack of increased fitness or weediness characteristics, and no effect on the health of other plants indicates that there is no apparent potential for significant impact on commercial use. MON 87460 has been shown to be compositionally and phenotypically equivalent to conventional corn and is expected to enter commerce as part of the normal trade flow of yellow corn to be utilized in the same manner as any other yellow corn.

Monsanto employs a rigorous product stewardship program that demonstrates respect for our customers, their markets, and the environment. Our market stewardship program considers many factors to ensure global integration and increased transparency. In keeping with past practice, MON 87460 will not be sold commercially without regulatory approvals from key corn import countries to ensure global compliance and the flow of international trade. Monsanto is committed to the industry practices on seed quality and control to prevent adventitious presence of unapproved traits. Before commercializing MON 87460, a detection method will be made available to grain producers, processors, and buyers. Our stewardship policy is the shared responsibility of Monsanto, our licensees, and our customers to insure that our products are used properly. Monsanto is also committed to legal and ethical obligations to ensure that our products and technologies are safe and environmentally responsible, and do not pose undue risks to human health or the environment during any stage of their life cycle. As such, Monsanto has policies in place to monitor its procedures as products are researched, developed, designed, manufactured, marketed, and discontinued through their life cycle.

## O.C.2. Health and Safety

### O.C.2.1. Food and Feed Safety

Data presented in this petition demonstrate the dietary safety of the drought tolerance trait expressed in MON 87460. The safety assessment of the CSPB and NPTII proteins included extensive protein characterization demonstrating the lack of similarity to known allergens and toxins and the long history of safe consumption of similar proteins (Section VI). In addition, data confirmed the CSPB and NPTII proteins digestibility *in vitro*, and the lack of acute oral toxicity in mice. From these data, it can be concluded with reasonable certainty that the CSPB and NPTII proteins have no meaningful toxic potential to humans, livestock, or other farm animals.

Compositional equivalence between corn improved through biotechnology-derived traits and conventional hybrids provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 1998). Compositional analyses of forage and grain from MON 87460 were conducted to assess the levels of key nutrients, anti-nutrients, and secondary metabolites for comparison to conventional corn. These results, based on evaluation of 77 different components (9 in forage and 68 in grain) confirmed that the forage and grain derived from MON 87460, and the intended foods and feeds derived from MON 87460 can be considered compositionally and nutritionally equivalent to those derived from conventional corn with a history of safe consumption (Section VII). As such, it can be concluded with reasonable certainty that dietary exposure to MON 87460 poses no meaningful risk to humans, livestock, or other farm animals.

### O.C.2.2. Worker Safety

As discussed in Sections IX.D.2.7 and IX.D.2.8, since it is agronomically and phenotypically equivalent to conventional corn, introduction of MON 87460 is not anticipated to have any impact on the management of diseases, insects and weeds. Therefore, worker safety issues related to the use of pesticides during agricultural production would not be affected.

### O.C.3. Gene Movement from MON 87460 into Sexually Compatible Relatives

In assessing potential risks associated with gene introgression from MON 87460 with its sexually compatible relatives, teosinte and gamma grass, there are two primary concerns: the potential for gene flow and introgression and the potential impact of the introgression.

As discussed in Sections II.C and II.D.5, corn and annual teosinte are genetically compatible, wind-pollinated and hybridize when in close proximity to each other, e.g., in areas of Mexico and Guatemala. However, in the U.S., hybridization in nature is extremely unlikely because geographical distributions of teosinte, found only as a small feral population in southern Florida, and corn do not overlap and because of differences in developmental morphology and reproductive timing between the two species (Section II.D.5). First generation corn-teosinte hybrids are generally less fit for survival and dissemination, and they show significantly reduced reproductive capacity. Therefore it is highly unlikely that gene introgression from MON 87460 into teosinte will occur or, if it did, that it would result in robust wild weed populations.

In contrast with corn and teosinte, which easily hybridize, it is only with extreme difficulty and special techniques that corn and gamma grass hybridize. Furthermore, the offspring of the cross show varying levels of sterility (Galinat, 1988; Mangelsdorf, 1974; Russell and Hallauer, 1980) (Section II.D.5). As no cases of gene flow between corn and its wild relatives are known to occur naturally in the U.S., there is no opportunity for an environmental impact. Therefore it is not anticipated that the introduction of MON 87460 will result in any increased potential for harm to the environment due to gene flow to sexually compatible wild relatives.

## O.C.4. Plant and Animal Communities including Threatened or Endangered Species

### O.C.4.1. Plants

Modern corn cannot survive as a weed due to intense selection during domestication, in which traits often associated with weediness such as seed dormancy, dispersal mechanisms, or the ability to form reproducing populations outside of cultivation have not been selected. Consequently corn is not capable of surviving without human assistance (Baker, 1965; Keeler, 1989; Galinat, 1988) and all corn hybrids, including MON 87460, have extremely low potential for weediness. Even when individual kernels of corn are distributed within a field or along transportation routes from the fields to storage or processing facilities, sustainable, volunteer corn populations are not found growing in fence rows, ditches, or road sides.

In comparative studies conducted between MON 87460 and a conventional control, dormancy and germination, growth and development, and reproductive characteristics were evaluated for changes that would impact plant pest potential, and in particular, plant weediness potential. No meaningful differences were detected between MON 87460 and control corn (Section VIII). Therefore, the introduction of the drought tolerance trait did not unexpectedly alter the assessed characteristics compared to the control. These data indicate that MON 87460 exhibits no characteristics that would improve the ability of this corn to survive without human intervention, and that its cultivation should not interfere with the cultivation of other corn hybrids or result in its uncontrolled spread into non-agricultural environments. Thus, the results support a conclusion of no increased weediness potential of MON 87460 compared to conventional corn. Additionally, APHIS has proposed to amend 7 CFR Part 340 to include its noxious weed authority. MON 87460 would not be considered a "noxious weed" as defined by the Plant Protection Act because the data show that it has no potential to cause direct injury or damage (physical harm) to any protected interest.

MON 87460 will be grown on agricultural acres managed by growers in a manner similar to conventionally bred drought tolerant corn. As discussed above, the weediness potential of MON 87460 has not been altered by the presence of the CSPB and NPTII proteins. Therefore it is highly unlikely that MON 87460 poses any more of a risk to threatened or endangered plant species than conventionally bred drought tolerant corn. Additionally the potential for gene flow from MON 87460 is limited to sexually compatible relatives (teosinte and *Tripsacum* (gamma grass) species. As discussed in Section IX.C.1, differences in flowering times, geographical separation, and developmental factors result in negligible opportunity for natural crosses in the U.S.

### O.C.4.2. Animals

MON 87460 is a product beneficial to agriculture with no pesticidal activity. As such all exposed organisms, including threatened or endangered species are considered to be NTOs. During the U.S. phenotypic field trials at multiple locations in 2006 and 2007, multiple observations for plant disease incidence as well as effects on several insect and arthropod species were made to assess whether the plant-disease or plant-insect

interactions of MON 87460 were altered compared to commercial corn (Section VIII.F.1). Of the more than 375 pest and non-pest arthropod evaluations, only two differences were observed between MON 87460 and the control. The differences detected were either within the range of the commercial reference hybrids or were isolated to a single study-site location. Because of the small number of differences that were not consistently observed, they are not considered to have biological significance. Out of the more than 425 disease stressor observations, no differences were detected between MON 87460 and the control. These results support the conclusion that MON 87460 does not have altered environmental interactions relative to other conventional corn, and plants that express the CSPB protein show no apparent impact on NTOs.

The potential for MON 87460 to pose a meaningful risk to threatened or endangered species was assessed in two ways. First, the likelihood of MON 87460 to be a plant pest impacting threatened or endangered species was considered. As described in Section VIII, evidence is presented demonstrating that MON 87460 has no increased weediness potential compared to conventional corn. Second, the possibility that threatened and endangered species were at risk through hazard and level of exposure was examined. As discussed in Sections VI and VIII.F.1, the CSPB and NPTII proteins are unlikely to pose a significant hazard to arthropods and to non-insect animals. Based on the nature of the proteins, their known activity, and the characteristics of the donor organisms, the CSPB and NPTII proteins have a history of safety to organisms exposed at levels found in MON 87460. In addition, compositional analyses of forage and grain tissues (Section VII) that might be consumed by NTOs, confirm that the levels of key nutrients and anti-nutrients in MON 87460 are comparable to those in conventional corn, and that the forage and grain derived from MON 87460 are compositionally equivalent to conventional corn. Thus, the potential for MON 87460 to have any effect on threatened and endangered species is negligible, regardless of exposure.

