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BASF Plant Science, L.P.

Petition for the Determination of Nonregulated Status for Imidazolinone-tolerant Soybean BPS-CV127-9

The purpose of this petition is to request a determination that the article should not be regulated under 7 CFR Part 340

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OECD Unique Identifier BPS-CV127-9

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Release of Information

As part of the regulatory requirement in the United States, BASF Plant Science, L.P. is submitting the information in this petition for review by the USDA. The submission of this information by BASF Plant Science, L.P. does not constitute an authorization to release any information contained within it 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, BASF Plant Science, L.P. expects that USDA will provide BASF Plant Science, L.P. 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 in advance of the release of the document(s). BASF Plant Science, L.P. authorizes the posting of the petition on the USDA Biotechnology Regulatory Services website when the Plant Pest Risk Assessment and the Environmental Assessment are published in the Federal Register for public comment. BASF Plant Science, L.P. understands that a copy of this information may be made available to the public by individual request, as part of a public comment period. Except in accordance with the foregoing, BASF Plant Science, L.P. does not authorize the release, publication or other distribution of this information (including website posting) without prior notice and consent by BASF Plant Science, L.P.

Certification

The undersigned certifies that, to the best knowledge 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.

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Abbreviations and Definitions

A.D.	*Anno Domini*
ADF	Acid Detergent Fiber
AHAS	Acetohydroxyacid synthase gene
AHAS	Acetohydroxyacid synthase large subunit
ai	Active ingredient
ANOVA	Analysis of variance
At	*Arabidopsis thaliana*
AtAHAS	Acetohydroxyacid synthase from *Arabidopsis thaliana*
AtSEC61γ	SEC 61 gamma subunit protein from *Arabidopsis thaliana*
B.C.	Before Christ
bp	Base-pair
ca	Circa
cDNA	Complementary DNA
CDS	Coding sequence
CFIA	Canadian Food Inspection Agency
CIP	Calf intestinal alkaline phosphatase
csr1-2	Gene from *Arabidopsis thaliana* encoding an imidazolinone-tolerance-conferring AHAS enzyme
CTAB	Cetyl-trimethyl ammonium bromide
CTP	Chloroplast transit peptide
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FAD	Flavin adenine dinucleotide
FDA	United States Food and Drug Administration
Fn	nth filial generation
gDNA	Genomic DNA
GM	Genetically modified
GmSEC61γ	SEC 61 gamma subunit protein from *Glycine max*
ha	Hectare
HC	Health Canada
ILSI	International Life Sciences Institute
kb	Kilobase pairs
LD_{50}	Dose that is lethal to 50% of treated animals
LOD	Limit of detection
M	Molar
MAFF	Ministry of Agriculture, Forestry and Fisheries of Japan
MHLW	Ministry of Health, Labour and Welfare of Japan
MOA	Ministry of Agriculture of China
mRNA	Messenger RNA
MT	Million metric tons
N	Normal (chemical concentration equal to 1 g/L)
NDF	Neutral Detergent Fiber
nt	Nucleotide
OECD	Organisation for Economic Co-operation and Development

ORF	Open reading frame
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidone
R272K	Mutation resulting in a substitution of an arginine residue (R) with a lysine (K) at amino acid 272 relative to the amino acid sequence of the AHAS protein of *Arabidopsis thaliana*
RLM-5'-RACE	RNA ligase-mediated rapid amplification of 5' complementary DNA ends
RT-PCR	Reverse transcriptase-polymerase chain reaction
S653N	Mutation resulting in a substitution of a serine residue (S) with an asparagine (N) at amino acid 653 relative to the amino acid sequence of the AHAS protein of *Arabidopsis thaliana*
SDS	Sodium dodecyl sulfate
SSC	Sodium chloride-sodium citrate buffer
T_0	generation resulting from regenerated transformed cells
T_4	Fourth transgenic generation
TAP	Tobacco acid phosphatase
TE	Tris-EDTA buffer
Tn	nth self-pollinated generation post-transformation
Tris	Tris(hydroxymethyl)aminomethane
USDA-APHIS	United States Department of Agriculture-Animal and Plant Health Inspection Service
UTR	Untranslated region
w/v	Weight per volume

Summary

BASF Plant Science is submitting this request to USDA-APHIS for a determination of nonregulated status for imidazolinone-tolerant BPS-CV127-9 soybean and for any progeny of BPS-CV127-9 soybean that are derived by conventional breeding practices with other soybeans, including conventional varieties and genetically modified varieties that have been granted nonregulated status under 7 CFR Part 340.

Soybean (*Glycine max* L.) plants that are tolerant to the imidazolinone class of agricultural herbicides have been developed by BASF Plant Science, L.P. (BPS) and EMBRAPA (Empresa Brasileira de Pesquisa Agropecuaria, Brasil). The herbicide-tolerant soybean plants BPS-CV127-9 (also referred to as CV127 in this petition) are derived from a single transformation event and were produced by introduction of the imidazolinone-tolerant acetohydroxyacid synthase large subunit (*AHAS*) gene *csr1-2*[1] with its native promoter from *Arabidopsis thaliana* into the soybean plant genome via biolistics transformation technology. The *csr1-2* gene from *A. thaliana* encodes an acetohydroxyacid synthase large subunit (AtAHAS) enzyme that is tolerant to imidazolinone herbicides due to a point mutation that results in a single amino acid substitution in which the serine residue at position 653 is replaced by asparagine (S653N). The AtAHAS catalytic subunit encoded by the *csr1-2* gene has altered herbicide binding properties such that imidazolinone herbicides do not bind to the enzyme while retaining its normal biosynthetic function in the plant (Pang *et al*., 2002).

The *Arabidopsis* AHAS (AtAHAS) is a member of the class of AHAS proteins found ubiquitously in plants. The AHAS enzyme catalyzes the first step in the biosynthesis of the branched-chain amino acids, valine, leucine, and isoleucine. Typically, inhibition of the AHAS enzyme by imidazolinone herbicides leads to a deficiency in branched-chain amino acids and other compounds derived from this pathway that are needed for plant growth and survival, and results in plant death. The herbicide tolerance in CV127 soybean will allow growers to treat the soybean crop with imidazolinone herbicides for weed control without causing injury to the soybean plant at normal field application rates. Soybean CV127 was developed for cultivation primarily in Brazil and Argentina, and the introduction of CV127 soybean varieties will offer soybean growers an additional tool for controlling weeds, as well as an important option for weed resistance management. Imidazolinone herbicides control a wide spectrum of grass and broadleaf weeds. The growing use of glyphosate with glyphosate-tolerant soybeans in Argentina and Brazil has led to a shift in the species of prevalent weeds with those that are more tolerant to glyphosate predominating. The most common weeds in this category include Benghal dayflower (*Commelina benghalensis* L.), morning glory (*Ipomoea* spp.), Brazil pusley (*Richardia brasiliensis*), and winged false buttonweed (*Spermacoce alata*). These weeds are sensitive to imidazolinone herbicides and the product concept of an imidazolinone-tolerant soybean fits well with the commercial needs for weed control in South America. Furthermore, it is expected that

[1] It should be noted that throughout this petition, the gene that resides within the genome of CV127 soybean that encodes the imidazolinone-tolerance trait is referred to as the *csr1-2* gene which is derived from *A. thaliana*; it is recognized that the *csr1-2* gene in CV127 soybean differs from the *csr1-2* gene in *A. thaliana* by a single nucleotide change that results in an R272K amino acid replacement. By convention, the amino acid numbering of plant AHAS enzymes are cross-referenced to the corresponding amino acid numbers of the AHAS from Arabidopsis, including the residues comprising the chloroplast transit peptide.

growers planting CV127 soybeans will be able to reduce the number of herbicides used to control weeds in their soybean fields and benefit from reduced weed control costs. During the 12-year period from 1996 through 2008, biotechnology-derived herbicide-tolerant soybeans alone accounted for a 50.45 million kg decrease in the amount of active ingredient applied (Brookes and Barfoot, 2010). The reduction in herbicide use is also expected to benefit the environment.

Several *AHAS* genes encoding AHAS enzymes that are tolerant to imidazolinone herbicides have been discovered in plants as naturally occurring mutations and through the process of chemically-induced mutagenesis. The S653N mutation in the *csr1-2* gene is among the five most common single-point mutations in *AHAS* genes that result in tolerance to imidazolinone herbicides in plants (Tan *et al*., 2005). For example, imidazolinone-tolerant maize (*Zea mays* L.), rice (*Oryza sativa* L.), bread wheat (*Triticum aestivum* L.), and oilseed rape (*Brassica napus* and *B. juncea* L. Czern.), were developed through mutagenesis, selection, and conventional breeding technologies and have been commercialized under the **Clearfield®** brand name since 1992, 2003, 2002, and 1996, respectively. Therefore, there has been a long history of safe production of crops containing an imidazolinone-tolerant AHAS with the same S653N amino acid substitution as found in the AtAHAS encoded by the *csr1-2* gene present in CV127 soybeans (Tan et al., 2005). In addition, these crops have been used to produce food and feed products that have proven to be as nutritious and as safe as similar products produced from conventional crops.

The environmental as well as food and feed safety of CV127 soybeans was confirmed based on results of a series of inter-related safety assessment studies. The detailed molecular characterization of CV127 soybean confirmed that CV127 contains a single functional *csr1-2* gene cassette integrated in the soybean genome. Biochemical characterization of the imidazolinone-tolerant AtAHAS protein expressed in CV127 soybean showed that the AtAHAS protein is typical of other AHAS proteins in this diverse protein family as well as most dietary proteins with a history of safe use in food and feed products and lacks any of the characteristics associated with known allergenic or toxic proteins. Extensive phenotypic and agronomic evaluations as well as ecological interactions of CV127 show that cultivation of CV127 soybean poses no different plant pest or weediness risk and no greater potential environmental impact than cultivation of conventional soybeans. Finally, the composition and nutritional equivalence of CV127 soybean compared to conventional soybeans was demonstrated by analysis of key nutrients and antinutrients in both grain and forage, and nutritional equivalence to conventional soybeans was further confirmed in a poultry feeding study. The results of these studies show that CV127 soybeans are as safe as conventional soybeans for environmental release as well as for food and feed uses.

Molecular characterization of CV127 soybean demonstrated that the *csr1-2* gene cassette that was inserted into the soybean genome by particle bombardment transformation is present as a single, intact copy. Furthermore, it was demonstrated that there are no additional fragments of DNA derived from the backbone of the plasmid used to produce the DNA transformation fragment inserted within the genome of CV127 soybeans. In the insert, there is also a 376 base-pair (bp) duplication of a portion of the *csr1-2* coding sequence directly before the 3' integration point. This duplicated 376 bp segment creates a 501 bp open reading frame (ORF) that extends

into the 3' flanking sequence. Reverse transcription-polymerase chain reaction (RT-PCR) results showed that this 501 bp ORF is not transcribed. It was also discovered that in addition to the *csr1-2* native gene promoter, the region upstream of (i.e. 5' to) the *csr1-2* coding sequence contains the complete coding sequence of the *A. thaliana* SEC61 (AtSEC61) gamma (γ) subunit protein, which is a component of the DNA fragment used for transformation. This protein is part of a multi-subunit secretory complex that is ubiquitous in all eukaryotes. The At*SEC61*γ 5' UTR, as annotated by The Arabidopsis Information Resource, begins 18 nucleotides downstream from the 5' transgene integration site. As such, it is extremely unlikely that the insert contains the complete native promoter for the At*SEC61*γ gene. Protein expression studies demonstrated that no detectable levels of the *A. thaliana* SEC61 gamma protein are produced in CV127 soybean leaf tissue or grain.

Southern blot analysis showed that the transgene insert in CV127 is stably integrated into the soybean genome across the breeding generations studied. This conclusion was confirmed in a study of the inheritance of the imidazolinone-tolerance trait over multiple breeding generations of CV127 soybean. Results of this study demonstrated that the trait is stably inherited according to classical Mendelian genetics, and results were consistent with the presence of a single dominant imidazolinone-tolerance gene in the soybean genome.

The environmental safety of CV127 was shown to be comparable to an isoline control and other conventional soybean varieties through evaluation of phenotypic, agronomic and ecological interaction characteristics of CV127 and control comparator plants. Field trials with CV127 soybeans were conducted at seven different locations in Brazil during the 2006/2007 growing season and at six trial locations in Brazil during the 2007 growing season. In all field trials, the CV127 soybeans were compared to a near-isogenic null segregant soybean line (referred to as the isoline control) that does not contain the *csr1-2* gene cassette and to two other conventional soybean varieties. The field trial sites were representative of the different regions within Brazil where soybean is commercially cultivated and each trial location consisted of four replications of CV127, the isoline control, and two conventional soybean varieties that were organized in a randomized block design. During the growing season, important agronomic characteristics including seed germination rate, days to reach key developmental stages, plant height, disease and insect susceptibility, and grain yield were evaluated. In addition, the impact of cultivating CV127 soybeans on factors relevant to the nitrogen fixation symbiosis with *Bradyrhizobium* was assessed and compared between CV127, the isoline control and two conventional comparator soybean varieties. Furthermore, laboratory and greenhouse-based studies of seed germination as well as pollen number and germination were also conducted and comparisons of these characteristics were made between CV127 soybean, the isoline control, and the other conventional soybean varieties. The results of these comparative studies demonstrated that other than tolerance to imidazolinone herbicides, there are no biologically meaningful differences between CV127 and the isoline control and/or the other conventional soybean varieties with respect to the parameters measured as described above. Therefore, these results reinforce the conclusion that the cultivation of CV127 soybeans poses no different plant pest or weediness potential and will have no different environmental impact than the cultivation of conventional soybean varieties.