### O.C.4.3. Biodiversity

As noted above, no potentially adverse effects on non-target organisms or threatened and endangered species were detected based on extensive characterization of MON 87460, which included phenotypic evaluation conducted in field studies over a broad range of environmental conditions. Furthermore, environmental interaction field studies, including evaluations of pest and non-pest arthropods and interaction with other biotic factor testing on MON 87460 revealed no potential to adversely impact NTOs. As such, the potential consequences to biodiversity are the same as with conventional corn. This conclusion is based on the history of safe use of the host plant, corn, the extensive characterization of MON 87460 compared to a conventional control, including: phenotypic assessments, compositional and nutritional equivalence, and the extensive characterization and history of safe use of the expressed CSPB and NPTII proteins.

The introduced drought tolerance trait confers a selective advantage only under specific conditions in the field, i.e., under water-limited conditions, which are predictable and spatially limited. This selective advantage is consistent with that shown by conventional drought tolerant corn hybrids currently planted in the U.S. This trait was shown not to

provide a meaningful selective advantage or disadvantage that altered the survival of MON 87460 volunteer plants in cultivated fields (Section VIII.F.3) or in areas that were not cultivated for agricultural production (Section VIII.F.4). Thus, it is concluded that the likelihood of MON 87460 spreading in the environment to non-agricultural lands and causing harm to ecosystem biodiversity is negligible, as corn is neither persistent nor invasive and these parameters are unaltered in MON 87460 compared to conventional corn.

### O.C.5. Cumulative Impacts

#### O.C.5.1. Potential for Cumulative Impacts from MON 87460 in Combined Trait Products Resulting from Conventional Breeding with Other Biotechnology-Derived Products

Corn hybrids produced by conventional plant breeding that combine multiple biotechnology-derived traits, have been commercialized since 2000 and have a demonstrated history of safety. For biotechnology-derived products, once single events are assessed and determined to be safe for human and animal consumption and safe to the environment, then combining the unrelated single events through conventional breeding should not pose any new characteristics which would change the safety assessment conclusions. Further, the use of conventional breeding to produce combined trait or combined event products would identify off-types and non-performing germplasm during development of new inbreds and hybrids and be removed from further development. Breeders use standard testing and assessment procedures to further examine and confirm the equivalence of the combined trait products to the single event products in terms of phenotypes, agronomic characteristics, and the efficacy of the traits.

Corn hybrids with various combinations of herbicide tolerance and insect protection traits have been grown commercially in the U.S. since 2000 with no reported incidence of increased weediness potential. MON 87460, which provides reduced yield loss under water-limited conditions, will likely be combined via conventional breeding techniques with one or more of these herbicide tolerance and insect protection traits. Similarly, MON 87460 presents negligible risk for increased pest potential or significant impact to the human environment or threatened and endangered species. In comparative studies conducted between MON 87460 and a conventional control, dormancy and germination, growth and development, and reproductive characteristics were evaluated for changes and none were observed (Section VIII). Additionally, abiotic stress studies for tolerance to cold, heat and salt were conducted with MON 87460 to evaluate the potential for increased tolerance to these adverse conditions and no differences from conventional corn were observed (Section VIII.F.2). Therefore, cumulative impacts are not anticipated from combining MON 87460 with one or more deregulated herbicide tolerance or insect protected traits.

#### O.C.5.2. Potential for Cumulative Impacts on Biodiversity, Preservation of Corn Germplasm Purity, and Specialty Corn Production

The petition has discussed, in great detail, the outcrossing potential of contemporary domestic corn varieties and their wild relatives. Sections VII and VIII presented data on the phenotypic, agronomic, environmental interactions, and compositional assessment of

MON 87460. Sections VII and II.D.5 examined the potential of weediness and gene flow of MON 87460 corn. These analyses concluded that (1) MON 87460 exhibits no characteristics that would cause increased weediness and is not likely to become invasive or form self-sustaining populations outside of agricultural fields; (2) unconfined cultivation should not lead to increased weediness of sexually compatible relatives (of which there are none in the U.S.); and (3) use of MON 87460 in breeding for improved corn hybrids will not reduce or limit the genetic diversity available to corn breeders in the future. In addition, the assessment in Section VIII.F.1 concluded that it is unlikely that MON 87460 would have long-term direct or indirect effects on non-target organisms common to agricultural production areas or on threatened and endangered species. Based on these analyses, there is negligible potential for adverse effects on biodiversity from MON 87460 compared to conventional and previously approved biotechnology-derived corn already under cultivation.

Since the development of hybrid corn systems in the 1930's, the corn industry has created and adopted systems to maintain and preserve the purity of corn germplasm developed for various uses including food, feed, processing, biofuels, and specialty uses such as popcorn, sweet corn, high oil corn, white corn, blue corn, and high-protein corn (Wych, 1988). These systems have been developed and implemented over several decades to maintain and meet industry standards on genetic purity and seed quality. Biotechnology-derived corn traits have been incorporated into these systems while achieving accepted purity standards for the corn industry and taking into consideration the cumulative effects of biotechnology-derived corn products.

To maintain the genetic purity of inbred and hybrid corn populations, production activities for each type are isolated from one another and from commercial grain production (Westgate et al., 2004; Wych, 1988). Isolation is achieved through various means but may include physical separation to prevent cross pollination, temporal isolation by planting at different times to stagger pollination times of different materials, detasseling, and the use of cytoplasmic male sterility. Additionally, production guidelines and operating procedures are used to ensure genetic purity and quality throughout the entire production process from planting and growth of the crop, through harvesting, transport, conditioning, packaging, storage and sale. With these operating procedures being widely used in the corn industry, the industry has been able to introduce new inbreds and hybrids, including biotechnology-derived corn, into the marketplace while still maintaining the desired levels of purity and quality of individual types. Thus, over the last 70 years, systems have been developed to maintain the purity and quality of the diverse corn types needed to meet the needs and desires of corn farmers and consumers. These systems have allowed the introduction of biotechnology-derived traits into the desired inbred lines while at the same time allowing for the maintenance of desired conventional inbred lines. Although 52% of the total U.S. corn acreage was planted to biotechnology-derived hybrids in 2005, organic and conventional corn production remains an option for growers as evidenced by the existence of numerous seed suppliers, a small subset of which is listed below (Table O-3). Therefore the introduction of MON 87460 is not expected to have any cumulative impact on the processes and practices that ensure the genetic purity of conventional and organic seeds.

**Table O-3. Organic and Conventional Seed Sources**

| Organic Corn Seed Sources[1] | Conventional Corn Seed Sources |
|---|---|
| Albert Lea Seed House | Garst Seed[2] |
| Blue River Hybrids | Heirloom Seed[3] |
| Golden Grains | Kruger Seed[4] |
| Great Harvest Organics | Monsanto (Asgrow/DeKalb)[5] |
| Merit Seeds | Pioneer[6] |
| Prairie Hybrids Seeds | |
| Welter Seed and Honey Company | |

[1]http://www.organicgrains.ncsu.edu/
[2]http://www.garstseed.com/GarstClient/Products/Corn/
[3]http://www.heirloomseeds.com/corn.htm
[4]http://www.krugerseed.com/index.php
[5]http://www.asgrowanddekalb.com/seedresourceguide/search/seeds
[6]http://www.pioneer.com/web/site/portal/

There has been a long history of preserving, protecting and enhancing corn germplasm. The introduction of biotechnology-derived corn has had little impact on genetic diversity and germplasm resources of corn, even considering the potential cumulative effect of multiple biotechnology-derived traits being introduced to corn. Plant breeders, institutions, government and non-government agencies take great strides to maintain corn germplasm resources. Corn genetic diversity is maintained through public and private plant breeding efforts, seed conservation in gene banks, germplasm collections maintained by the International Maize and Wheat Improvement Center (CIMMYT) and other governmental agencies, on-farm conservation and enhancement activities in Latin America, and conservation of corn wild relatives in Mexico and Central America. These efforts maintain and preserve corn genetic diversity necessary for improving the base genetics of corn, separate from, or in combination with, the breeding practices used to introduce biotechnology-derived traits into new corn inbreds and hybrids. Thus, the genetic diversity of corn will continue to be maintained.

The introduction and large scale cultivation of biotechnology-derived corn in the past decade did not have any negative impact on organic corn production or other specialty corn production in the U.S. In 2005, 0.16% of U.S. corn acreage was devoted to organic production. It is worthwhile to note that states with the greatest percentage of organic corn production often have above-average penetration of biotechnology-derived crops. For example, the leading organic corn-growing states are Iowa, Minnesota, and Wisconsin. Of these, Iowa and Minnesota have above-average penetration of biotechnology-derived crop plantings (32% and 36%, respectively, of total corn plantings relative to the U.S. average of 26% in 2001) (Brookes and Barfoot, 2005).

Seed manufacturers and growers of specialty use corn, such as organic corn, sweet corn and popcorn have been using the same established processes and methods (e.g., isolation, detasseling, and use of cytoplasm male sterility) as described above to ensure the genetic purity of their corn products. Organic producers use process-based standards outlined in

the USDA's National Organic Program to assure that products produced meet a standard set by the industry or the purchasers of the products. No claims or assumptions regarding the quality or content of organic products may be made beyond the fact that the product was produced under these standards. Due to well-recognized segregation practices, seed producers have numerous organic corn seed hybrids available to them and AOSCA has developed protocols by which a seed developer can produce 99% "non-GMO" seed. Therefore, it is not anticipated that the introduction of MON 87460 will have a negative impact on specialty and organic farming.

In conclusion, measures of preservation of corn germplasm purity including temporal and spatial isolation, detasseling, and the use of cytoplasmic male sterility have been well established and have a track record of effectiveness over many years. The adoption and large scale cultivation of biotechnology-derived corn in the past decade did not have any negative impact on biodiversity, genetic purity of corn, or the production of specialty corn including organic corn in the U.S. There is no evidence to suggest that the introduction of MON 87460 corn will adversely impact biodiversity, genetic purity of corn germplasm, or small scale specialty use corn production even considering the potential cumulative effect of multiple biotechnology-derived traits. It is also concluded based on past experience and standards that the introduction of MON 87460 will not have an impact on the production of specialty corn products, including organic corn.

### O.C.5.3. Potential for Cumulative Impacts on Land Use and Agronomic Practices

The introduction and rapid adoption of biotechnology-derived corn products in the past decade have had no significant impact on cropland acreage in the U.S. The total crop area in the U.S. has remained relatively steady for decades, as is the case for field corn acreage. From 1996 to 2006, the total annual field corn acres fluctuated around 78 million acres (Table IX-3), while in the same time frame the adoption rate for biotechnology-derived corn products increased from zero to 61%. In 2007, a significant increase (16%) in corn acreage to 93M acres occurred with a corresponding increase of 73% in the adoption rate for biotechnology-derived corn products (USDA-NASS, 2007). This increase in total corn acreage resulted primarily from fewer acres of soybean planted in the Corn Belt and the Great Plains (USDA-NASS, 2007). Farmers rotate crops and adjust planting acreages of different crops based on market demand and commodity prices. Currently U.S. and global corn market demand remains relatively high for food, feed, ethanol production, and other industrial uses.

From the information above it is clear that starting with first biotechnology-derived product, MON 810 originally cultivated in 1997, the addition of herbicide tolerant and coleopteran resistant events have not significantly altered the total number of acres dedicated to agriculture or corn production in the U.S The cultivation of biotechnology-derived products has substantially reduced chemical insecticide and herbicide use and has supported the increase in no-till corn acreage resulting in less soil erosion and less runoff of pesticides and water (Brookes and Barfoot, 2005; Carpenter and Gianessi, 2001; Fawcett and Towery, 2002; Carpenter et al., 2004; Sankula, 2006; NCGA, 2007). However since MON 87460 does not contain a pesticide trait, its introduction is not anticipated to provide additional benefits in terms of decreased pesticide use.

Cumulative changes to seed production, crop rotation and tillage practices, or weed, disease, and insect management practices in the Great Plains are not expected from the introduction of MON 87460. Conventionally bred, drought tolerant hybrids have been planted in the U.S. for decades without any documentation of such effects. MON 87460 is not phenotypically and agronomically different from conventional corn, with the exception of reduced yield loss under water-limited conditions (Section VIII). It is foreseeable that increased drought tolerance in corn from the expressed trait in MON 87460 and continued improvements in drought tolerance from conventional breeding may reduce yield loss with or without the added benefits of irrigation. Over time, this may result in a cumulative benefit by decreasing the amount of water used for agriculture in the Great Plains.

### O.C.5.4. Potential for Cumulative Impacts on Non-agricultural Environments and Threatened and Endangered Species

Basic to all evaluations of biotechnology-derived corn's potential for cumulative impact to the environment or threatened and endangered species is the fact that corn cannot persist as a weed. It is an annual, wind-pollinated crop which lacks sexually compatible wild relatives (including threatened or endangered plant species) in the U.S., except for an occasional botanical garden specimen or small feral populations of *Zea mexicana* in Florida, Alabama and Maryland or *Zea perennis* in South Carolina (http://plants.usda.gov). Corn exhibits extremely limited seed dormancy, has no weedy characteristics, and volunteers are easily controlled. It is not capable of establishing persistent populations in unmanaged environments. As demonstrated previously, the presence of the CSPB protein in no way alters the weediness potential or gene flow potential of MON 87460.

Lack of toxicity of the CSPB protein has also been demonstrated (Section VI). The safety assessment of the CSPB protein included extensive protein characterization demonstrating the lack of similarity to known allergens and toxins and the long history of safe consumption of similar proteins. In addition, data confirmed the CSPB protein digestibility *in vitro*, and the lack of acute oral toxicity in mice. Collectively, these data establish the safety of the CSPB protein and indicate that the CSPB protein has no meaningful toxic potential to exposed organisms in the environment.

Compositional equivalence between corn improved through biotechnology-derived traits, and conventional hybrids provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 1998). Compositional analyses of forage and grain from MON 87460 were conducted to assess the levels of key nutrients, anti-nutrients, and secondary metabolites for comparison to conventional corn. These results confirmed that the forage and grain derived from MON 87460, and the intended foods and feeds derived from MON 87460, can be considered compositionally equivalent to those derived from conventional corn with a history of safe consumption (Section VII). As such, it can be concluded with reasonable certainty that environmental exposure to MON 87460 poses no meaningful risk to organisms in the environment including humans.

Given these attributes and the fact that MON 87460 has a mechanism of action different from previously deregulated biotechnology-derived products, it is highly unlikely that MON 87460 when combined through traditional breeding methods with insect protected and/or herbicide tolerant traits will have a cumulative impact on threatened and endangered species or non-agricultural environments.

### O.C.5.5. Potential for Cumulative Impacts on Economic and Environmental Interests

The introduction and adoption of biotechnology-derived corn products in the past decade has had a negligible impact on cropland use as evidenced by the fact that from 1996 to 2006, the total annual field corn acres fluctuated around 78 million acres (**Table IX-3**), while during the same period, the adoption rate for biotechnology-derived corn products increased from zero to 61%. Economics factors largely influence total crop acreage, as in 2007, when corn acreage increased to 93 million acres in response to increased demand which resulted in substantially higher prices for corn. However in 2008, as corn prices declined, total corn acreage also fell to 86 million acres.

Biotechnology-derived products have contributed to increased corn yields, reduced mycotoxin levels in corn grain, enhanced simplicity and flexibility of insect pest control and weed management, reduced chemical insecticide and herbicide use, increased no-till corn acreage which resulted in less soil erosion and less runoff of pesticides and water (Koziel et al., 1993; Martin and Hyde, 2001; Carpenter and Gianessi, 2001; Gianessi et al., 2002; Shelton et al., 2002; Fawcett and Towery, 2002; Hyde et al., 2003; Carpenter et al., 2004; Sankula and Blumenthal, 2004; Sankula et al., 2005; Sankula, 2006; Wu, 2006; NCGA, 2007).

In a recent study, economists Brookes and Barfoot (2008) quantified the cumulative economic and environmental impacts of biotech crops grown during the past eleven years (1996-2006). The authors report that biotech crops have resulted in substantial global economic and environmental benefits. Over the past 11 years biotechnology-derived crop adoption has positively impacted the environment by reducing greenhouse gas emissions from agriculture and reducing pesticide spraying. This technology has also contributed to higher yields for many farmers contributing to increased grower incomes. A new study issued by the National Center for Food Agricultural Policy reported an increased net return to U.S. growers of $2.6B (http://www.ncfap.org) for 2006 alone. The estimated farm income benefit to growers worldwide for all biotechnology-derived crops is $30B (Brookes and Barfoot, 2008). Additionally, there is speculation that, without wide adoption of this technology, world prices for corn and soybean could be even higher than the current prices. In 2004, the United Nations Food and Agriculture Organization noted that agricultural biotechnology is a complementary tool to traditional farming methods that can help poor farmers and consumers and improve food security (UN-FAO, 2004).

These benefits are from biotechnology crops that were designed to be tolerant of or resistant to biotic environmental stressors such as weeds (via novel herbicide tolerances)

and insects. The introduction of MON 87460 marks the first attempt to turn biotechnology towards the amelioration of abiotic environmental stressors such as drought. Globally, drought is the most important limiting abiotic factor of corn yield (Barker et al., 2005; Campos et al., 2006). Significant progress has been made to reduce corn yield loss in water-limited environments through breeding and cultural practices, but there remains potential for additional improvement. Positive impacts on yield and reduced yield loss in water-limited environments will continue to provide value to producers, consumers, and the environment.

Given the above, Monsanto fully anticipates that drought tolerant corn MON 87460, particularly when combined with other biotechnology-derived traits, will add to the positive cumulative impact of biotechnology products on the U.S. economy and the environment.