The environmental as well as food and feed safety of the AtAHAS protein expressed in CV127 soybeans was demonstrated in studies that confirmed that the AtAHAS protein is equivalent to other AHAS proteins found ubiquitously among plant species that have a history of environmental safety as well as safe use in food and feed products. Tissue samples were collected from CV127, the isoline control, and two conventional soybean varieties at different growth stages at two field locations in the Brazilian field trials during two growing seasons. In addition, leaf samples and grain samples were collected from all trial locations from young plants at the vegetative growth stage V2 and at harvest, respectively. These samples were analyzed by enzyme-linked immunosorbent assay (ELISA) to determine the quantity of AHAS enzyme in the tissues. As expected, the levels of AHAS in CV127 were higher than in the isoline control since the former contains the *csr1-2* gene that encodes the imidazolinone-tolerant AtAHAS as well as the genes encoding the endogenous soybean AHAS proteins. Generally, the level of AHAS was higher in young, actively-growing tissues than in older, mature tissues and very low or undetectable amounts were measured in grain. In all cases the levels of AHAS protein were less than 1 ppm (part per million) on a tissue dry weight basis. The results of this study demonstrate that total AHAS levels in tissues of CV127 are relatively very low.

The safety of the AtAHAS protein expressed in CV127 soybean was demonstrated in several different studies. The AtAHAS protein is structurally homologous to endogenous AHAS proteins found ubiquitously among plant species, including crop plants with a history of safe use. The AtAHAS protein was readily degraded in simulated mammalian digestive conditions, typical of most dietary proteins with a history of safe use in food and feed products. Also, the AtAHAS protein expressed in CV127 soybean does not share immunologically relevant amino acid sequence segments or structure with known allergens or have significant sequence homology to known protein toxins. Also, the AtAHAS protein showed no acute toxicity when administered to mice. Results of these studies show that the AtAHAS protein expressed in CV127 soybean tissues does not possess any attributes of known protein food allergens, is not toxic to mammals, and therefore confirm the environmental as well as food and feed safety of AtAHAS.

Composition analyses of the grain and forage demonstrated that CV127 is compositionally and nutritionally equivalent to and as safe as the isoline control as well as other conventional soybeans. Samples of grain or forage were harvested from CV127, the isoline control, and two conventional varieties from multi-location replicated field trials conducted in Brazil. Grain samples were collected from trials conducted in two separate growing seasons and analyzed for a comprehensive range of important nutrients and antinutrients of soybean. Forage samples were collected from trials in one growing season and were analyzed for proximates and fiber content. Statistical analysis of composition data from grain and forage samples demonstrated that the composition of grain and forage from CV127 soybeans is comparable to that of the isoline control and conventional soybean varieties. Furthermore, the nutritional equivalence of CV127 soybean to the isoline control and conventional soybean varieties was confirmed in a poultry feeding study. A 42-day feeding trial with broiler chickens was conducted to compare the performance of the animals fed with soybean meal from CV127 soybeans to those fed soybean meal from conventional soybean grain. Results of this study demonstrated that the performance of the animals fed a feed containing soybean meal from CV127 soybean was comparable to those fed a feed containing soybean meal from the grain of the isoline control and two conventional soybean varieties. Collectively, these results demonstrate that the grain and forage

produced by CV127 soybean are compositionally equivalent to, and as nutritious as, grain and forage produced by the isoline control and other conventional soybean varieties.

In summary, an extensive range of studies of the agronomic and environmental safety characteristics of CV127 soybeans and the safety and nutritional qualities of grain and forage produced by CV127 soybean have been conducted. In each of these studies, CV127 soybean was compared to the isoline control and in most cases to two other conventional soybean varieties. The results of these studies demonstrated that the agronomic and environmental qualities of CV127 soybeans are comparable to those of conventional soybeans that have a long history of safe cultivation and consumption. The grain produced by CV127 was also demonstrated to be as nutritious and as safe as the soybean grain produced by conventional varieties of soybeans. Collectively, these studies demonstrate that, other than tolerance to imidazolinone herbicides, CV127 soybeans possess the same agricultural and nutritional characteristics as conventional soybeans that have a long history of cultivation and safe use in food and feed products.

There are many similarities in agronomic practices used in soybean production between the U.S. and Brazil, including weed, insect and disease control practices. The Maturity Groups of Brazilian soybean cultivars are common to those appropriate for cultivation in the southern U.S. Furthermore, environmental conditions during the growing season, including average temperatures and rainfall, are comparable between U.S. and Brazilian soybean production. Details of these similarities are presented in section IX-C.4 of this petition. Therefore, in the event that CV127 soybean were to be introduced into the U.S. environment, the data generated from field studies conducted in Brazil to support the environmental as well as food and feed safety of CV127 soybeans are equally applicable to the environmental, food and feed safety assessment of CV127 in the U.S. environment.

I. Rationale for the Development of Imidazolinone-tolerant Soybean BPS-CV127-9

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) is responsible under the Plant Protection Act (7 U.S.C. § 7701-7772) for preventing the introduction or dissemination of plant pests into the United States. Under APHIS regulation 7 CFR § 340.6 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 and unrestricted introduction of the article is permitted.

BASF Plant Science, L.P. is submitting safety assessment data for imidazolinone herbicide-tolerant soybean BPS-CV127-9, hereafter referred to as CV127 soybean, and requests a determination from APHIS that CV127 soybean and all progeny derived from CV127 soybean by traditional breeding methods with nonregulated soybean varieties be considered nonregulated articles under 7 CFR 340.

B. Rationale for the Development of Imidazolinone-tolerant Soybean CV127

Soybean is the leading oilseed crop produced and consumed in the world today. The current world production of soybean greatly exceeds that of all other edible oilseed crops (Wilcox, 2004; Soy Stats, 2011). In 2011, 258.4 million metric tons (MMT) of soybeans were produced worldwide representing 58% of the world's total oilseed production (Soy Stats, 2011). Soybean has been the dominant oilseed crop produced in the world since the 1960s (Smith and Huyser, 1987) and in the past twenty years (1989 through 2009) soybean production has increased by 116 MMT (FAOSTAT, 2011). For the past 50 years, the United States (U.S.) has been the world's leading producer of soybeans and in 2010 approximately 90.6 MMT were produced in the U.S., or about 35% of the world's total production (Soy Stats, 2011). The second and third largest producers of soybean are Brazil and Argentina who produced 70.0 and 49.5 MMT of soybeans, respectively, in 2010 (Soy Stats, 2011). Soybean production in Brazil has increased dramatically in recent years with a 31 MMT increase in production between 2000 and 2010 (Soy Stats, 2008). The two largest soybean producers in Asia are the People's Republic of China (15.2 MMT in 2011) and India (9.6 MMT). Soybean yield can greatly impact the economic value of the crop, and increased yield can be achieved by recommended weed control and other crop management practices and by using soybean varieties that have enhanced yield potential.

For many years, genetically modified (GM) soybean varieties have constituted the largest percentage of genetically modified crops planted worldwide. In 2010 the area planted to GM soybeans was 73.3 million hectares or 50% of the global area planted to soybeans (James, 2010). The vast majority of this acreage was planted with herbicide-tolerant soybean varieties. Herbicide-tolerant soybeans were planted on 30.0 million of the total 31.6 million hectares planted to soybeans in the U.S. in 2010 (James, 2010). In Argentina, virtually the entire area planted to soybeans in 2010, equal to 19.5 million hectares, was planted with herbicide-tolerant

GM soybeans (James, 2010). Cultivation of herbicide-tolerant GM soybeans is also increasing rapidly in Brazil accounting for about 75% of the total area planted to soybean in 2010 (James, 2010). The rapid and widespread adoption of herbicide-tolerant soybeans by growers worldwide has been due to increased yields, the reduced cost of effective weed control, and a simplified, more flexible weed control program (Reddy, 2001; Gianessi, 2005) that is offered by the use of herbicide-tolerant crops.

The primary herbicide-tolerant soybeans grown today are tolerant to the herbicide glyphosate. Glyphosate-tolerant soybeans were first grown commercially in 1996 and the cultivation of this crop has steadily increased every year since introduction (Gianessi and Reigner, 2006). In recent years, several weed species have evolved resistance to glyphosate (Heap, 2011; Nandula *et al*., 2005). It should be noted that most of the cases of glyphosate-resistant weeds occurred in non-transgenic crops. Exposure to glyphosate started well before the introduction of glyphosate-tolerant crops and thus attributing the development of resistance to glyphosate in weed species solely to the use of glyphosate-tolerant crops is not warranted (Dill, 2005).

In addition, soybeans that are tolerant to sulfonylurea herbicides due to a mutation in the acetohydroxyacid synthase (*AHAS*) large subunit gene have been developed by conventional mutagenesis procedures (Sebastian *et al*., 1989). The STS™, or sulfonylurea-tolerant soybean, technology does not provide cross-tolerance to imidazolinone herbicides and it is currently marketed in the United States as a stacked trait together with glyphosate tolerance by NK®, among others. Use of STS™ soybeans allows effective weed control by sulfonylurea herbicides in soybean production areas where glyphosate-tolerant weeds are present.

The introduction of imidazolinone herbicide-tolerant soybeans will provide the grower with the opportunity to cultivate soybeans that are tolerant to a different herbicide with a different mode of action than glyphosate as a means to control weeds that may be tolerant to glyphosate and to help prevent the continued evolution of more weed species that are glyphosate tolerant. The rotation of herbicide-tolerant crops that are resistant to different herbicide classes in combination with the application of the corresponding herbicides is an effective strategy for managing the development of herbicide tolerance in weed populations.

Although soybeans are naturally tolerant to some imidazolinone herbicides due to an ability to metabolize specific imidazolinones (Tecle *et al*., 1993), there are certain imidazolinone compounds, for example imazapyr and imazapic, that are active ingredients in a number of imidazolinone herbicide products and are not readily metabolized in soybeans. As a result, conventional soybeans are very sensitive to imazapyr and imazapic and the product concept for CV127 soybeans includes the use of imidazolinone herbicides containing these active ingredients for effective weed control. Imidazolinone herbicide tolerance has been developed in several different crops by either conventional seed mutagenesis procedures or by natural mutation. For example, imidazolinone-tolerant bread wheat, canola, rice, lentils, and sunflower are marketed by BASF under the **Clearfield®** brand name. However, multiple attempts by BASF to introduce commercial levels of tolerance to postemergence applications of imidazolinone herbicides containing imazapic by seed mutagenesis methods were unsuccessful in soybeans (unpublished data). Therefore, BASF used plant genetic engineering technology to introduce the imidazolinone herbicide-tolerant gene *csr1-2* from *Arabidopsis thaliana* into soybeans to

develop the imidazolinone-tolerance trait. BASF is developing the imidazolinone herbicide-tolerant soybean CV127 for cultivation primarily in Brazil and Argentina. The major weeds in soybean cultivation in these countries are sensitive to the imidazolinone herbicides containing imazapyr and imazapic, making this product concept an attractive proposition for soybean cultivation in South America. Therefore, regulatory approvals for this product are sought in Brazil and Argentina for production as well as for food and feed uses, and in the U.S. and other countries for importation of grain from CV127 soybean for food, feed, and processing uses.

CV127 soybeans have been compared to an isoline control and two conventional soybean varieties with respect to phenotypic and agronomic characteristics as well as specific ecological parameters in multi-location field trials in Brazil in two separate growing seasons (Section VIII, page 109). Results of these studies show that CV127 soybeans pose no greater weediness or plant pest risk and no different potential environmental impact than conventional soybean varieties.

The introduction of CV127 soybeans in Argentina and Brazil will offer growers excellent weed control options which in turn is expected to provide enhanced soybean yield potential. Furthermore, cultivation of CV127 soybean is expected to further build on the environmental benefits realized as a result of cultivation of herbicide-tolerant soybeans. The imidazolinone herbicides possess several environmentally beneficial characteristics compared to other herbicide classes (Tan *et al*., 2005). Imidazolinone herbicides control a wide spectrum of grass and broadleaf weeds. The growing use of glyphosate with glyphosate-tolerant soybeans in Argentina and Brazil has led to a shift in the species of prevalent weeds with those that are more tolerant to glyphosate predominating. The most common weeds in this category include Benghal dayflower (*Commelina benghalensis* L.), morning glory (*Ipomoea* spp.), Brazil pusley (*Richardia brasiliensis*), and winged false buttonweed (*Spermacoce alata*). These weeds are sensitive to imidazolinone herbicides. Imidazolinone herbicides are effective at low application rates (Shaner and Singh, 1998) and possess residual activity in the soil resulting in fewer herbicide applications for effective weed control (Bovey and Senseman, 1998). For example, in Brazil, the recommended application rate and frequency that are proposed for imidazolinone herbicides used with CV127 soybean is one application per season at a rate of approximately 70 g ai/hectare. The imidazolinone herbicides are rapidly absorbed by both roots and shoots and subsequently rapidly translocated, accumulating in actively-growing tissue (Shaner and Singh, 1998); they are among the most potent herbicides on the market, with effective weed control at application rates of 32 to 125 g ai/ha (Shaner and Singh, 1998). As a reference, the application rate of other conventional herbicides ranges from 10 to 1300 g ai/ha with glyphosate commonly applied at a rate of 750 g ai/ha (Bonny, 2008).