**O.D. Highly Uncertain, Unique, or Unknown Risks**

None of the effects on the human environment previously discussed are highly uncertain or involve unique or unknown risks. Any impacts would be similar to those already observed for current biotechnology-derived and conventional corn.

**O.E. Conclusions**

MON 87460 has been shown to be no different from conventional corn in its phenotypic, environmental, and compositional characteristics, with the exception of the drought tolerance trait. As to the drought tolerance trait in MON 87460, it is similar to that found in conventionally bred, drought tolerant corn hybrids currently planted in the U.S., both in terms of functional characteristics and lack of potential environmental impact. Thus, MON 87460 is expected to be similar in its agronomic characteristics and environmental impact to conventional corn.

No adverse impacts to threatened or endangered species, biodiversity, specialty and organic farming, and non-agricultural environments is envisioned since corn cannot establish and persist outside an agricultural environment and MON 87460 has been demonstrated to be phenotypically and compositionally equivalent to conventional corn. Any potential changes in agricultural practices would be consistent with the commercial production of conventional hybrids with good drought tolerance, thus these agricultural practices would not be significantly impacted by the introduction of MON 87460.

The drought tolerance trait of MON 87460 has a mechanism of action unique from other deregulated biotechnology-derived traits. Therefore, there are no anticipated cumulative impacts from combining MON 87460 with deregulated herbicide tolerant, insect protected, or nutritionally enhanced biotechnology-derived products, when viewed in the context of pre-existing agricultural practices. For the same reason, cumulative impacts on biodiversity, threatened and endangered species, specialty and organic corn production are also not anticipated. Because MON 87460 reduces yield loss under water-limited conditions, it is foreseeable that continued improvements in drought tolerance for corn, including the introduction of MON 87460, may result in the cumulative benefit of decreasing water demands for irrigation in the Great Plains. This trend is already

underway due to the continued improvements in conventionally bred drought tolerant corn, and the introduction of MON 87460 is not anticipated to have a significant impact.

MON 87460 has been thoroughly characterized and the extensive information presented in Sections I through X of this petition demonstrate that MON 87460 does not present a plant pest risk. On the basis of this analysis, the requested action of deregulation in whole does not present a significant environmental impact and should lead to a Finding of No Significant Impact.

**O.F. References**


Baker, H.G. 1965. Characteristics and modes of origin of weeds. Page 147-172 in The Genetics of Colonizing Species. Baker, H.G. and G.L. Stebbins (eds.). Academic Press, New York.

Barker, T., H. Campos, M. Cooper, D. Dolan, G. Edmeades, J. Habben, J. Schlusser, D. Wright, and C. Zinselmeier. 2005. Improving Drought Tolerance in Maize. Pages 173-253 in Plant Breeding Reviews. Vol 25, J. Janick, (ed.) John Wiley and Sons, Inc., Hoboken, New Jersey.

Brookes, G. and P. Barfoot. 2008. GM Crops: The global socio-economic and environmental impacts 1996–2006. PG Economics, Ltd., Dorchester, UK.

Campos, H., M. Cooper, G.O. Edmeades, C. Loffler, J.R. Schussler, and M. Ibanez. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. Maydica. 51:369-381.

Carpenter, J.E., and L.P. Gianessi. 2001. Agricultural biotechnology: Updated benefits estimates. In Report published by the National Center for Food and Agricultural Policy, Washington, D.C.

Carpenter, J.E., S. Sankula, C.E. Silvers, and L.P. Gianessi. 2004. Insecticidal *Bacillus thuringiensis* plants versus chemical insecticides. ACS Symposium Series 866 - Agricultural Biotechnology. Chapter 3:37-51.

Fawcett, R., and D. Towery. 2002. Conservation tillage and plant biotechnology: How new technologies can improve the environment by reducing the need to plow. Report of Conservation Technology Information Center, Purdue University, West Lafayette, IN.

Galinat, W.C. 1988. The origin of corn. Pages 1-31 in Corn and Corn Improvement. 3rd ed., Sprague, G.F. and J.W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Gianessi, L.P., C.S. Silvers, S. Sankula, and J.E. Carpenter. 2002. Plant biotechnology: current and potential impact for improving pest management in U.S. agriculture. an analysis of 40 case studies. National Center for Food & Agricultural Policy, Washington, D.C.

Hart, C. E. 2006. Feeding the ethanol boom: where will the corn come from? Iowa Agriculture Review. 12:3-5.

Hyde, J., M.A. Martin, P.V. Preckel, L.L. Buschman, C.R. Edwards, P.E. Sloderbeck, and R.A. Higgins. 2003. The value of Bt corn in southwest Kansas: A Monte Carlo simulation approach. Journal of Agricultural and Resource Economics. 28:15-33.

James, C. 2006. Global Status of Commercialized Transgenic Crops. ISAAA Brief No. 35. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, New York.

Keeler, K. 1989. Can genetically engineered crops become weeds? BioTechnology. 7:1134-1139.

Koziel, M.G., G.L. Beland, C. Bowman, N.B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Lewis, D. Maddox, K. McPherson, M.R. Meghji, E. Merlink, R. Rhodes, G.W. Warren, M. Wright, and S.V. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. BioTechnology. 11:194-200.

Mangelsdorf, P.C. 1974. Corn: its Origin, Evolution, and Improvement. Harvard University. Press. Cambridge, Massachusetts.

Martin, M.A., and J. Hyde. 2001. Economic considerations for the adoption of transgenic crops: The case of Bt corn. Journal of Nematology. 33:173-177.

NCGA. 2007. No-till Corn on the Rise, National Corn Growers Association Notes (3-26-07). http://www.ncga.com/news/notd/2007/march/032607.asp

OECD. 1998. Report of the OECD Workshop on the Toxicological and Nutritional Testing of Novel Foods. Organization for Economic Co-operation and Development. Paris, France.

Russell, W.A. and A.R. Hallauer. 1980. Corn. Pages 299-312 in Hybridization of Crop Plants. Fehr, W.R. and H.H. Hadley (eds.). American Society of Agronomy, and Crop Science Society of America, Publishers, Madison, Wisconsin.

Sankula, S. 2006. Quantification of the impacts on US agriculture of biotechnology-derived crops planted in 2005. National Center for Food and Agriculture Policy. http://www.ncfap.org/whatwedo/pdf/2005biotechimpacts-finalversion.pdf

Sankula, S., and E. Blumenthal. 2004. Impacts on US agriculture of biotechnology-derived crops planted in 2003: an update of eleven case studies. www.ncfap.org/whatwedo/biotech-us.php

Sankula, S., G. Marmon, and E. Blumenthal. 2005. Biotechnology-derived crops planted in 2004 – impacts on US agriculture. www.ncfap.org/whatwedo/biotech-us.php

Shelton, A.M., J.Z. Zhao, and R.T. Roush. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annual Review of Entomology. 47:845-81.

UN-FAO. 2004. The state of food and agriculture 2003-2004: Agricultural Biotechnology – Meeting the Needs of the Poor, Part 1. 39 pp.

USDA-ARS. 2000. National Organic Program. Final Rule. 7 CFR Part 205. 65 Federal Registration. 80548.

USDA-ERS. 2007. Organic Production. United States Department of Agriculture, Economic Research Service http://www.ers.usda.gov/Data/Organic

USDA-FSA-CRP. 2007. News Release: USDA Announces Results of Intentions to Re-enroll and Extend CRP Contracts from United States Department of Agriculture, Farm Service Agency, Conservation Reserve Program (USDA-FSA-CRP).

USDA-NAAS. 2006. USDA-NASS. 2006. National Agricultural Statistics Service, United States Department of Agriculture. Acreage Report, Cr Pr2-5 (6-06) a. Accessed June 30, 2006.

USDA-NASS. 2007. National Agricultural Statistics Service, United States Department of Agriculture. Acreage Report, Cr Pr 2-5 (6-07) Accessed June 30, 2008.

USGC. 2007. Value Enhanced Corn Report 2005/2006. United States Grain Council, Washington, D.C., http://www.grains.org/galleries/technical_publications/USGC%20Value%20Enhanced%20Corn%20Report%202006%20%20(English).pdf

Westgate, M.E., M.E. Otegui, and F.H. Andrade. 2004. Physiology of the Corn Plant. in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

Wu, F. 2006. Mycotoxin reduction in *Bt* corn: potential economic, health, and regulatory impacts. Transgenic Research. 15:277-289.

Wych, R.D. 1988. Production of hybrid seed corn. Pages 565-608 in Corn and Corn Improvement. 3rd ed., Sprague, G.F., and J. W. Dudley, (eds.). American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, WI.