In addition, imidazolinone herbicides have a very favorable toxicology profile. Due to the fact that imidazolinones are rapidly excreted before they can accumulate in blood or tissues (Gagne *et al.*, 1991) and since animals do not possess the AHAS enzyme that is the target for imidazolinones, imidazolinone herbicides have very low mammalian toxicity. Subchronic and chronic toxicity tests with rats showed no adverse effects at doses up to 10,000 mg/kg body weight (Gagne *et al.*, 1991). At the highest levels tested, imidazolinone herbicides have shown no toxicity to birds (LD_{50}>2,150 mg/kg), fish (LD_{50}>100 mg/liter), or honey bees (LD_{50}>100 µg/bee) (Gagne *et al*., 1991). Imidazolinone herbicides are readily degraded in the soil to non-

toxic, naturally occurring compounds by the activity of soil microbes (Basham and Lavy, 1987; Flint and Witt, 1997). Furthermore, due to the relatively mild climate during the winters in Brazil and Argentina, there is increased microbial activity in the soils that leads to a shorter half-life and lower carry-over activity from the application of imidazolinones (Ulbrich *et al.*, 2005). Due to the characteristics of the imidazolinone herbicides described above, it is anticipated that the use of imidazolinone herbicides with imidazolinone-tolerant CV127 soybeans will provide a safe and environmentally beneficial system of weed control for soybean growers.

Grower adoption of imidazolinone-tolerant CV127 soybeans is expected to have other benefits to the environment. The adoption of herbicide-tolerant soybeans has resulted in a significant reduction in the adverse environmental impacts of intensive agricultural practices (Fawcett and Towery, 2002; Brookes and Barfoot, 2008; Brookes and Barfoot, 2010; Holland, 2004). One of the greatest negative environmental impacts from agriculture comes from the practice of tilling the soil. Soil tillage is practiced to reduce weeds in crops but it leads to reductions in topsoil by promoting erosion from wind and water, loss of soil moisture, and soil compaction (Holland, 2004). The use of a broad-spectrum herbicide to control weeds without affecting the crop has facilitated the adoption of reduced- or zero-tillage practices in which seeds are planted directly into unplowed soil. A survey conducted by the American Soybean Association (ASA, 2001) demonstrated that in a five-year period in the U.S. when the use of herbicide-tolerant soybeans increased from 1.7% of the U.S. soybean acres to 74%, there was a dramatic increase in the adoption of no-tillage and reduced-tillage practices (ASA, 2001). Reduced tillage practices have resulted in improved soil structure, reduced soil erosion, and reduced risk from runoff and pollution of surface waters with sediment, nutrients, and pesticides (Fawcett and Towery, 2002; Holland, 2004). This survey also found that 53% of U.S. soybean farmers made fewer tillage passes per year through their soybean fields since the adoption of herbicide-tolerant soybeans, with an average reduction of 1.8 tillage passes across all farmers (ASA, 2001), resulting in an estimated savings of $385 million per year in reduced tillage costs (Duke and Cerdeira, 2005). In Argentina there has also been a documented increase in the use of no-tillage agriculture in soybeans concomitant with the cultivation of herbicide-tolerant soybeans. This has been shown to reduce the loss of topsoil from erosion from 10 tons of topsoil lost per hectare per year in conventional soybeans to 2.5 tons lost annually in fields cultivated in herbicide-tolerant soybean varieties using reduced tillage practices (Penna and Lema, 2003). It is anticipated that grower adoption of imidazolinone-tolerant CV127 soybeans will similarly promote the adoption of no-tillage and reduced-tillage practices by soybean growers.

The use of herbicide tolerant crops, including soybean, has also resulted in a documented reduction in the consumption of tractor fuel (Brookes and Barfoot, 2010). The amount of time spent by farmers on their tractors in fields of herbicide-tolerant crops compared to fields of conventional crops has been reduced since fewer herbicides are being applied and due to the adoption of reduced- or no-tillage practices (Bonny, 2008). During the 12-year period from 1996 through 2008, Brookes and Barfoot (2010) reported an 8.4% reduction in pesticide use due to the cultivation of biotechnology-derived crops. Biotechnology-derived herbicide-tolerant soybeans alone accounted for a 50.45 million kg decrease in the amount of active ingredient applied over this same time period (Brookes and Barfoot, 2010).

The adoption of no-till farming practices has been estimated to save 3.9 gallons of fuel per acre compared to conventional tillage (CTIC, 2002; Jasa *et al.*, 1991). The reduced fossil fuel usage due to no-till and low-till farming translates into an estimated reduction of carbon dioxide release into the environment of 88.81 kg/ha and 35.66 kg/ha, respectively, relative to conventional practices (Brookes and Barfoot, 2010). Carbon dioxide is one of the greenhouse gases that have been implicated in the global warming phenomenon. In addition, no-tillage and reduced-tillage farming utilizes less plowing and results in an increase in organic matter and carbon sequestration in the field, which also leads to less carbon dioxide release into the atmosphere (Holland, 2004). Brookes and Barfoot (2010) estimated that the increase in soil carbon sequestration in the United States due to reduced tillage soybean production prevented the atmospheric release of a maximum of 38,057 million kg of carbon dioxide over the period of 1996 through 2008. In 2008, the permanent combined reduction in carbon dioxide emissions due to fuel reduction and increased soil carbon storage was approximately 15.6 billion kg which is equivalent to removing 6.9 million cars from the road for a year (Brookes and Barfoot, 2010). Similarly, grower adoption of imidazolinone herbicide-tolerant soybeans is expected to promote reductions in fuel use and tillage with concomitant reductions in carbon dioxide emissions.

C. Submissions to Other Regulatory Agencies

Imidazolinone herbicide-tolerant CV127 soybean is within the scope of the 1992 Food and Drug Administration's (FDA) policy statement addressing the regulation of products derived from new plant varieties, including those developed through biotechnology (FDA, 1992). Therefore, BASF Plant Science, L.P. has submitted a summary of food and feed safety and nutritional assessment for CV127 soybean to the FDA. Also, an import tolerance petition and supporting residue data were submitted to the U.S. Environmental Protection Agency (EPA) on September 29, 2010 for the use of imazapyr and imazapic on imported CV127 soybeans (Federal Register volume 76, number 24; Federal Register volume 76, number 60, respectively).

Regulatory submissions for the cultivation, as well as for food and feed uses, of CV127 soybeans were made in Brazil and Argentina where cultivation of CV127 is anticipated. Regulatory approval for CV127 was obtained in Brazil in December 2009. Regulatory submissions for the importation of CV127 soybeans and processed soybean products for food and feed uses have been or will be made in countries that import significant quantities of soybeans or processed soybean products from Brazil or Argentina and that have implemented functional regulatory review processes. These include submissions to a number of governmental regulatory agencies including, but not limited to, the Ministry of Health, Labour and Welfare (MHLW) and the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan, the Canadian Food Inspection Agency (CFIA) and Health Canada (HC), the European Commission of the European Union, the Ministry of Agriculture (MOA) of China, Food Standards Australia New Zealand (FSANZ), and the Taiwanese Department of Health (DOH). Submissions have also been completed to the Rural Development Administration (RDA) of the Republic of Korea, for determination of environmental and feed safety of CV127 soybean, and to the other Korean Regulatory Authorities including the Korea Food and Drug Administration (KFDA), the National Institute of Environmental Research (NIER), the National Fisheries Research and Development Institute (NFRDI), and the Korean Center for Disease Control and Prevention (KCDC). The Bureau of Plant Industry, Republic of the Philippines, granted regulatory approval for food, feed, and

processing uses of CV127 soybeans in October 2010. The Comisión Federal para la Protección contra Riesgos Sanitarios (COFEPRIS), Mexico, granted approval for CV127 import for food and feed uses as well as processing in May 2011.

II. Production and Biology of Soybean

The biology of soybean described herein is based upon the consensus documents for *Glycine max* (L.) Merr. prepared by the Organisation for Economic Co-operation and Development (OECD, 2000), and a biology document published by the CFIA Plant Biosafety Office (CFIA, 1996), as well as recent literature on the topic.

A. Soybean as a Commercial Crop

Soybean is an important crop for the production of oil and protein. The current world production of soybean greatly exceeds that of all other edible oilseed crops (Wilcox, 2004; Soy Stats, 2011). In 2011, 258.4 million metric tons (MMT) of soybeans were produced worldwide representing 58% of the world's total oilseed production (Soy Stats, 2011). Soybean has been the dominant oilseed produced in the world since the 1960s (Smith and Huyser, 1987) and in the past twenty years soybean production has increased by 116 MMT (FAOSTAT, 2011). Soybean is grown as a commercial crop in over 35 different countries (OECD, 2001). For the past 50 years, the United States (U.S.) has been the world's leading producer of soybeans and in 2010 approximately 90.6 MMT were produced in the U.S., or about 35% of the world's total production (Soy Stats, 2011). The second and third largest producers of soybean are Brazil and Argentina who produced 70.0 and 49.5 MMT of soybeans, respectively, in 2010 (Soy Stats, 2011). The fourth and fifth largest soybean producers in the world are the People's Republic of China (15.2 MMT in 2010) and India (9.6 MMT), respectively (Soy Stats, 2011). In addition to being the world's largest producers of soybeans, the U.S., Brazil, and Argentina lead the world in the export of soybeans (43.3, 32.5, and 11.0 MMT, respectively in 2010), (Soy Stats, 2011).

Palm oil and soybean oil are the leading vegetable oils consumed in the world today with 2010 production of 48.7 and 42.1 MMT, respectively, representing 33 and 29 percent of the world's vegetable oil production (Soy Stats, 2011). Soybeans also dominate the production of protein meals for animal feed. Worldwide consumption of soybean meal in 2010 was 178.6 MMT, representing 69% of the total protein meal consumed. Due to its large animal production industry, the U.S. exported only 8.4 MMT of soybean meal in 2010, ranking third worldwide behind Argentina (29.35 MMT) and Brazil (13.9 MMT).

The major commodity products of soybean include the seeds and the processed products, soybean oil and meal. There are only limited food and animal feed uses for unprocessed soybeans due to their anti-nutrient content, including trypsin inhibitors and lectins. However, heat treatment inactivates the anti-nutrient factors and allows the use of soybeans to produce soy sprouts, roasted soybeans and the traditional soy foods, for example miso, soy milk, soy sauce, and tofu (OECD, 2001). It is estimated that soybean oil accounts for 94% of soybean ingredients in the human diet (OECD, 2001). Examples of the use of soybean oil in food products include purified oil for use in margarines, shortenings, and cooking and salad oils (USDA-ERS, 2006a). Soybean oil is the second largest source of vegetable oil worldwide (Soy Stats, 2011). In addition, fatty acids, sterols and lecithin which are generated from soybean are used in various food products. Soybean meal is the most valuable processed product of soybean, accounting for roughly 50-75% of its overall value (USDA-ERS, 2006a). Most soybean meal (97%) is used in feed rations for livestock, including poultry, swine, and dairy and beef cattle, and is also used in

pet food (OECD, 2001). The utility of soybean meal for animal feed is partly due to its high content of essential amino acids, particularly lysine, leucine, and isoleucine, which are required supplements in animal feed (OECD, 2001).

Soybeans are used to manufacture soaps, inks, paints and disinfectants (CFIA, 1996). Industrial uses of soybean have been summarized by Cahoon (2003), and the United Soybean Board (USB, 2003). Aside from human consumption, refined soybean oil is used in numerous technical applications (OECD, 2001).

B. The History of Soybean

Soybeans are native to North and Central China (Hymowitz, 1970; OECD, 2000). Historic and archeological evidence suggests that the soybean [*Glycine max* (L.) Merr.] first emerged as a domesticate during the Zhou dynasty in northeastern China (Hymowitz, 2004). Soybean domestication most likely occurred during the Shang dynasty (ca. 1500-1100 B.C.) or earlier. It is thought that by the first century A.D., the soybean had spread to central and south China and Korea. The subsequent movement of soybean germplasm within the primary gene center was probably associated with the development and consolidation of territories and the collapse of the Chinese dynasties (Ho, 1969; Hymowitz, 1970).

Due to the development of land races, the cultivation of soybeans spread to other regions in East Asia, including Japan, Indonesia, the Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal, and north India from the first century A.D. to the 15th to 16th centuries (Hymowitz, 1990). The establishment of trade routes over sea and land during this time facilitated the movement of soybeans from China and the rapid acceptance of seeds as a staple food by other cultures (Hymowitz and Newell, 1981; Hymowitz, 1990). Beginning in the late 16th century and during the 17th century, European visitors to China and Japan noted the various food products produced from soybeans (Hymowitz, 1990). Soy sauce was commonly traded between the East and Europe by the late 1600s (Hymowitz, 1990).

The soybean was first introduced into North America in 1765 by Samuel Bowen who obtained the soybean seed while he was a seaman employed by the East India Company on a trade ship sailing between China and London (Hymowitz, 1990). He enabled the planting of the first soybeans in North America on a farm near Savannah, Georgia. Nearly a century later, in 1851, soybean was first introduced into Illinois and from there it spread throughout the Midwestern U.S. (Hymowitz, 1990).