MONSANTO

MONSANTO COMPANY
800 NORTH LINDBERGH BLVD.
ST. LOUIS, MISSOURI 63167
http://www.monsanto.com

June 18, 2010

David S. Reinhold
Assistant Director
Environmental Risk Analysis Programs
Biotechnology Regulatory Services
USDA, APHIS
4700 River Road, Unit 147
6B-01G
Riverdale, MD 20737

RE: Addendum to the Petition for the Determination of Non-Regulated Status for MON 87460; Petition # 09-055-01p

Dear Dr. Reinhold,

As discussed during a May 28, 2010 phone conversation with Dr. Subray Hegde, USDA/APHIS/BRS, Monsanto is submitting an addendum to the Petition for the Determination of Non-Regulated Status for MON 87460, Petition # 09-055-01p. The addendum contains additional information and data regarding water use, root development and potential land use impacts of MON 87460 and is intended to support the development of an Environmental Assessment.

Section 1 of the addendum provides a brief review of information presented in the Petition and goes on to describe the results of soil moisture depletion analyses from two years of field trials. The addendum also presents the results of a root development assay conducted under well-watered and water-limited conditions. The data presented in Section 1 indicate that MON 87460 does not exhibit altered soil moisture depletion patterns or altered root growth compared to conventional corn. The results of these studies further support data presented in Section VIII of the Petition demonstrating that MON 87460 does not differ agronomically or phenotypically from conventional corn and are consistent with the conclusion in Appendix O of the Petition that MON 87460 is expected to be similar to conventional corn with respect to its total water use and potential environmental impact.

Section 2 of the addendum augments the information presented in the Petition by focusing on land use considerations that are specific to the Western Great Plains, the area of the U.S. where MON 87460 is expected to provide the greatest benefit. As described in Section IX.D, and Appendix O of the Petition, MON 87460 is not expected to have a significant impact on land use or

organic and specialty corn grain production throughout the U.S. Information summarized in this addendum in combination with data presented in Section VIII of the Petition supports the conclusion that MON 87460 is unlikely to have a significant impact on agronomic practices, crop rotation patterns or the number of acres planted with biotechnology-derived corn varieties in the Western Great Plains.

Should you have any questions concerning this letter, or wish to set up a meeting for further discussion, please contact Dr. Russell Schneider, Monsanto Senior Director U.S. Regulatory Affairs & Policy, Washington DC, at 202-383-2866, or me at 314-694-2662.

Sincerely,

William R. Reeves, Ph.D.
Regulatory Affairs, Corn

cc: Subray Hegde/USDA/APHIS/BRS
Russell Schneider/Monsanto
Regulatory files/07-CR-191U

MONSANTO

# Addendum to the Petition for the Determination of Non-Regulated Status for MON 87460

The undersigned submits this addendum to the petition under 7 CFR Part 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

June 18, 2010

OECD Unique Identifier: MON-87460-4
Monsanto Petition Number: 07-CR-191U

**Submitted by**

William R. Reeves, Ph.D.
Regulatory Affairs Manager
Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167
Phone: (314) 694-2662
Fax: (314)-694-3080
E-mail: william.r.reeves@monsanto.com

**Prepared by**

William R. Reeves, Ph.D.

**Contributors and/or Principal Investigators**

C. Bonin, Ph.D., B. Sammons, Ph.D., L. Stork, Ph.D., J. Whitsel

## Release of Information

Monsanto is submitting the information in this addendum for review by the USDA as part of the regulatory process. By submitting this information, Monsanto does not authorize its release to any third party. In the event the USDA receives a Freedom of Information Act request, pursuant to 5 U.S.C., § 552 and 7 CFR Part 1, covering all or some of this information, Monsanto expects that, in advance of the release of the document(s), USDA will provide Monsanto with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, e.g., responsiveness, confidentiality, and/or competitive concerns. Monsanto expects that no information that has been identified as CBI (confidential business information), will be provided to any third party. Monsanto understands that a CBI-deleted copy of this information may be made available to the public in a reading room and by individual request, as part of a public comment period. Except in accordance with the foregoing, Monsanto does not authorize the release, publication or other distribution of this information (including website posting) without Monsanto's prior notice and consent.

**Addendum to USDA Petition No. 09-055-01p for Determination of Non-regulated Status for MON 87460**
**June 18, 2010**

## Section 1
## Supplementary Text on Impacts of MON 87460 on Water Relations

Section I.D of Petition 09-055-01p presents data indicating that cold shock protein B (CSPB) does not alter water relations (i.e., relative water content, leaf water potential, leaf osmotic potential) compared to conventional corn. Subsequent studies of soil moisture depletion and root biomass confirm the water relations data and demonstrate that MON 87460 is not significantly different from conventional corn in terms of soil moisture depletion over the course of the growing season or in terms of root biomass and root:shoot ratio. Taken together, these results further support the conclusion in Appendix O of Petition 09-055-01p that MON 87460 is expected to be similar to conventional corn with respect to its overall water utilization and potential environmental impact.

### 1.1. Data from Petition 09-055-01p

Table I-4 of Petition 09-055-01p (p. 49) presents relative water content data for MON 87460, other CSPB-containing events and a conventional control grown under water-limited conditions in the greenhouse. The data show that the average relative water content of MON 87460 (75%) is numerically similar that of the control (78%) and that other corn events containing the same genetic construct as MON 87460 exhibit a range of average relative water content values (75 – 82%) that bracket the value in the control plants.

Figure I-22 of Petition 09-055-01p (p. 69) presents total water potential data from three events containing the same construct as MON 87460 and a control grown under water-limited conditions in the field. The data show that the total water potential of corn transformation events containing the same genetic construct as MON 87460 is not significantly different from the control across multiple time points throughout the day.

Figure I-23 of Petition 09-055-01p (p. 70) presents osmotic potential data from three events containing the same construct as MON 87460 and control grown under water-limited conditions in the field. The data show that the osmotic potential of corn transformation events expressing the same genetic construct as MON 87460 is not significantly different from the control across multiple time points throughout the day.

Collectively, these data presented in Petition 09-055-01p support a conclusion that expression of CSPB in corn does not alter plant water relations after the imposition of water-limited conditions.

### 1.2 Soil Moisture Depletion Analysis

Subsequent to the submission of Petition 09-055-01p, data on the potential for MON 87460 to deplete soil moisture were assembled and analyzed. The two years of data collected on plants grown under well-watered and water-limited field conditions demonstrate that MON 87460 does not deplete soil moisture differently from conventional corn. This observation is consistent with the conclusion in Appendix O of Petition 09-055-01p (Petitioner's Environmental Assessment) that MON 87460 is expected to be similar to conventional corn with respect to its agronomic

characteristics and environmental impact. Additionally, these data indicate that the yield benefit observed in MON 87460 plants under water-limited conditions is not accompanied by a withdrawal of more water from the soil than occurs with conventional corn. Likewise, the data also demonstrate that MON 87460 is unlikely to withdraw more water from the soil than conventional corn under conditions where soil moisture is sufficient to produce adequate yields.

Data for this analysis were collected from field trials conducted in California during 2007 and 2008. The 2007 data are from the same field trial in which the yield data presented in Table I-3 (p. 48 of Petition 09-055-01p) were generated. Under well-watered conditions, yields for MON 87460 and the control were not significantly different (297 bushels/acre and 295 bushels/acre, respectively). Under water-limited conditions, MON 87460 exhibited a 9.6% yield advantage over the control (206 bushels/acre vs. 188 bushels/acre) that was statistically significant ($p<0.05$). Similarly in 2008, yields for MON 87460 and the control were not significantly different under well-watered conditions (242 bushels/acre vs. 243 bushels/acre, respectively). Under water-limited conditions in 2008, MON 87460 exhibited a 7.6% yield advantage over the control (156 bushels/acre vs. 145 bushels/acre) that was statistically significant ($p<0.05$).

*Materials and methods*

In the 2007 study, ten replicates each of MON 87460 and the control were grown under water-limited conditions from the V7 to R2 growth stages. Five replicates each of MON 87460 and the control were grown under well-watered conditions. Each replicate consisted of four six-row plots with 20.5 foot rows planted with 50 kernels per row. Border rows were grown between treatments to ensure water did not migrate from the well-watered to the water-limited plots. The plots in each irrigation treatment was planted using a separate randomized complete block design with test and control positioned adjacent one another (paired) to minimize variability.

In the 2008 study, twenty replicates each of MON 87460 and the control were grown under water-limited conditions from the V10 to V14 growth stages. The same design was used for the well-watered treatment. Each replicate consisted of four eight-row plots with 20.5 foot rows planted with 50 kernels per row. Border rows were grown between treatments to ensure water did not migrate from the well-watered to the water-limited plots. Each irrigation treatment was planted using a separate randomized complete block design.

In both 2007 and 2008, capacitance soil moisture probes (SenTek, EnviroSmart$^{TM}$) were utilized to characterize the depletion of moisture from the soil profile during the growing season. The probes have five sensors at soil depths of 20, 30, 50, 70 and 100 cm that monitor the available soil moisture within a 10 cm radius from each sensor. Within the water-limited block the probes were installed within the plots from ten replicates of MON87460 and ten replicates of the control. MON 87460 and the control were matched within each of the irrigation runs. Within the well-watered block, probes were installed within five replicates each for MON 87460 and control.

Prior to installation of the soil moisture probes, each sensor was normalized against air and water to set relative readings for moisture content. Probes were installed within the plant rows – in between two adjacent corn plants at the middle of the plot. At installation, each probe was outfitted with radio telemetry devices that allow data to be transmitted wirelessly. As the moisture in the soil is depleted, the electrical output signals from the sensors decline in

proportion. The data are generated in scale frequency units and converted to mm soil moisture using a soil type-specific calibration equation obtained from the manufacturer. Soil moisture depletion was calculated by subtracting later readings from earlier readings.

Cumulative soil moisture depletion calculations were made by adding the daily soil moisture depletion values over the course of the growing season. Statistical analyses were made between MON 87460 and the control across the growing season using analysis of variance. The paired design assumes equal variance at each pair (replicate) and standard error will therefore be equal across entries (test and control). The standard errors are calculated across all of the replicates within each of the two irrigation treatments, leading to a single standard error value for both the test and control.

*Results*

Table 1-1 summarizes the cumulative soil moisture depletion across the entire 2007 growing season under well-watered and water-limited conditions. The data demonstrate that soil moisture depletion for MON 87460 and the control was not significantly different under well watered (319.81 mm vs. 377.28 mm, respectively) or water-limited conditions (272.29 mm vs. 284.19 mm, respectively).

Table 1-2 summarizes the cumulative soil moisture depletion across the entire 2008 growing season under well-watered and water-limited conditions. The data demonstrate that soil moisture depletion for MON 87460 and the control was not significantly different under well-watered (193.84 mm vs. 178.15 mm, respectively) or water-limited conditions (182.87 mm vs. 195.52 mm, respectively).

Collectively, these data presented show that MON 87460 does not deplete soil moisture differently from conventional corn when grown under well-watered or water-limited conditions. They further demonstrate that the yield advantage of MON 87460 observed under water-limited conditions is not accompanied by a withdrawal of more water from the soil than occurs with conventional corn.

### 1.3 Root Biomass Comparisons

Under both well-watered and water-limited conditions, key measures of root biomass do not differ between MON 87460 and conventional corn. The results of this study further support data presented in Section VIII of Petition 09-055-01p demonstrating that MON 87460 does not differ phenotypically from conventional corn. This lack of differences also supports the conclusion that MON 87460 is unlikely to significantly alter agronomic practices including water use. Comparisons of root biomass provide an understanding of whether roots from MON 87460 are likely to have extended deeper into the soil profile beyond the range of the soil moisture probes used in the field studies described above.

Direct measures of root biomass collected under field conditions are hampered by difficulties in separating intact roots from the soil matrix. To reduce these difficulties, MON 87460 and the conventional control were established in pots in a greenhouse and grown under well-watered or water-limited conditions. Measurements were taken of growth stage, plant height, root and shoot biomass and water consumption. Water-limited conditions were imposed from the V7 to VT/R1 growth stages.

*Materials and methods*

The test substance, MON 87460, and a conventional isogenic control were established in 3-foot tall, 8-inch diameter pots filled with Turface Field and Fairway™, an inert, clay-based medium which provided good capillary action to distribute water evenly throughout the pots and washed off roots easily. The study was planted in a greenhouse in a complete factorial treatment structure (2×2) in a randomized complete block design with 12 replications. The factors were irrigation treatment (well-watered or water-limited) and plant substance type (MON 87460 test or control).

Irrigation treatments were managed according to volumetric water content (VWC). The well-watered treatment was maintained at 0.56 VWC from planting through the duration of the experiment. The water-limited treatment maintained 0.56 VWC from planting through approximately the V7 growth stage, at which point the treatment was maintained at 0.3 – 0.4 VWC for the remainder of the experiment. Plant fertility was maintained through the use of Hoagland's nutrient solution for every irrigation ranging from 0.5X concentration for well-watered plants up to 2X for water-limited plants. Plants were harvested when plants in the well-watered treatment reached the VT – R1 growth stage, 25 days after imposition of drought stress on the water-limited treatment.

Plants were evaluated within each treatment for plant height and growth stage three times during the experiment: prior to treatment, 13 days after treatment (DAT) when plants in the water-limited treatment exhibited leaf rolling (an indicator of drought stress), and at the conclusion of the experiment 25 DAT. Growth stage was determined using methods described by Iowa State University (2008). Fresh and dry weights of shoots and roots were measured at the conclusion of the experiment, root:shoot ratio, and total water used were calculated. Growth stage data were categorical and were not statistically analyzed.

*Results*

Table 1-3 presents the comparisons between MON 87460 and the conventional control. No statistically significant differences were detected between MON 87460 and the control for any endpoint, including root weight and ratios of root:shoot biomass. Root:shoot ratio was calculated to assess the partitioning of biomass into above- and below-ground biomass in response to drought stress. MON 87460 did not differ from the control in its partitioning of biomass into roots and shoots under well-watered or water-limited conditions. In addition, MON 87460 was not significantly different from the control in the total amount of water consumed under well-watered or water-limited conditions. The results of these measurements used to assess root biomass and development and water use support a conclusion that MON 87460 does not differ from conventional corn in total water use, root biomass, or the proportion of total biomass partitioned into the roots under both well-watered and water-limited conditions.

## 1.4 Summary

The data presented in this addendum to Petition 09-055-01p indicate that MON 87460 does not exhibit altered soil moisture depletion patterns or altered root biomass compared to conventional corn. Across two years of field studies, MON 87460 depleted soil moisture to a similar extent as

™ Turface Field and Fairway is a registered trademark of Profile Products LLC.

the conventional control under both well-watered and water-limited conditions. Within the water-limited treatments, MON 87460 exhibited statistically significant yield advantages in 2007 and 2008 that exceeded the 6% target increase described in Section I of Petition 09-055-01p. Additionally, key measures of root biomass were not significantly different between MON 87460 and conventional corn. The results of these studies further support data presented in Section VIII of Petition 09-055-01p demonstrating that MON 87460 does not differ agronomically or phenotypically from conventional corn and are consistent with the conclusion in Appendix O of Petition 09-055-01p that MON 87460 is expected to be similar to conventional corn with respect to its water use characteristics and potential environmental impact.

**References**

Iowa State University. 2008. How a Corn Plant Develops, Special Report No. 48. Ames, IA.

**Table 1-1. Full Season Cumulative Soil Moisture Depletion from MON 87460 and Control Plots in a 2007 California Field Trial**

| Treatment | Entry | Cumulative Soil Moisture Depletion (mm water ± standard error) | p-value |
|---|---|---|---|
| Well-watered | MON 87460 | 319.81 ± 61.75 | 0.546 |
| | Control | 377.28 ± 61.75 | |
| Water-limited | MON 87460 | 272.29 ± 12.71 | 0.525 |
| | Control | 284.19 ± 12.71 | |

Note: No statistically significant differences (α=0.05) were detected within treatment between MON 87460 and the control using analysis of variance.

**Table 1-2. Full Season Cumulative Soil Moisture Depletion from MON 87460 and Control Plots in a 2008 California Field Trial**

| Treatment | Entry | Cumulative Soil Moisture Depletion (mm water ± standard error) | p-value |
|---|---|---|---|
| Well-watered | MON 87460 | 193.84 ± 21.94 | 0.640 |
| | Control | 178.15 ± 21.94 | |
| Water-limited | MON 87460 | 182.87 ± 9.02 | 0.347 |
| | Control | 195.52 ± 9.02 | |

Note: No statistically significant differences (α=0.05) were detected within treatment between MON 87460 and the control using analysis of variance.

**Table 1-3. Plant Growth Parameters of MON 87460 and the Control Prior to Irrigation Treatment and at 13 and 25 Days After Treatment (DAT)**

| | | Well-Watered | | Water-Limited | |
|---|---|---|---|---|---|
| | **Characteristic** | **MON 87460** | **Control** | **MON 87460** | **Control** |
| Prior to treatment | Height (cm) | 104.8 | 103.3 | 105.3 | 105.8 |
| | Growth stage[1] | V7-V8 | V6-V8 | V7 | V7-V8 |
| Post-treatment (13 DAT) | Height (cm) | 166.2 | 166.4 | 133.2 | 134.8 |
| | Growth stage | V12-VT | V12-VT | V10-V12 | V9-V12 |
| Post-treatment (25 DAT) | Height (cm) | 198.1 | 197.6 | 178.9 | 178.8 |
| | Growth stage | VT-R2 | VT-R2 | VT | VT-R1 |
| | Shoot fresh weight (g) | 557.1 | 537.5 | 279.1 | 263.2 |
| | Root fresh weight (g) | 405.4 | 411.9 | 241.1 | 234.6 |
| | Shoot dry weight (g) | 104.5 | 102.3 | 58.1 | 55.8 |
| | Root dry weight (g) | 38.2 | 37.4 | 25.9 | 25.8 |
| | Root:shoot ratio[2] | 0.36 | 0.37 | 0.45 | 0.47 |
| | Total water consumed[3] (L) | 35.0 | 34.0 | 19.6 | 19.1 |

Note: No statistically significant differences ($\alpha$=0.05) were detected within treatment between MON 87460 and the control using analysis of variance.