Soybeans were first introduced from the U.S. into Brazil in 1882 (Dall'Agnol, 2004). However, the germplasm that was introduced in the state of Bahia at latitude 12°S was not adapted to such low latitudes and did not perform well (Dall'Agnol, 2004). A decade later and after the development of later-maturing varieties, soybeans were planted further south in the state of Sao Paulo at 23°S and improved production was realized. In the first years of the 20th century, soybeans were grown at 30°S in Rio Grande do Sul, the most southerly state in Brazil, with climatic conditions similar to those in the southern U.S. Commercial production of soybeans in Brazil increased dramatically in the mid-1950's following a government decision that provided incentives to grow wheat. Since soybeans are a good rotational crop with wheat and use similar

farm machinery and infrastructure, this policy also led to increased cultivation of soybeans in Brazil. Soybean cultivation has expanded and is now possible in all regions of Brazil from 5°N to 33°S latitude except in the Amazon and Pantanal regions due to environmental protection restrictions and in the eastern areas due to the mountainous terrain (Guidelines for GAP, 2002). The primary centers of soybean cultivation in Brazil range from southern Brazil to the savannas (Cerrado) of central Brazil. Soybeans are typically planted from mid-October to mid-December with harvest from mid-March to late April, depending on latitude. Today Brazil is the second largest producer of soybeans behind the U.S.

C. The Taxonomy and Genetics of Soybean

Glycine max (L.) Merr. is a diploidized tetraploid (2n=40) that belongs to the family Fabaceae and is further classified taxonomically as follows:

Kingdom	Plantae -- Plants
Subkingdom	Tracheobionta -- vascular plants
Division	Magnoliophyta -- angiosperms, flowering plants
Class	Magnoliopsida -- dicots
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	Glycine Willd. -- soybean
Species	Glycine max (L.) Merr. -- soybean

The above taxonomic information for soybean was obtained from the Integrated Taxonomic Information System (http://www.itis.gov/) and soybean is assigned the taxonomic serial number 26716.

The genus *Glycine* Willd. contains two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Glycine* comprises 22 wild perennial species that are indigenous to Australia, islands in the west, central and southern Pacific Ocean, China, Russia, Japan, Indonesia, Korea, Papua New Guinea, the Philippines, and Taiwan (Hymowitz, 2004) A list of the recognized species of the genus *Glycine* Willd. is presented in Table II-1.

The cultivated soybean, *G. max* (L.) Merr. and its wild annual relatives from Asia, *G. soja* Sieb. and Zucc. are classified in the subgenus *Soja*. *Glycine soja* is an annual that grows in the wild in fields, hedgerows, roadsides, and riverbanks in many countries of East Asia (OECD, 2000). This plant has a slender build with narrow trifoliate leaves. The flowers are purple, or on rare instances white, and are found on short, slender racemes. The pods are short and tawny with hirsute pubescence and contain oval-oblong seeds (Hermann, 1962).

In addition to *G. max* and *G. soja*, the subgenus *Soja* also contains a form known as *G. gracilis*. This semi-cultivated or weedy plant is found only in northeast China and is intermediate in morphology between *G. max* and *G. soja*. *G. gracilis* is a variant of *G. max* (Hermann, 1962; Wang, 1976; Shoemaker *et al*., 1986). The three species of the subgenus *Soja* are capable of cross-pollination and the hybrid seed that is produced can germinate normally and produce plants

with fertile pollen and seed (Singh and Hymowitz, 1989). The wild, weedy relatives of *G. max, G. soja* and *G. gracilis* are indigenous to Asia and do not occur naturally in North America (CFIA, 1996) or Brazil. Therefore, there is no potential for outcrossing of the imidazolinone herbicide-tolerant trait from CV127 soybean to weedy relatives in Brazil where the crop will be cultivated nor in the U.S.

Within the tribe Phaseoleae, the genus *Glycine* is the only genus containing species that have diploid chromosome numbers of 40 and 80 and not 20 (Lackey, 1980). Based on taxonomic, cytological, and molecular systematics evidence, it has been proposed that the unique chromosome number of *Glycine* is most likely derived from an unknown progenitor species with a chromosome base number of 11. From this ancient progenitor, a putative ancestor of *Glycine* arose in Southeast Asia with 2n=20 (Kumar and Hymowitz, 1989; Singh and Hymowitz, 1999; Lee and Hymowitz, 2001; Singh *et al*., 2001). Tetraploidization (2n = 2x = 40) through auto- or allopolyploidy of this ancestor species occurred at some time to produce a species in which 2n = 40. The sequence of events in the development of *G. max* from the ancient progenitor species is proposed by Singh *et al*. (2001) to be the following: wild perennial (2n = 4x = 40; unknown or extinct) to wild annual (2n = 4x = 40; *G. soja*) to soybean (2n = 4x = 40; *G. max*). Soybean is regarded as a stable tetraploid with a diploidized genome (Gurley *et al*., 1979; Lee and Verma, 1984; Skorupska *et al*., 1989).

Table II-1. List of Species in the Genus *Glycine* Willd.
Includes 2n Chromosome Number, Genome Symbol, and Distribution (from Hymowitz, 2004).

		2n	Genome[†]	Distribution
Subgenus *Glycine*				
1.	*G. albicans* Tind. & Craven	40	Il	Australia
2.	*G. aphyonota* B. Pfeil	40	?	Australia
3.	*G. arenaria* Tind.	40	HH	Australia
4.	*G. argyrea* Tind.	40	A2A2	Australia
5.	*G. canescens* F.J. Herm.	40	AA	Australia
6.	*G. clandestina* Wendl.	40	A1A1	Australia
7.	*G. curvata* Tind.	40	C1C1	Australia
8.	*G. cyrtoloba* Tind.	40	CC	Australia
9.	*G. dolichocarpa* Tateishi & Ohashi	80	?	(Taiwan)
10.	*G. falcate* Benth.	40	FF	Australia
11.	*G. hirticaulis* Tind. & Craven	40	H1H1	Australia
		80	?	
12.	*G. lactovirens* Tind. & Craven.	40	IlIl	Australia
13.	*G. latifolia* (Benth.) Newell & Hymowitz	40	B1B1	Australia
14.	*G. latrobeana* (meissn.) Benth.	40	A3A3	Australia
15.	*G. microphylla* (Benth.) Tind.	40	BB	Australia
16.	*G. peratosa* B. Pfeil & Tind.	40	?	Australia
17.	*G. pindanica* Tind. & Craven	40	H3H2	Australia
18.	*G. pullenii* B. Pfeil, Tind. & Craven	40	?	Australia
19.	*G. rubiginosa* Tind. & B. Pfeil	40	?	Australia
20.	*G. stenophita* B. Pfeil & Tind.	40	B3B3	Australia
21.	*G. tabacina* (Labill.) Benth.	40	B2B2	Australia
		80	Complex[‡]	Australia, West Central and South Pacific Islands
22.	*G. tomentella* Hayata	38	EE	Australia
		40	DD	Australia, Papua New Guinea
		78	Complex[¶]	Australia, Papua New Guinea
		80	Complex[§]	Australia, Papua New Guinea, Indonesia, Philippines, Taiwan
Subgenus *Soja* (Moench) F.J. Herm.				
23.	*G. soja* Sieb. & Zucc.	40	GG	China, Russia, Taiwan, Japan, Korea (Wild Soybean)
24.	*G. max* (L.) Merr.	40	GG	Cultigen (Soybean)

[†] Genomically similar species carry the same letter symbols.

[‡] Allopolyploids (A and B genomes) and segmental allopolyploids (B genomes).

[¶] Allopolyploids (D and E, A and E, or any other unknown combination).

[§] Allopolyploids (A and D genomes, or any other unknown combination).

D. Morphology of Cultivated Soybean

The following section dealing with the morphology of cultivated soybean draws heavily on the 2000 OECD consensus document on the biology of *Glycine max* (L.) Merr. (soybean).

The cultivated soybean is an annual that has an erect, sparsely branched, bushy growth habit and can reach a height of 1.5 meters (OECD, 2000). The primary leaves are unifoliate, opposite and ovate, the secondary leaves are trifoliate and alternate, and compound leaves with four or more leaflets are occasionally present. The nodulated root system consists of a taproot from which emerges a lateral root system. The plants of most cultivars are covered with fine trichomes, but glabrous types also exist (OECD, 2000). The purple, pink, or white flowers are situated on short axillary racemes or reduced peduncles. The flower consists of a tubular calyx of five sepals, a corolla of five petals (one banner, two wings and two keels), one pistil and nine fused stamens with a single separate posterior stamen (OECD, 2000). The pod is straight or slightly curved, varies in length from two to seven centimeters, and consists of two halves of a single carpel which are joined by a dorsal and ventral suture (OECD, 2000). The shape of the seed, usually oval, can vary amongst cultivars from almost spherical to elongate and flattened (OECD, 2000). The pods typically produce one to three seeds.

There are three types of growth habit found amongst *G. max* (L.) Merr. soybean cultivars: determinate, semi-determinate and indeterminate (OECD, 2000). Determinate growth is characterized by the cessation of vegetative activity of the terminal bud when it becomes an inflorescence at both axillary and terminal racemes (OECD, 2000). Determinate genotypes are typically grown in Brazil and the southern United States (Maturity Groups V to X). Indeterminate genotypes continue vegetative activity throughout the flowering period and are grown primarily in central and northern regions of North America (Maturity Groups 000 to IV), [OECD, 2000]. Semi-determinate types have indeterminate stems that terminate vegetative growth abruptly after the flowering period (OECD, 2000).

E. Reproductive Biology and Hybridization with Cultivated Soybean and Related Species

Soybean is a self-pollinating species that is propagated by seed (OECD, 2000). The flowers consist of a tubular calyx of five sepals, a corolla of five petals, one pistil, and nine fused stamens with a single separate posterior stamen (OECD, 2000). One day before pollination, the stamens elongate and form a ring around the stigma that is receptive to pollen for a period from approximately 24 hours before to 48 hours after flowering (OECD, 2000). The anthers, following maturation in the bud, directly pollinate the stigma within the same flower (OECD, 2000). Pollination typically occurs on the same day that the flower opens and depending on environmental conditions, anthesis normally occurs in the late morning. Pollen typically comes in contact with the stigma during the process of anthesis. Pollen viability lasts for a short time of two to four hours and no viable pollen is present by late afternoon. Natural or artificial cross-pollination is only possible during the short time when the pollen is viable. Accordingly, soybean exhibits a very low level of cross-pollination (below one percent) and is primarily self-pollinating (Caviness, 1966; Yoshimura *et al*., 2006).

Due to the strong propensity for self-fertilization described above, the frequency of cross-pollination is very low. This is true even under conditions designed to optimize cross-fertilization including bringing synchronous flowers into close proximity to one another. Between plants in the field located in adjacent rows, outcrossing has been measured in the range of 0.03 to 3.62% (Caviness, 1966; Beard and Knowles, 1971; Yoshimura *et al*., 2006). When plants are located more than 4.5 meters from one another, natural cross-pollination in soybean is undetectable or extremely low (less than 0.02%), [Caviness, 1966]. More recent studies of soybean cross-pollination were conducted by Ray *et al*. (2003) and Yoshimura *et al*. (2006) and they report similar findings. Plants grown in close proximity to each other (15 cm) were found to have average outcrossing rates of 1.8%, while plants separated by distances of 0.9 m and 5.4 m had outcrossing rates of 0.41 and 0.03%, respectively (Ray *et al*., 2003). Soybeans are generally not a preferred plant for insect pollinators and insect activity has been found not to increase the outcrossing rate (Erickson, 1975; Erickson, 1984). The regulations governing the production of certified Foundation soybean seed are consistent with the low outcrossing rate recognized for soybean. These regulations place no restriction on the separation distance between different cultivars in the field provided that the distance is sufficient to prevent mechanical mixing during harvest.

Due to the low level of genomic similarity among species of the genus *Glycine*, *G. max* is unable to cross with other plants outside of the subgenus *Soja* and so intergeneric hybridization does not occur. Since *G. max* is the only *Glycine* species found in either Brazil or in the United States, there is no possibility for cross pollination between *G. max* and *G. soja* in these countries.

F. Weediness Potential of Cultivated Soybean

Soybean plants are not weedy and are not found outside of cultivation (OECD, 2000). Soybeans are annuals that reproduce solely from seeds. Cultivated soybean rarely displays any dormancy characteristics (TeKrony *et al*., 1987) and are sensitive to cold temperatures (Raper and Kramer, 1987); thus, their potential to survive in the U.S. from one growing season to the next is very low. Soybean seeds normally germinate quickly under the appropriate environmental conditions that include adequate moisture and moderate temperatures and thus, could potentially grow as a volunteer. However, any volunteers that grow after harvest would be destroyed by the low and freezing temperatures encountered during the following winter. In the event that volunteers were to become established, they would not compete well with succeeding crops and they could be controlled by either mechanical or chemical means (OECD, 2000).

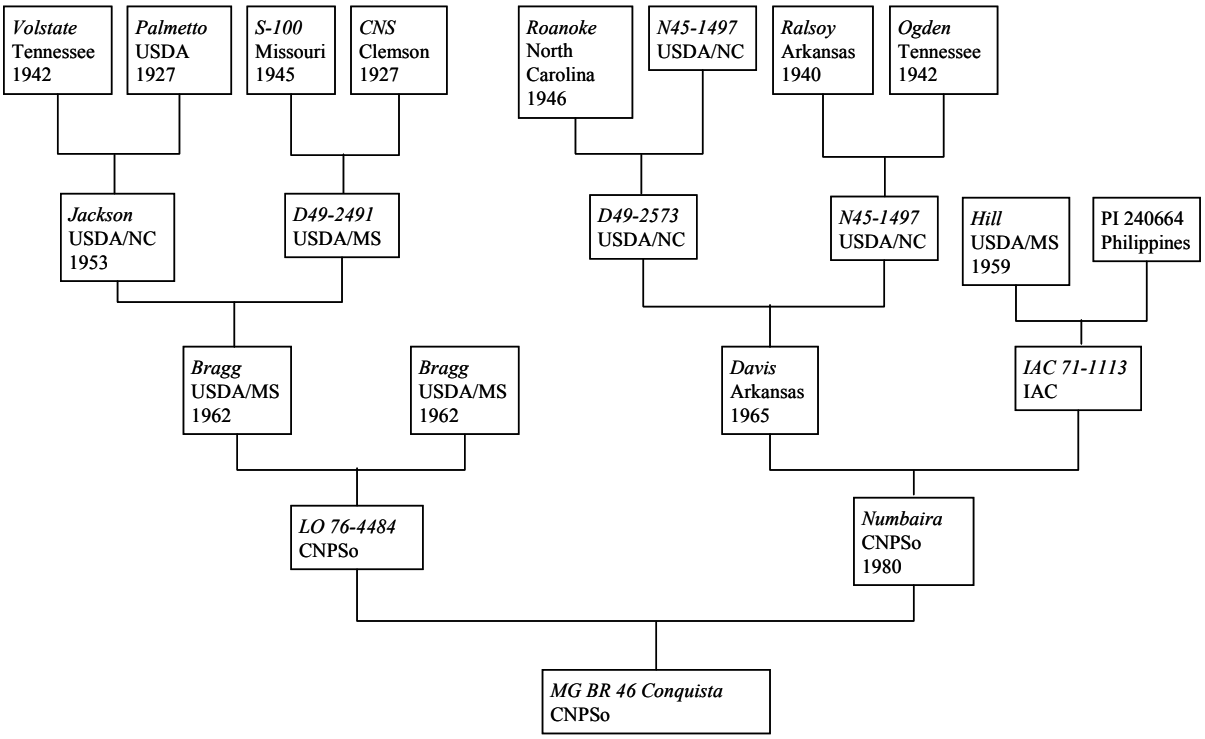
G. Characteristics of the Recipient Soybean Cultivar

The soybean variety that was used as a recipient in transformation experiments to create the imidazolinone-tolerant CV127 soybean was the commercial variety 'Conquista'. Conquista (also known as MG BR 46) is a highly productive, conventional Brazilian soybean variety in maturity group VIII. It is well adapted to cultivation in regions of less than 25 degrees latitude and was developed by Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) with release for commercial cultivation in Brazil in 1995 (Pardey *et al*., 2004). A partial pedigree of Conquista (Figure II-1) shows that a significant portion of its genetic background is derived from varieties developed in the U.S. From the 1950s to the 1970s, a substantial amount of breeding was conducted by USDA researchers using soybean varieties adapted to the southern U.S. states to develop commercially successful soybean varieties (Pardey *et al*., 2004). These varieties were also adapted to the tropical latitudes of Brazil such as the Cerrados region (Pardey *et al*., 2004) where many soybeans are cultivated today. The soybean variety Conquista was a product of this breeding effort and, as a result, it is closely related to soybean varieties that are currently cultivated in the U.S.

H. Soybean Comparators Used in this Petition

In the studies and experiments conducted to support the safety assessments of CV127 presented in this petition, appropriate control soybean varieties were used as comparators. In field and laboratory studies, near-isogenic null segregants or, in some instances, the parental control variety Conquista were used as comparator varieties in order to minimize all differences except for the presence of the *csr1-2* gene cassette in CV127 soybean which conferred imidazolinone-herbicide tolerance. For those studies that used the AtAHAS protein expressed in CV127 soybean, this protein was derived either from CV127 soybean tissues or from protein produced and purified from an *E. coli* fermentation process. In the field studies, two other conventional varieties of soybean that are also typical of commercial varieties cultivated in Brazil were also used as reference varieties to establish a range of responses typical of soybean varieties that are cultivated in Brazil.

Figure II-1. Partial Pedigree for Soybean Variety Conquista (largely derived from Pardey *et al*., 2004). Where available, information on the name of the breeding line (in italics), institution, and year of release are included.



III. Description of the Transformation and Development of CV127 Soybean

A DNA fragment of plasmid pAC321 containing the *csr1-2* gene that confers tolerance to imidazolinone herbicides was used to transform embryogenic axis tissue derived from the apical meristem of a single soybean seed of the commercial variety 'Conquista'. This variety and tissue type was chosen for insertion of the *csr1-2* gene because it responds well to particle bombardment transformation and tissue culture regeneration. In addition, Conquista is a highly productive Brazilian soybean variety with wide adaptation to different Brazilian growing environments. Biolistic transformation (also known as microprojectile or particle bombardment) (Aragão *et al*., 1996) was used to produce soybean transformation events containing the *csr1-2* gene. This DNA delivery system is well documented to transfer and integrate new DNA into a plant genome (Klein *et al.,* 1987; Sanford *et al.,* 1993; Lee *et al*., 1996). Prior to bombardment, a purified DNA fragment containing the *csr1-2* gene was precipitated onto microscopic gold particles. The precipitated DNA and particles were then placed onto a plastic macrocarrier and accelerated at high velocity. A stopping screen in the flight path of the particles retained the macrocarrier while the particles with DNA were permitted to continue their flight and eventual penetration and incorporation into the soybean plant cells. These cells were transferred to a selective media containing the equivalent of 50 g ai/ha imazapyr (i.e. 500 nM, Aragão *et al*., 2000), an imidazolinone herbicide, and only those cells transformed with the *csr1-2* gene continued to grow. From this process a tolerant T_0 generation plant was identified and named Soybean Event 127 (CV127).

The T_0 generation plants resulting from the transformation of Conquista were advanced to the fourth transgenic generation (T_4) via four cycles of self-pollination (Figure III-1). Some T_4 plants were then backcrossed to Conquista to reduce the number of copies of the transgenic insert to a single locus (Figure V-4). The resulting progeny, the first filial (F_1) generation, were advanced to the eighth filial (F_8) generation by successive rounds of self-pollination and selection. The homozygous F_8 line developed from this process was identified as CV127 line 603 (Line 603), (Figure III-1).

Line 603 was crossed to a conventional soybean breeding line which was known only by its pedigree, Conquista^3 x BRI98-641. One of the parental lines in the pedigree, BRI98-641, is a conventional soybean line with insect tolerance. BRI98-641 was never released in Brazil as a commercial variety, but it was used as a parental line in Embrapa's commercial soybean breeding program. The other parental line in the Conquista^3 x BRI98-641 pedigree, Conquista, is the commercial variety used in the original transformation to develop Line 603. As indicated by the superscript (3) in the pedigree, breeding line Conquista^3 x BRI98-641 was developed in a backcrossing program in which Conquista was used as the recurrent parent for three backcrosses to transfer the insect tolerance trait from BRI98-641 into the high-performing Conquista variety. Thus, the genetic background of breeding line Conquista^3 x BRI98-641 is approximately 94% Conquista. The cross of Line 603 with this breeding line, Conquista^3 x BRI98-641, produced a line identified as CV127 line 127 (Line 127) whose genetic background is approximately 97% Conquista (Figure III-1).

A schematic diagram depicting the development of CV127 soybeans is shown in Figure III-2.

Figure III-1. Breeding History of CV127 Soybean.
The original transformation event is designated as the T_0 generation and the fourth self-pollinated generation as T_4. Key molecular and inheritance data was collected at the points in the history as indicated in the legend. Two elite lines are shown. CV127 line 603 was the product of a cross between the T_4 generation and the conventional soybean variety Conquista. CV127 line 127 is the product of a cross between CV127 line 603 and a backcross-derived conventional soybean line known as 'Conquista^3 x BRI98-641'.

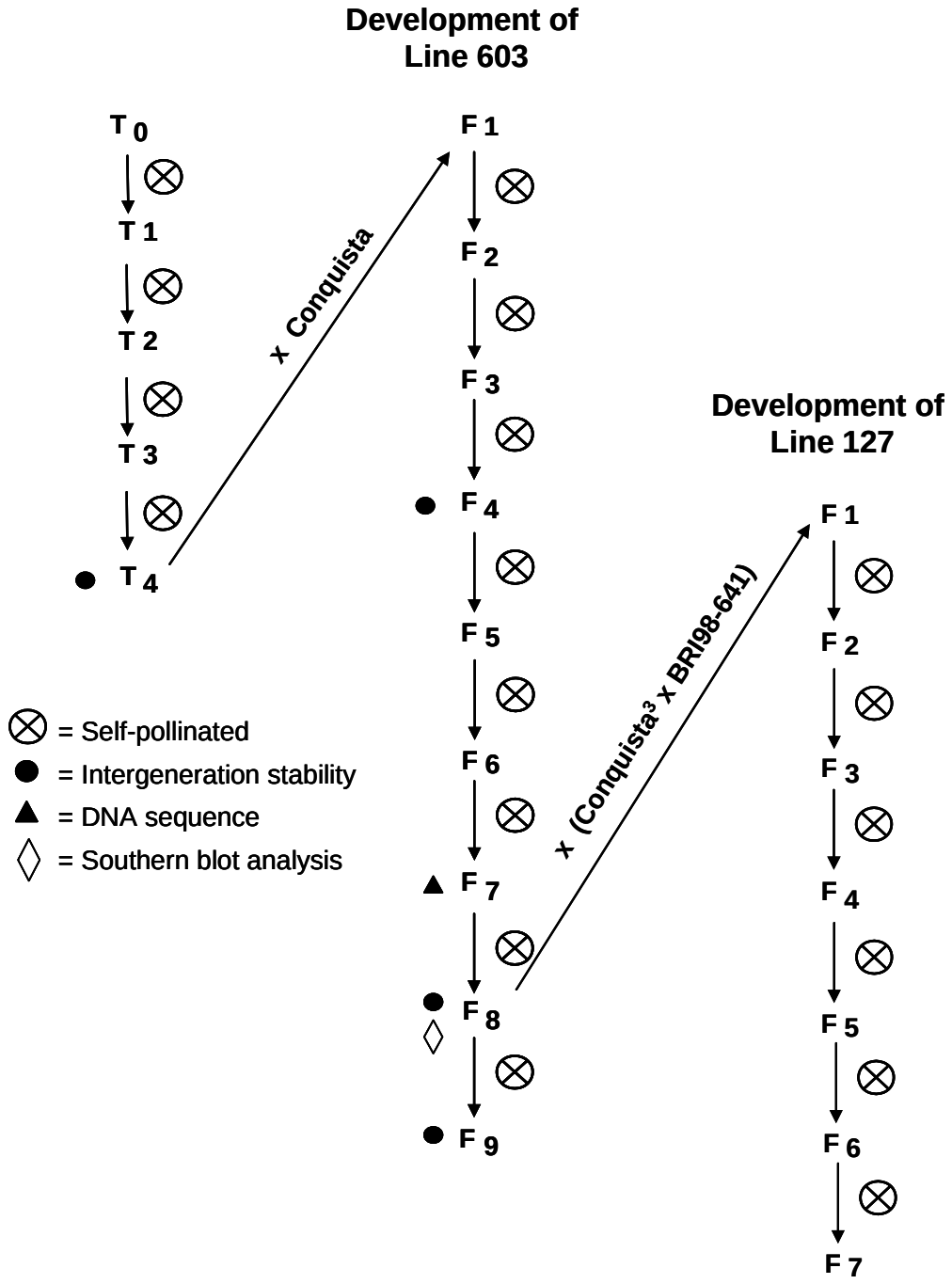
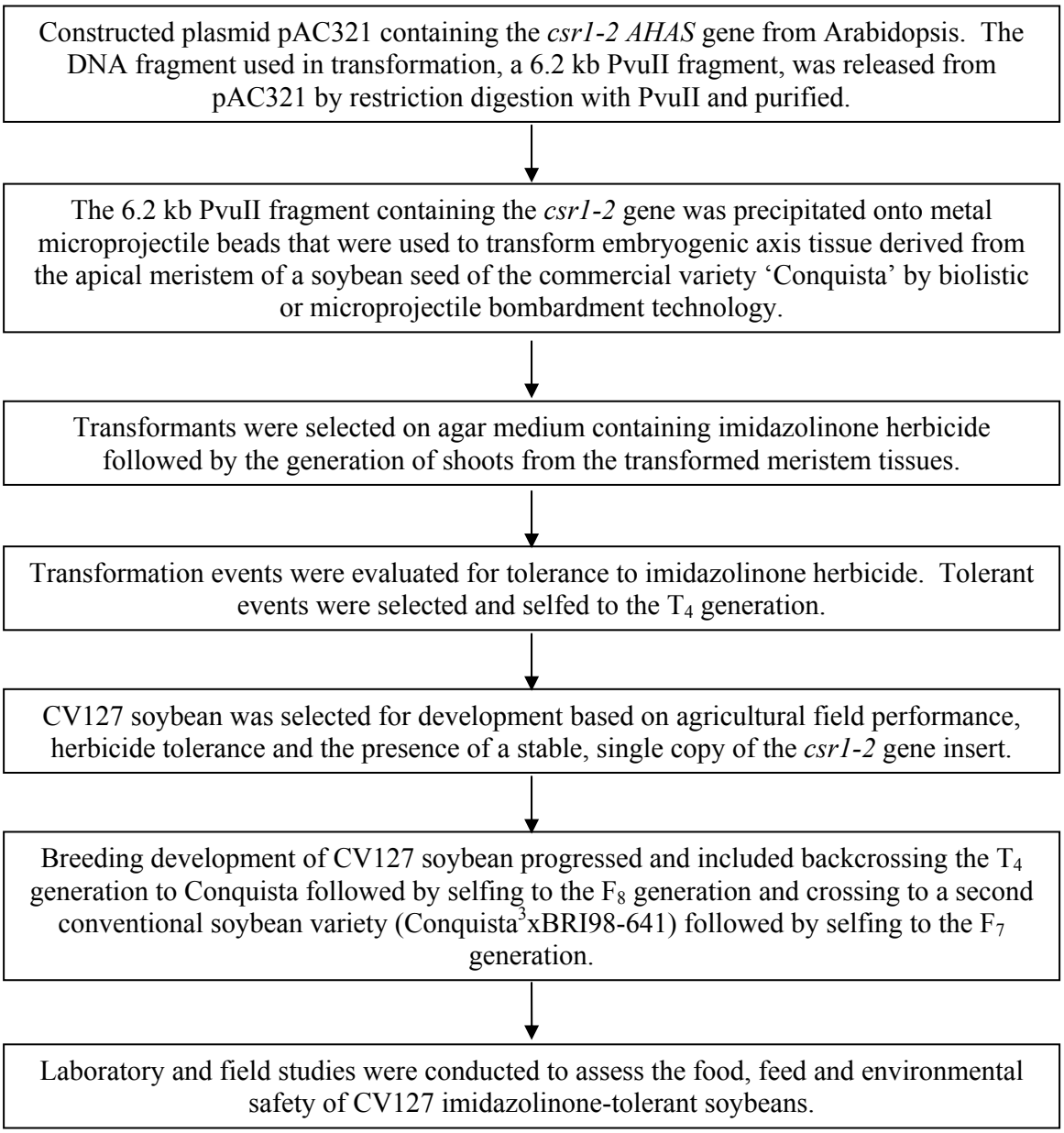