[1] Growth stage was determined using methods described by Iowa State University (2008).

[2] Root: shoot ratio was calculated by root biomass divided by shoot biomass.

[3] Total water consumed was calculated by subtracting the water remaining in the pot at the conclusion of the experiment from the total amount of water applied throughout the experiment.

**Section 2**
**Supplementary Text on Impacts of MON 87460 on Land Use**

As described in Section IX.D (pages 240-261), and Appendix O (Sections O.C.5.2 and O.C.5.3, pages 535-539) of Petition 09-055-01p, MON 87460 is not expected to have a significant impact on land use throughout the U.S. Information summarized in this addendum further supports this conclusion specifically for the Western Great Plains, the region where MON 87460 is expected to provide the greatest benefit. The statements in Section IX.D and Appendix O of the Petition are based on data presented in Section VIII of the Petition demonstrating that MON 87460 does not differ agronomically or phenotypically from conventional corn and is no different from conventional corn in its plant pest potential. This lack of differences combined with the information provided here also supports the conclusion that MON 87460 is unlikely to significantly alter agronomic practices, crop rotation patterns and their impacts, or the number of acres planted with biotechnology-derived corn varieties in the Western Great Plains.

## 2.1 Agronomic Practices

Agronomic practices are an important determinant of crop productivity and profitability. As described below, agronomic practices such as hybrid selection, reduced tillage and altered planting strategies have commonly been employed to optimize corn yields in areas where water use efficiency is a primary concern. MON 87460 is expected to be incorporated into these practices similarly to conventionally bred drought tolerant varieties and is expected to comprise one part of a risk management strategy that emphasizes conventional breeding and agronomics in addition to biotechnology. For this reason, MON 87460 is not expected to have any significant impact on individual growers' choices to implement their preferred agronomic practices.

Drought tolerance imparted through conventional breeding allows corn production to occur throughout areas of the Western Great Plains that typically experience dry conditions during portions of the growing season. Approximately 25% of the hybrids offered by DEKALB are rated as having excellent drought tolerance and are recommended for fields that regularly experience drought stress; an additional 50-65% of the hybrids offered for planting in these areas were rated as having very good performance in fields that regularly experience some drought stress (Monsanto Company, 2010). Other seed companies also breed corn for drought tolerance and provide drought tolerance ratings for their hybrids (DAS, 2010; PHII, 2010).

Reduced tillage and no-till practices are well-known for improving water management in dryland crop production and improving yield potential (Croissant et al., 2008). Research conducted by the University of Nebraska demonstrated that plots managed under no-till conditions over the long term had better soil structure, better rainfall infiltration rates and less runoff compared to conventionally tilled plots (Pryor, 2006). Accordingly, yields in the no-till plots were greater than those in conventionally tilled plots. The combination of reduced labor and increased yield also produced a corresponding increase in projected profits per unit area. MON 87460 is not expected to have a significant impact on the number of acres being managed using reduced till or no-till practices because such practices are expected to complement the drought tolerance trait.

Plant density also impacts yield potential with optimum planting densities in dryland cropping systems determined by the amount of available soil moisture at planting. Recommended planting densities for dryland corn in Nebraska, for example, range from 8,000 to 16,000 plants per acre. By altering plant density to reflect the amount of available soil moisture at planting, it is possible to maximize profits per unit area while also mitigating the risk of crop failure (Klein and Lyon, 2003). As MON 87460 provides a yield benefit compared to conventional corn when water is limiting during later growth

stages, its introduction is not expected to have a significant impact on plant density decisions based on soil moisture at planting.

Skip row planting is a specific method for altering plant density and improving corn drought tolerance. As its name implies, skip row planting involves skipping one or two rows of corn for every one or two rows planted. The underlying idea is to prevent developing plants from exhausting soil moisture early in the season leaving a reserve of soil moisture to support reproductive development (Klein, 2006). Accordingly, no-till management is recommended for growers employing skip row practices. Research on skip row planting indicates that the greatest benefit occurs when yields obtainable through standard planting methods are less than 100 bushels/acre (Lyon et al., 2009). For example, in dry years when standard planting methods produced 16-21 bushels/acre, skip row planting produced 25-50 bushels/acre (Klein, 2006). Skip row planting, therefore, can allow corn growers to maintain profitability in locations where soil moisture could otherwise preclude a profitable crop.

## 2.2 Crop Rotation

MON 87460 is not expected to significantly alter crop rotation patterns because recent trends in crop rotations already favor corn. Farmers rotate crops and adjust planting acreages of different crops based on market demand and commodity prices. From 1996 to 2006, the total number of field corn acres in each year was approximately 78 million acres (see Table IX-1 in Petition 09-055-01p). In 2007, the number of corn acres increased significantly to 93 million acres as corn grain prices increased. This increase in total corn acreage resulted primarily from growers choosing to plant corn rather than soybean in the Corn Belt and the Great Plains (USDA NASS, 2007).

Rotation with other crops is advantageous for corn yield. Rotation benefits corn production by allowing alternate weed, insect and disease management strategies and improving soil structure (Hicks and Thomison, 2004). Croissant et al. (2008) also advise that dryland growers avoid following a particular crop with itself to reduce problems with insects, disease and volunteer plants. In the U.S. Corn Belt, soybean is a common rotational crop although sorghum is also used. In the Western Great Plains, winter wheat, sorghum and soybean may be planted in rotation with corn. Wheat-fallow rotations have been employed to store soil moisture by eliminating the water use that would normally occur if a crop was grown during the fallow season. Recent research has shown, however, that wheat-fallow rotations may actually reduce the amount of soil moisture available to winter wheat crops because fallow season management may require tillage to prevent weed growth (Croissant et al., 2008). In no-till systems, the cost of herbicides to control weeds during fallow periods can exceed profits from the subsequent winter wheat crop. According to Croissant et al. (2008), in order to maximize the return on any stored water, crops such as corn, sorghum or millet should be grown in rotation with winter wheat. Rotation choices in the Western Great Plains are therefore expected to be made primarily in the context of crop profitability, water management and the need to control weeds, diseases and pests. While MON 87460 is expected to provide increased yield under water-limited conditions, its use is not expected to change the current rotational practices of Western Great Plains growers because current crop rotation trends already favor corn and crop rotation provides benefits in terms of weed, disease and pest management.

Input recommendations for various crops span broad and overlapping ranges as a result of crop type, expected yields, soil type, rainfall patterns, existing levels of nitrogen and phosphorous, soil organic matter and rotation history. The impact of crop rotation choices in the Western Great Plains therefore will vary according to these factors. Table 2-1 summarizes recommended nitrogen and phosphorous inputs for corn, sorghum, soybean and winter wheat, four crops commonly used in Western Great Plains rotations. The table also presents transpiration ratios, the weight of water transpired to produce an equal weight of above ground dry matter. Depending on the specific conditions of a given field,

corn and sorghum may potentially require more nitrogen and phosphorous than soybean and winter wheat. Conversely, the amount of water needed to produce a pound of above-ground dry matter is lower for corn and sorghum than for soybean and winter wheat. Overall, the data in Table 2-1 illustrate the wide range of inputs that may be required in the Western Great Plains to produce a profitable crop. MON 87460 is not expected to have a significant impact on the impact of crop rotation decisions because MON 87460 is not expected to change current rotation patterns and input requirements vary by crop and by location.

## 2.3 Adoption of Biotechnology-derived Corn Varieties

Appendix O of Petition 09-055-01p examines data for the U.S. as a whole and concludes that MON 87460 is not likely to alter the total number of acres planted with biotechnology-derived corn varieties or impact the ability of organic and specialty corn growers to maintain their current practices. Appendix O also notes that states with the greatest number of acres planted with organic corn also have the greatest number of acres planted with biotechnology-derived corn. Additional information presented here, specific to the Western Great Plains states, demonstrates that these states already have a significant percentage of their corn acres planted with biotechnology-derived crops and the conclusions for the U.S. presented in the Petition also apply to the Western Great Plains specifically. Table 2-2 presents the percent of total corn acres in states comprising Western Great Plains planted with biotechnology-derived varieties in 2009 (USDA ERS, 2009). For the Western Great Plains states where state-specific data are available, the percentage of acres planted with biotechnology-derived corn varieties is near or above the national average (85%). For all other states where state-specific data are not available, 78% of corn acres were planted with biotechnology-derived varieties. The percent of total U.S. corn acres planted with biotechnology-derived varieties has been increasing since the beginning of the last decade when 25% of corn acres were planted with biotechnology-derived varieties. MON 87460 is expected to be cultivated as a combined-trait product with herbicide tolerance and insect resistance traits and is therefore the acres planted with MON 87460 are expected to be the same acres already planted with biotechnology-derived corn varieties.