Figure III-2. Schematic Diagram Depicting the Process Followed in the Development of Imidazolinone-Tolerant CV127 Soybeans.



IV. Donor Genes and Regulatory Sequences

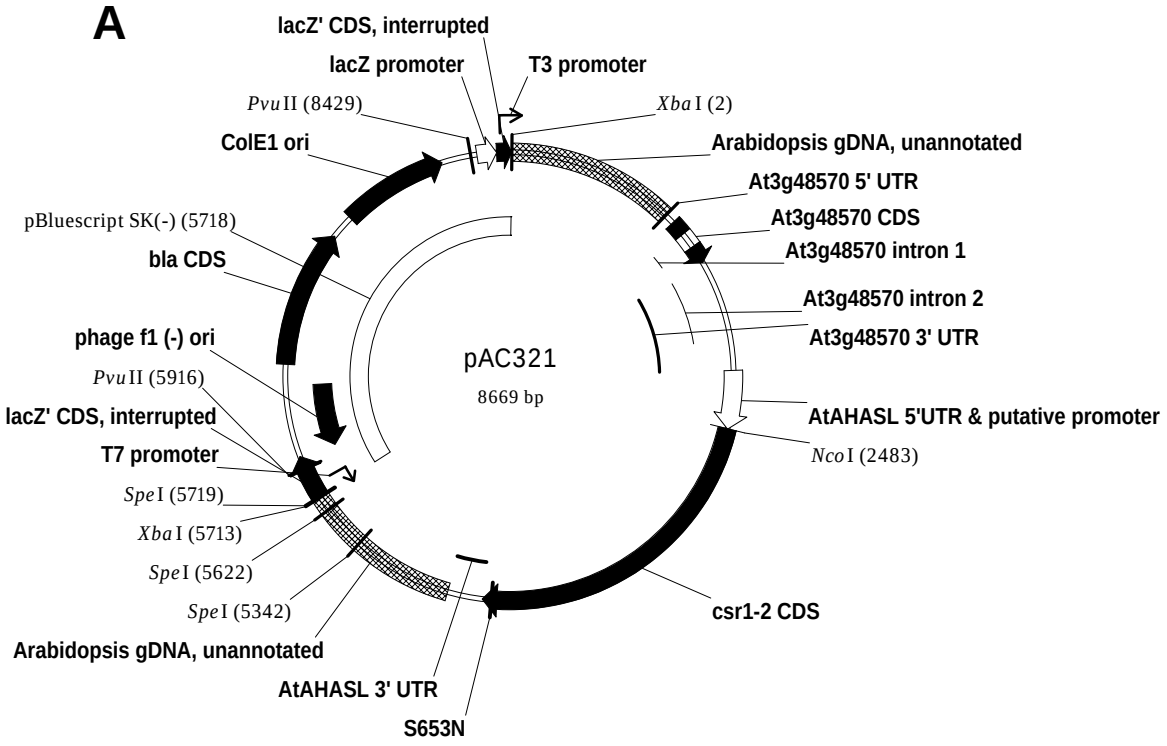
This section describes the donor genes and regulatory elements used in the development of CV127 and the deduced amino acid sequence of the AtAHAS protein produced in CV127.

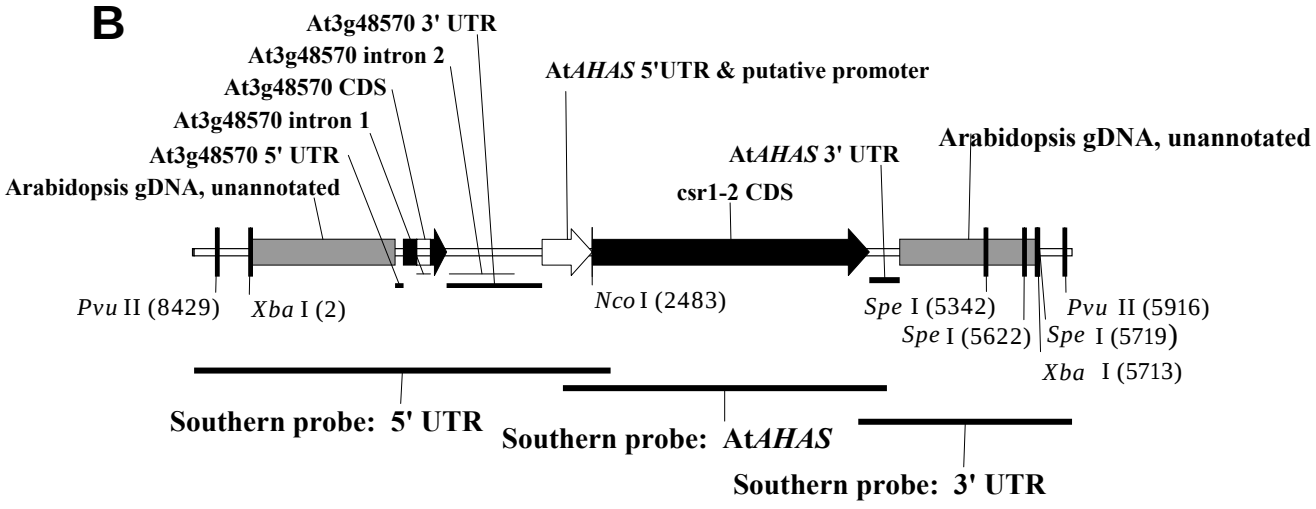
A. Vector pAC321

Molecular maps of the DNA fragment containing the *csr1-2* gene that was used in the transformation of soybean tissues and the plasmid pAC321 from which it was derived are shown in Figure IV-1. The genetic elements of plasmid pAC321 are described in Table IV-1. Plasmid pAC321 is approximately 8.7 kb and the DNA fragment derived from it that was used in the transformation of soybean tissues is an approximately 6.2 kb PvuII fragment. This fragment was cleaved from plasmid pAC321 by restriction with the restriction endonuclease PvuII and was purified prior to use in transformation.

Plasmid pAC321 consists primarily of the *E. coli* cloning plasmid pBluescript SK(-) (Stratagene, La Jolla, CA) with a 5.7 kb XbaI fragment containing the *csr1-2* gene cassette from *Arabidopsis thaliana* cloned into it. The *csr1-2* gene cassette consists of the *csr1-2* gene encoding the acetohydroxyacid synthase large subunit (AtAHAS) protein which is responsible for the imidazolinone herbicide tolerance trait in CV127 soybeans, with its native promoter and 5'- and 3'-untranslated regions (UTR) from *Arabidopsis thaliana*. In addition to the *csr1-2* native gene promoter, the 5' region upstream of the *csr1-2* coding sequence contains the complete coding sequence of the *A. thaliana* SEC61 (AtSEC61) gamma subunit protein, which is a component of the DNA fragment used for transformation. Characterization of this coding sequence is discussed in more detail in Sections IV.C. and VI.E. of this petition. The backbone section of plasmid pAC321, (the section outside of the borders of the transformation fragment) consists of genes and genetic elements derived from the *E. coli* cloning plasmid pBluescript SK(-).

Figure IV-1. Circular Map of Plasmid pAC321 and the Linear Map of the DNA Fragment Derived from the Plasmid Used for Plant Transformation.
A) Circular map of plasmid pAC321. B) Linear map of the PvuII fragment of pAC321 containing the At*AHAS* promoter and 5' UTR, *csr1-2* coding sequence and At*AHAS* 3' UTR that was used for transformation. The restriction sites of the enzymes (NcoI, XbaI, SpeI) and DNA probes used for Southern blot analyses of copy number, absence of backbone and intergenerational stability are indicated.





Fragment of pAC321 with probes

Table IV-1: DNA Components of Plasmid pAC321

Genetic Element	Range (bp)	Function
Arabidopsis gDNA, unannotated	1-1051	*Arabidopsis thaliana* genomic DNA: no genes currently annotated in this region
Arabidopsis locus At3g48570	1052-2119	Coding and regulatory sequences for protein translocation complex SEC61 GAMMA CHAIN-LIKE protein from *Arabidopsis thaliana*
At3g48570 5' UTR	1052-1113	5' untranslated region for putative Arabidopsis *SEC61 GAMMA CHAIN*
At3g48570 coding sequence (CDS)	1114-1207, 1307-1422	Putative Arabidopsis *SEC61 GAMMA CHAIN* coding sequence
At3g48570 intron 1	1208-1306	Putative Arabidopsis *SEC61 GAMMA CHAIN* intron 1, interrupts CDS
At3g48570 3' UTR	1423-1442, 1916-2119	3' untranslated region for putative Arabidopsis *SEC61 GAMMA CHAIN*
At3g48570 intron 2	1443-1915	Putative Arabidopsis *SEC61 GAMMA CHAIN* intron 2
At *AHAS* putative promoter and 5' UTR	2120-2483	Putative promoter and 5' untranslated region for Arabidopsis *ACETOHYDROXYACID SYNTHASE LARGE SUBUNIT*
csr1-2 CDS	2484-4496	Coding sequence for *Arabidopsis thaliana acetohydroxyacid synthase large subunit* with (S653N) point mutation (csr1-2) which confers tolerance to imidazolinones (Sathasivan *et al*., 1990)
At *AHAS* 3' UTR	4497-4714	3' untranslated region for Arabidopsis *ACETOHYDROXYACID SYNTHASE LARGE SUBUNIT*
Arabidopsis gDNA, unannotated	4715-5717	*Arabidopsis thaliana* genomic DNA: no genes currently annotated in this region[1]
pBluescript SK(-) phagemid	5718-8669	Stratagene Corporation; La Jolla, CA. (Short *et al*., 1988) [2]
T7 promoter transcription initiation site	5805	Bacteriophage T7 promoter transcription initiation site; allows *in vitro* synthesis of RNA from DNA cloned in phagemid by T7 RNA polymerase
phage f1 (-) ori	5986-6442	Bacteriophage f1 origin of replication; allows single-strand DNA production in *E. coli* strains containing the F' episome when a helper phage is present
bla CDS	6573-7433	*E. coli* β-lactamase coding sequence; confers resistance to β-lactam antibiotics such as ampicillin and carbenicillin
ColE1 ori	7581-8248	*E. coli* plasmid replication origin ColE1; derived from pUC19
lacZ promoter	8468-8589	*E. coli lacZ* promoter; drives transcription of the alpha fragment of β-galactosidase (*lacZ'*).
lacZ' CDS, interrupted	8590-8669, 5718-5994	*E. coli* β-galactosidase alpha fragment coding sequence, interrupted by Arabidopsis genomic DNA in pAC321; allows blue-white screening for DNA insertions in pBluescript SK(-) multiple cloning site by alpha-complementation
T3 promoter transcription initiation site	8632	Bacteriophage T3 promoter transcription initiation site; allows *in vitro* synthesis of RNA from DNA cloned in phagemid by T3 RNA polymerase

[1] The sequence of pAC321 differs from the Arabidopsis genome sequence data available at www.Arabidopsis.org by a single nucleotide within the unannotated region at nucleotide 5073 of pAC321. pAC321 is missing an A residue relative to the public sequence.