Table 2-2 also presents the number of acres in each Western Great Plains state planted with organic corn for grain or seed. As observed for the U.S. as a whole, the states in the Western Great Plains that plant the greatest number of organic corn acres also have the highest percentages of their total corn acres planted with biotechnology-derived varieties. For example, 143,074 acres of organic corn for grain or seed were planted in the U.S. in 2008 and the Western Great Plains state with the greatest number of organic corn acres was Nebraska with 8,991 acres (USDA NASS, 2010). Ninety-one percent of the total corn acres Nebraska are planted with biotechnology-derived varieties (USDA ERS, 2009) indicating that the cultivation of biotechnology-derived corn varieties is not necessarily incompatible with the cultivation of organic corn.

## 2.4 Summary

This document augments the information presented in Petition 09-055-01p by focusing on land use considerations that are specific to the Western Great Plains, the area of the U.S. where MON 87460 is expected to provide the greatest benefit. As described in Section IX of the Petition, MON 87460 is unlikely to significantly alter agronomic practices, crop rotation patterns or the number of acres planted with biotechnology-derived corn varieties. Growers in the Western Great Plains have selected conventionally-bred drought tolerant hybrids, minimized tillage and altered planting density for many years to manage soil moisture and maximize yields under conditions that would otherwise be unfavorable for corn production. MON 87460 is expected to be incorporated into these practices similarly to conventionally bred drought tolerant varieties. Additionally, given the importance of

economic factors and local conditions in determining crop choices, MON 87460 is not expected to have a significant impact on crop rotation patterns or their impacts. Crop rotation provides significant benefits and decisions about which crops to plant in a given rotation are made in the context of crop profitability, water management and the need to control pests rather than the availability of a single trait. Finally, it is unlikely that MON 87460 will significantly alter the number of acres planted with biotechnology-derived corn varieties in the Western Great Plains, given the current significant percentage of acres in the Western Great Plains already planted with corn containing biotechnology-derived traits. As a result, MON 87460 is not expected to have a significant impact on land use within the Western Great Plains specifically or the U.S. as a whole.

## References

Croissant, R.L., G.A. Peterson, and D.G. Westfall. 2008. Dryland cropping systems. Publication 0.544. Colorado State University Extension. www.ext.colostate.edu/pubs/crops/00516.html. Accessed May 7, 2010.

DAS. 2010. Dow AgroSciences LLC. Mycogen Grain Corn Maturity 111-112. http://www.dowagro.com/mycogen/grain/111-112.htm. Accessed June 10, 2010.

Davis, J.G. and D.G. Westfall, 2009. Fertilizing winter wheat. Publication 0.544. Colorado State University Extension. www.ext.colostate.edu/pubs/crops/00544.html. Accessed May 7, 2010.

Ferguson, R.B., C.A. Shapiro, A.R. Dobermann, C.S. Wortmann. 2006. Fertilizer recommendations for soybean. Publication G859. University of Nebraska – Lincoln Extension, Institute of Agriculture and Natural Resources. http://www.ianrpubs.unl.edu/sendIt/g859.pdf. Accessed May 7, 2010.

Hicks, D.R., and P.R. Thomison. 2004. Corn Management. in Corn: Origin, History, Technology, and Production, C.W. Smith, J. Betran and E.C.A. Runge, (eds.) John Wiley and Sons, Hoboken, New Jersey.

Klein, R.N. 2006. Skip row corn for improved drought tolerance in rain fed corn. Publication EC196-12. University of Nebraska – Lincoln Extension, Institute of Agriculture and Natural Resources. http://www.ianrpubs.unl.edu/sendIt/ec196.pdf. Accessed May 7, 2010.

Klein R.N. and D.J. Lyon. 2003. Recommended seeding rates and hybrid selection for rainfed (dryland) corn in Nebraska. Publication G03-1528-A. University of Nebraska – Lincoln Extension, Institute of Agriculture and Natural Resources.

Lyon, D. J., Pavlista, A. D., Hergert, G. W., Klein, R. N., Shapiro C. A., Knezevic, S., Mason, S. C., Nelson, L. A., Baltensperger, D. D., Elmore, R. W., Vigil, M. F., Schlegel, A.J., Olson, B. L., and Aiken, R. M. 2009. Skip-row planting patterns stabilize corn grain yields in the central Great Plains. Crop Management. http://www.agronext.iastate.edu/corn/contact/roger_elmore/docs/skip.pdf. Accessed May 25, 2009.

Monsanto Company. 2010. Asgrow and DEKALB seed resource guide. http://www.asgrowanddekalb.com/seedresourceguide/search/seeds. Accessed May 7, 2010.

PHII. 2010. Pioneer Hi-Bred International. Characterizing Pioneer® brand Hybrids for Drought Tolerance. http://www.pioneer.com/web/site/portal/menuitem.b98005ee4490c1a5d6c1f492d10093a0/. Accessed June 9, 2010.

Pryor, R. 2006. No-till farming in dryland cropping systems. Publication EC196-2. University of Nebraska – Lincoln Extension, Institute of Agriculture and Natural Resources. http://www.ianrpubs.unl.edu/sendIt/ec196.pdf. Accessed May 7, 2010.

Shapiro, C.A., R.B. Ferguson, G.W. Hergert, C.S. Wortman, D.T. Waters. 2008. Fertilizer suggestions for corn. Publication EC117. University of Nebraska – Lincoln Extension, Institute of Agriculture and Natural Resources. http://www.ianrpubs.unl.edu/sendIt/ec117.pdf. Accessed May 7, 2010.

USDA ERS, 2009. Adoption of genetically engineered crops in the U.S.: corn varieties. http://www.ers.usda.gov/Data/BiotechCrops/ExtentofAdoptionTable1.htm. Accessed May 7, 2010.

USDA NASS, 2007. Online databases for corn and soy at the United States Department of Agriculture, National Agricultural Statistics Service (USDA NASS). http://www.nass.usda.gov Accessed June 30, 2008.

USDA NASS, 2010. 2008 Organic Production Survey. United States Department of Agriculture, National Agricultural Statistics Service (USDA NASS). http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Organics/index.asp. Accessed June 9, 2010.

Wortmann, C.S., A.R. Dobermann, R.B. Ferguson, G.W. Hergert, C.A. Shapiro, D. Tarlakson. 2006. Fertilizer Suggestions for Grain Sorghum. Publication G1669. University of Nebraska – Lincoln Extension, Institute of Agriculture and Natural Resources. http://www.ianrpubs.unl.edu/sendIt/g1669.pdf. Accessed May 7, 2010.

**Table 2-1. Inputs for corn, sorghum, soybean and winter wheat under dryland conditions**

| Crop | Nitrogen (lb N/acre) | Phosphorous (lb $P_2O_5$/acre) | Transpiration ratio[1,2] |
|---|---|---|---|
| Corn[3] | 0 – 280 | 0 – 80 | 349 |
| Sorghum[4] | 0 – 200 | 0 – 80 | 304 |
| Soybean[5] | 0 – 100 | 0 – 65 | 646 |
| Winter wheat[6] | 0 – 75 | 0 – 40 | 528 |

[1]Pounds of water transpired/pound above-ground dry matter produced
[2]Croissant et al., 2008
[3]Shapiro et al., 2008
[4]Wortmann et al., 2006
[5]Ferguson et al., 2006
[6]Davis and Westfall, 2009

**Table 2-2. Percent of total corn acres planted with biotechnology-derived varieties and the number of acres planted with organic corn in states comprising the U.S. Western Great Plains**

| State | Percent of total acres within each state planted with biotechnology-derived varieties[1] | Acres of organic corn planted for grain or seed in each state[2] |
|---|---|---|
| Colorado | 78[3] | 1,117 |
| Kansas | 91 | 3,746 |
| Montana | 78[3] | NR |
| Nebraska | 91 | 8,991 |
| New Mexico | 78[3] | NR |
| North Dakota | 93 | 1,189 |
| Oklahoma | 78[3] | NA |
| South Dakota | 96 | 2,694 |
| Texas | 84 | 2,985 |
| Wyoming | 78[3] | 331 |

[1] Data are for 2009 and were obtained from USDA ERS, 2009.

[2] Data are for 2008 and were obtained from USDA NASS, 2010, Table 26. The total reported for the U.S. was 143,074 acres of organic corn planted for grain or seed.

[3] Values not available for these states. The value presented in the table (78%) is the aggregate percentage for all other states included in USDA's corn estimating program. The average for the U.S. as a whole is 85%.

NR – Values not reported for Montana and New Mexico in USDA NASS, 2010 to avoid disclosing data for individual farms.

NA – No data provided for Oklahoma in USDA NASS, 2010.