[2] The pBluescript SK(-) sequence in pAC321 differs from accession number X52324 at nucleotide 7751 of pAC321. Nucleotide 7751 of pAC321 is T (in agreement with the pUC19 origin of replication sequence, accession number L09137) while the X52324 sequence contains a C residue at this position. This difference does not occur within the region of pAC321 that was used for transformation (the 6156 bp PvuII fragment).

B. The *csr1-2* Gene Coding Sequence and the AtAHAS Protein

The mechanism of action of imidazolinone herbicides on weeds and non-tolerant plants is by inhibition of the AHAS enzyme and the resulting inhibition of branched-chain amino acid biosynthesis. AHAS catalyses the first common step in branched-chain amino acid biosynthesis that is specific to plants and microorganisms (Stidham and Singh, 1991). The enzyme catalyzes the condensation of two molecules of pyruvate to form acetolactate, the precursor of valine and leucine, or the condensation of a molecule of pyruvate with a molecule of 2-ketobutyrate to form 2-aceto-2-hydroxybutyrate, an intermediate in isoleucine biosynthesis (Delfourne *et al*., 1994; Singh and Shaner, 1995; Duggleby and Pang, 2000). AHAS is the key control enzyme within the biosynthetic pathway and is regulated by feedback inhibition by the end-product amino acids, valine, leucine and isoleucine. Imidazolinone herbicides readily inhibit the activity of AHAS by binding to the active site of the protein resulting in plant death (Duggleby and Pang, 2000; Tan *et al*., 2005). Studies have shown that specific single-nucleotide mutations in the *AHAS* genes confer tolerance to imidazolinone herbicides by altering the binding site for these herbicides on the mutant AHAS enzymes (Tan *et al*., 2005), but have no effect on feedback regulation by branched-chain amino acids and the normal biosynthetic function of the enzyme (Newhouse *et al*., 1992).

CV127 soybean contains the *csr1-2* gene from *Arabidopsis thaliana* that encodes an imidazolinone-tolerant AHAS enzyme (AtAHAS) (Sathasivan *et al*., 1990). The AtAHAS protein encoded by *csr1-2* gene is functionally identical to the native AtAHAS, except for its tolerance to imidazolinone herbicides. The herbicide tolerance is due to a single point mutation of a guanine to adenine in the coding sequence for AtAHAS that results in a substitution of a serine with an asparagine at residue 653 (S653N). This amino acid change in plant AHAS proteins is known to prevent the binding of imidazolinone herbicides and thereby to result in tolerance to these herbicides with no effect on feedback regulation by branched-chain amino acids or the enzyme's normal biosynthetic function (Newhouse *et al*., 1992). In addition, a second mutation was discovered in the *csr1-2* gene integrated in the genome of CV127 soybean. This mutation, in which an arginine residue at position 272 is replaced by lysine, does not impact the enzymatic function of the AHAS enzyme or its herbicide tolerance properties. A study was conducted to compare the AtAHAS large subunit protein, containing both the S653N and R272K mutations, as expressed in CV127 with the AtAHAS large subunit protein encoded by the *csr1-2* gene that contains only the S653N mutation. In this study it was demonstrated that both AtAHAS enzymes had equivalent levels of catalytic activity and tolerance to imidazolinone herbicides. Feedback regulation of AHAS activity by the branched-chain amino acids is effected through the AHAS small subunit (AHASS) (Weinstock *et al*., 1992; Hershey *et al*., 1999). The AtAHAS enzyme encoded by the *csr1-2* gene in CV127 interacts with the endogenous soybean AHAS small subunit protein to achieve this regulation. Therefore, the feedback regulation of the AtAHAS encoded by the *csr1-2* gene in CV127 is expected to be identical to that of the endogenous soybean AHAS.

The *csr1-2* gene encodes a single polypeptide of 670 amino acids that includes the *A. thaliana* native chloroplast transit peptide, predicted to consist of 85 amino acids (Mazur *et al*., 1987). During transport into the chloroplast, it is predicted that the chloroplast transit peptide is

removed to produce the mature AtAHAS enzyme that is approximately 64 kD and consists of 585 amino acids (Mazur *et al*., 1987). The amino acid residues of AtAHAS are counted from the N-terminus of the 670 amino acid full-length protein containing the chloroplast transit peptide. The *csr1-2* gene is expressed from the native *A. thaliana* promoter and it also contains the native 3'-untranslated region (UTR) that contains the polyadenylation signal. The deduced amino acid sequence of the AtAHAS enzyme encoded by the *csr1-2* gene in CV127 is presented in Figure IV-2.

Figure IV-2. The Deduced Amino Acid Sequence of the AtAHAS Protein in CV127 Soybean Including the Predicted Chloroplast Transit Peptide (Underlined).
The lysine (R272K) and asparagine residues (S653N) that are changed from the wildtype AtAHAS protein are in bold text.

```
1         10         20         30         40         50         60
MAAATTTTTT SSSISFSTKP SPSSSKSPLP ISRFSLPFSL NPNKSSSSSR RRGIKSSSPS

          70         80         90        100        110        120
SISAVLNTTT NVTTTPSPTK PTKPETFISR FAPDQPRKGA DILVEALERQ GVETVFAYPG

        130        140        150        160        170        180
GASMEIHQAL TRSSSIRNVL PRHEQGGVFA AEGYARSSGK PGICIATSGP GATNLVSGLA

        190        200        210        220        230        240
DALLDSVPLV AITGQVPRRM IGTDAFQETP IVEVTRSITK HNYLVMDVED IPRIIEEAFF

        250        260        270        280        290        300
LATSGRPGPV LVDVPKDIQQ QLAIPNWEQA MKLPGYMSRM PKPPEDSHLE QIVRLISESK

        310        320        330        340        350        360
KPVLYVGGGC LNSSDELGRF VELTGIPVAS TLMGLGSYPC DDELSLHMLG MHGTVYANYA

        370        380        390        400        410        420
VEHSDLLLAF GVRFDDRVTG KLEAFASRAK IVHIDIDSAE IGKNKTPHVS VCGDVKLALQ

        430        440        450        460        470        480
GMNKVLENRA EELKLDFGVW RNELNVQKQK FPLSFKTFGE AIPPQYAIKV LDELTDGKAI

        490        500        510        520        530        540
ISTGVGQHQM WAAQFYNYKK PRQWLSSGGL GAMGFGLPAA IGASVANPDA IVVDIDGDGS

        550        560        570        580        590        600
FIMNVQELAT IRVENLPVKV LLLNNQHLGM VMQWEDRFYK ANRAHTFLGD PAQEDEIFPN

        610        620        630        640        650        660
MLLFAAACGI PAARVTKKAD LREAIQTMLD TPGPYLLDVI CPHQEHVLPM IPNGGTFNDV

        670
ITEGDGRIKY
```

C. The *Arabidopsis thaliana* SEC61 Gamma Subunit Protein

The linear DNA fragment used in the transformation contains a 2.5 kb segment upstream of the *csr1-2* coding region that was originally annotated as the *AHAS* promoter and 5' untranslated region (UTR). More-recent sequence analysis has revealed that this segment also contains a previously unannotated Arabidopsis gene encoding the gamma subunit of SEC61 (AtSEC61γ), a multimeric transport protein of the endoplasmic reticulum that is ubiquitous in all plants and other eukaryotes (Hartmann *et al*., 1994). The CV127 insert contains the majority of the At*SEC61*γ subunit gene including the complete coding sequence. The At*SEC61*γ 5' UTR, as annotated by The Arabidopsis Information Resource, begins 18 nucleotides downstream from the 5' junction of the transgene and soybean genomic DNA flanking the integration site. As such, it is extremely unlikely that the insert contains the complete native promoter for the At*SEC61*γ gene. The possible production of the Arabidopsis AtSEC61γ subunit protein in CV127 was evaluated and results showed that no AtSEC61γ subunit protein was detected in tissues of CV127 soybean above the levels of detection of the assay. (No AtSEC61γ subunit protein was detected at levels > 30 or >110 ppb, respectively, for leaf and grain). These studies are described in more detail in Section VI.E.

D. Regulatory Sequences

Transcriptional regulation of the *csr1-2* gene in CV127 is under the control of its native *A. thaliana* promoter that is located immediately 5' to the *csr1-2* 5' UTR and coding sequence and 3' to the At*SEC61*γ gene. Termination of transcription of the *csr1-2* gene is also effected by the native *A. thaliana csr1-2* gene transcription termination sequences contained within the 3'-untranslated region located downstream of the *csr1-2* gene.

E. Genetic Elements of pAC321 Outside of the Transformation Fragment

The plasmid pAC321 consists primarily of the transformation fragment cloned into the *E. coli* cloning plasmid pBluescript SK(-) (Stratagene, La Jolla, CA). This plasmid contains the ColE1 origin of replication for maintenance of the plasmid in *E. coli* and the *E. coli* gene encoding β–lactamase which confers ampicillin resistance and is used as a selectable marker for the plasmid in *E. coli*. In addition, the plasmid encodes the alpha fragment of the *E. coli lacZ* gene whose expression is regulated by the *lacZ* gene promoter. The *lacZ* gene encodes the *E. coli* β-galactosidase enzyme. The *lacZ* alpha fragment gene in plasmid pAC321 is disrupted by the insertion of the 5.7 kb XbaI fragment comprising the *csr1-2* gene cassette. The genetic elements within the plasmid pAC321 that are not part of the transformation fragment are listed and described in Table IV-1. Since only the 6.2 kb PvuII fragment containing the *csr1-2* gene cassette was used in the transformation, very few of the sequences derived from the plasmid backbone of pAC321 were included within the transformation fragment. No plasmid backbone DNA was detectable in Southern blot analyses with probes specific for these fragments (Section V of this petition).

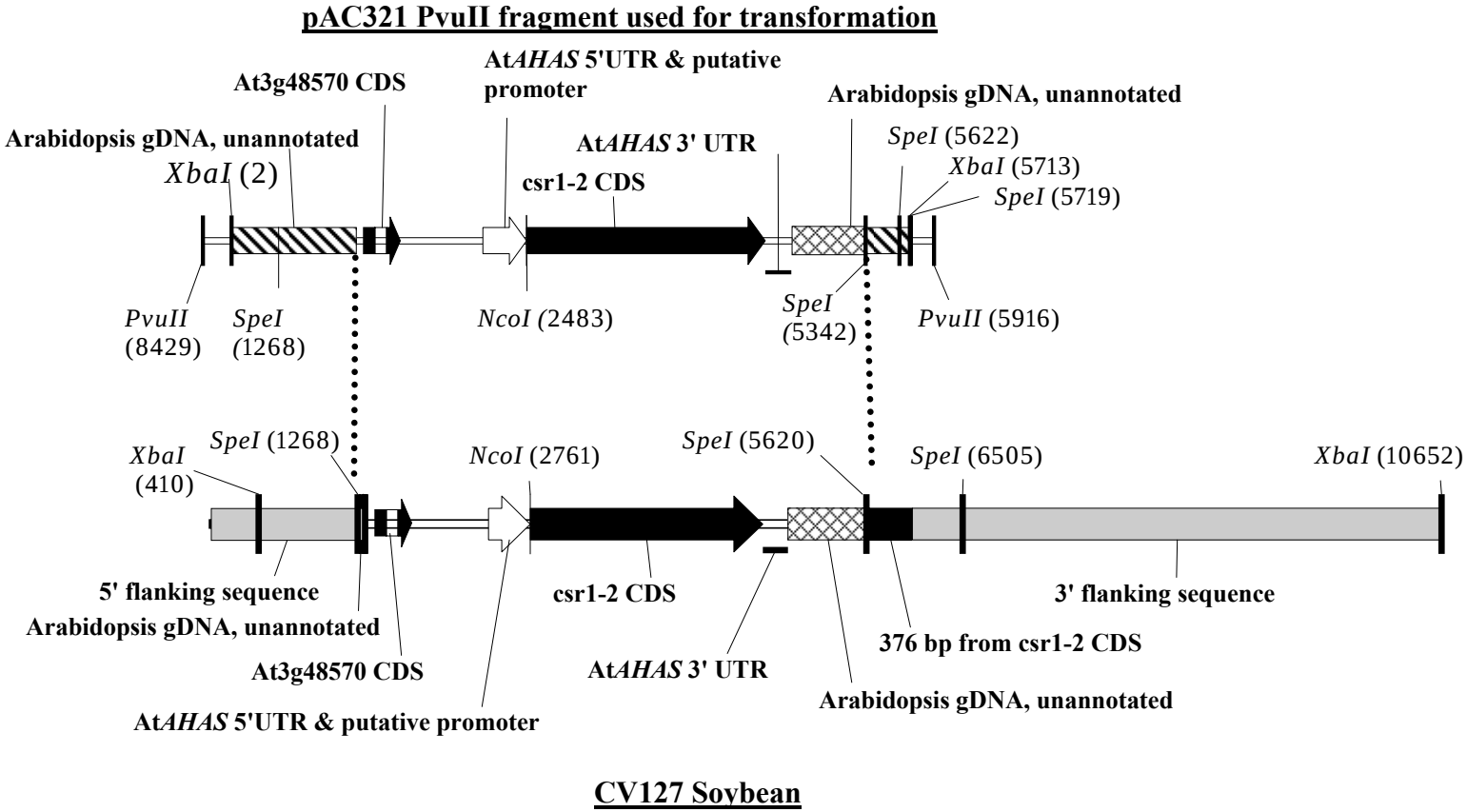
V. Genetic Analysis of the Insertion in CV127 Soybean

A complete genetic analysis of the transgene insert and flanking region of soybean CV127 has been conducted and the results of these analyses are presented in this section. The results of these analyses demonstrated that the *csr1-2* gene expression cassette was integrated at a single genetic locus in the soybean genome and that no DNA sequences from the backbone of the transformation vector were detected in the genome. The *csr1-2* gene cassette in CV127 soybean contains three point mutations relative to the transformation plasmid with one mutation in the At*AHAS* coding sequence and the other two downstream of the 3' untranslated region (UTR) of the *csr1-2* gene. The G to A mutation in the *csr1-2* coding sequence, which caused an amino acid change of R_{272} to K_{272} does not affect the desired herbicide tolerance phenotype conferred by the *csr1-2* gene. Southern blot analysis and sequence verification of the point mutation indicate that the insert is stably integrated in the soybean genome across the nine breeding generations studied. Also, trait inheritance studies across breeding generations confirmed the expected trait segregation ratios, further demonstrating that imidazolinone herbicide tolerance in CV127 soybean is the result of a single functional dominant *csr1-2* gene stably integrated in the soybean genome.

In the transgene insert, there is also a 376 base-pair (bp) duplication of a portion of the *csr1-2* coding sequence directly before the 3' integration point of the insert. This duplicated 376 bp segment creates a 501 bp open reading frame (ORF) that extends into the 3' flanking sequence. The results of reverse transcription-polymerase chain reaction (RT-PCR) analyses of total RNA extracted from leaf, root, leaf bud, and seed from BPS-CV127-9 soybean showed the absence of a detectable ORF 501-specific PCR product, suggesting that this 501 bp ORF is not expressed. The insert also contains the majority of the Arabidopsis *SEC61*γ subunit gene locus (refer to Table IV-1 for genetic element At3g48570), which is a component of the DNA fragment used for transformation. This gene is discussed in more detail in section V.E. A more detailed description of these analyses and the results follows. The materials and methods used in these analyses are presented in Appendix A.

The genetic elements contained within the 5.7 kb XbaI fragment that was derived from the genome of Arabidopsis and integrated into plasmid pAC321 are listed in Table IV-1 beginning with nucleotide 1 and continuing to nucleotide 5717. The hybridization probes used in the Southern blot analyses and the map of plasmid pAC321 containing the transformation fragment used to generate CV127 soybean are presented in Figure IV-1. The DNA sequence data (not presented) and other results from the molecular characterization of the insert were used to construct a linear map of the transgene insert and the flanking soybean genomic DNA (Figure V-1). The pAC321 transformation fragment is shown aligned with the DNA insert in CV127 soybean to identify the portions of the fragment that are present in the soybean CV127 insert in Figure V-1.

Figure V-1. Alignment of pAC321 Transformation Fragment with the Transgene Insert in CV127 Soybean[1].



[1] The PvuII fragment from plasmid pAC321 that was used to transform soybean is shown in the upper portion of the figure. Parts of this fragment that are not contained within the transgene insert in CV127 soybean are indicated by boxes filled with diagonal stripes. Characteristics of the transgene insert and the flanking genomic soybean DNA in CV127 soybean are shown in the lower portion of the figure. The DNA between the vertical dotted lines (drawn between the maps of the transformation fragment and the transgene insertion region) is common to DNA from both sources. Restriction sites relevant to the Southern blot analysis are indicated. The numbering system of the PvuII transformation fragment corresponds to that of the pAC321 plasmid map in Figure IV-1. The numbering system for the CV127 soybean DNA insert region begins with the first nucleotide at the 5' end of the soybean genomic flanking sequence (flanking sequences indicated by gray boxes).

A. Copy Number and Insert Integrity

The copy number and integrity of the DNA insert in CV127 soybean were evaluated by Southern blot analysis of genomic DNA from plants of the F_8 generation of line 603 digested with NcoI, SpeI and XbaI restriction enzymes. (For a description of specific generations of CV127, a breeding diagram is presented in Figure III-1.) Blots were hybridized separately with three different probes (the At*AHAS* 5' UTR, the *csr1-2* coding sequence for the AtAHAS protein, and the At*AHAS* 3' UTR) that spanned the entire DNA fragment used for transformation (Figure IV-1). The *csr1-2* coding sequence probe is referred to as the "AHAS" probe in Figure V-2, Figure V-4, and Table V-1. Genomic DNA from the non-transgenic cultivar Conquista as well as Conquista genomic DNA spiked with one- and two-genome copy equivalents of pAC321 plasmid DNA was included in the Southern blots as controls.

Non-transgenic Conquista DNA digested with all three restriction enzymes and hybridized with the three probes did not show any hybridizing signal, indicating that neither the endogenous soybean *AHAS* nor the endogenous soybean *SEC61*γ subunit genes are detected at the Southern blot stringency conditions used (Figure V-2, lanes 1, 5 and 9). The pAC321 DNA, spiked at one- and two-genome copy equivalents into non-transgenic Conquista DNA (Figure V-2, lanes 2, 3, 6, 7, 10 and 11), was detected at the expected band sizes (Table V-1) with a fairly strong intensity suggesting that the Southern method employed was sensitive enough to detect a single-copy insert in the transgenic event. DNA samples from the CV127 soybean F_8 generation treated with the different restriction enzyme and probe combinations all gave single hybridizing bands (Figure V-2, lanes 4, 8 and 12) except for the SpeI digest hybridized with the *csr1-2* coding sequence probe, which had an additional small band of approximately 800 to 900 bp (Figure V-2B, lane 8). This small *csr1-2*-hybridizing SpeI fragment is consistent with the observation that a small fragment of the *csr1-2* coding sequence was duplicated at the 3' flanking sequence junction in CV127 soybean (see Section V.D.) thereby creating an 885 bp SpeI fragment. All major bands had signal intensities similar to the one-genome copy equivalent of pAC321. Due to the homozygous state of the material analyzed, it would be reasonable to expect band intensities closer to the two genome copy equivalent control. The quantification of plasmid DNA versus genomic DNA coupled with the large dilution required for loading the plasmid DNA, can result in variations of intensity. However, the DNA sequence information for the insert as well as genomic flanking DNA (data not presented), and the number of bands observed in the Southern blot substantiate the conclusion that a single copy of the insert is present in CV127 soybean.

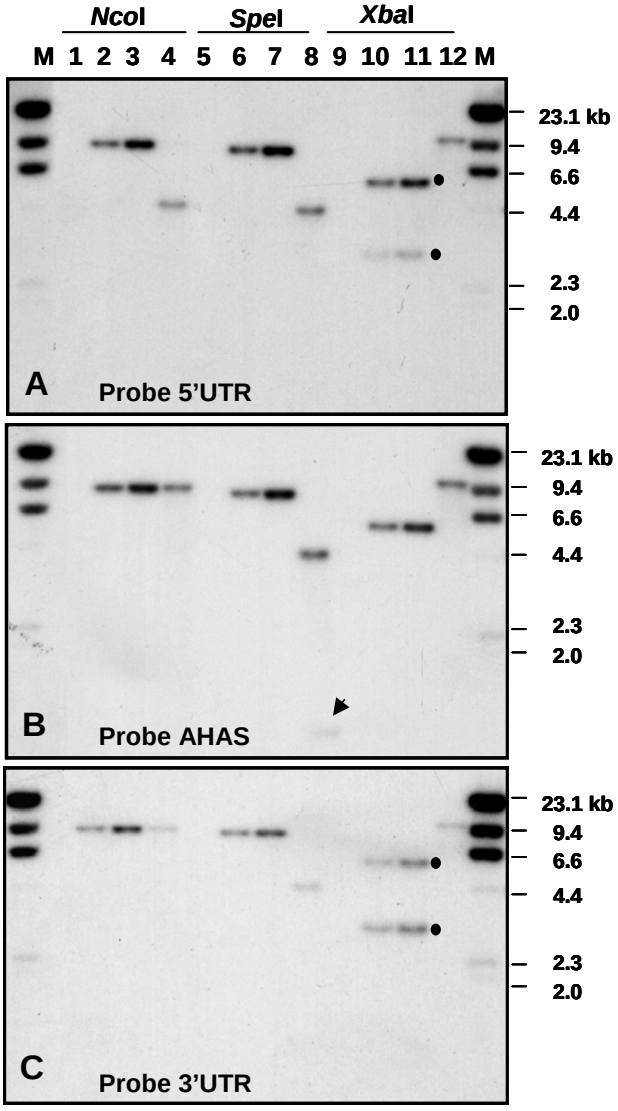
Genomic DNA from CV127 soybean that was digested with NcoI and probed with At*AHAS* 5' UTR produced a hybridizing band approximately 4.5 kb in size (Figure V-2). The size of this band is consistent with the production of a single DNA fragment defined by the NcoI site within the insert (nt 2761, Figure V-2 panel D) and an NcoI site approximately 4.5 kb upstream in the 5' genomic soybean flanking DNA sequence. The same digest probed with either the *csr1-2* coding sequence or At*AHAS* 3'UTR produced a hybridizing band approximately 9.0 kb in size. The size of this band is consistent with a single DNA fragment defined by the NcoI site in the insert (nt 2761, Figure V-2 panel D) and an NcoI site approximately 9.0 kb downstream in the 3' soybean genomic flanking DNA sequence.

Digestion of CV127 genomic DNA with SpeI and probed with At*AHAS* 5' UTR produced a hybridizing band of approximate size 4.4 kb. This is consistent with the production of a single DNA fragment from a SpeI site in the insert (nt 5620, Figure V-2, panel D) and a SpeI restriction site approximately 4.4 kb upstream in the 5' DNA flanking sequence of the soybean genome (nt 1268, Figure V-2, panel D). The presence of this upstream SpeI site was confirmed in the analysis of the 5' DNA flanking sequence of CV127 soybean (data not presented). The same digest probed with either the *csr1-2* coding sequence or the At*AHAS* 3' UTR also produced a 4.4 kb hybridizing band corresponding to the same fragment described above. In addition, a hybridizing band of approximate size 800 to 900 bp was detected when the SpeI digest was probed with the *csr1-2* coding sequence, consistent with a single SpeI DNA fragment containing the duplicated 376 bp segment of the *csr1-2* gene at the 3' flanking DNA sequence junction. This hybridizing fragment was produced from the SpeI site in the DNA insert and a SpeI site 885 bp downstream in the soybean genome (nt 5620 – 6505, Figure V-2, panel D). The 885 bp hybridizing band was not detected by the At*AHAS* 3' UTR probe, indicating that At*AHAS* 3' UTR DNA was not included in the 885 bp fragment, and the SpeI nt 5620 site in the insert is adjacent to the duplicated 376 bp segment of the *csr1-2* gene. Therefore, SpeI restriction enzyme sites at nucleotide 5622 and 5719 in the linear PvuII fragment of plasmid pAC321 used for transformation (shown in Figure V-1) were not included in the DNA insert in the CV127 genome. This was confirmed by DNA sequence analysis of the DNA insert (refer to Section V.D.). The smaller predicted SpeI fragments of 280 and 97 bp in the pAC321-spiked controls were not detected because these fragments were expected to produce low-intensity hybridizing signals below the level of detection of the analysis.

CV127 soybean genomic DNA when digested with XbaI and probed with At*AHAS* 5' UTR shows a single hybridizing band of approximate size 10 kb. Based on the positions of the XbaI restriction sites in the linear DNA used for transformation (Figure V-1), a hybridizing band of approximately 5.7 kb was expected to be produced from within the DNA insert in the CV127 soybean genome. However, DNA sequence analysis of the DNA insert in CV127 showed that neither of the XbaI restriction sites in the linear transformation DNA were included in the DNA insert. Therefore, the 10 kb hybridizing band was produced from XbaI restriction sites within the 5' and 3' DNA sequences flanking the insert (nt 410 and 10652, Figure V-2, panel D). Accordingly, the same digest probed with either the *csr1-2* coding sequence or At*AHAS* 3' UTR produced the same 10 kb hybridizing band corresponding to the same DNA fragment described above.

Analysis of the number and size of all hybridizing bands on the Southern blots shown in Figure V-2 is consistent with the integration of a single DNA insert in the CV127 soybean genome containing a single functional copy of the *csr1-2* gene, as well as coding sequence for the AtSEC61γ subunit protein on the 5' end of the *csr1-2* gene, and a single DNA fragment containing a 376 bp duplicated segment of the *csr1-2* gene at the 3' end of the transgene insert.

Figure V-2. Southern Blot Analysis of Insert Copy Number in CV127 Soybean.



Genomic DNA of non-transgenic soybean variety Conquista (lanes 1, 5 and 9); Conquista spiked with 1- (lanes 2, 6 and 10); or 2-genome copy equivalents of pAC321 (lanes 3, 7 and 11); and genomic DNA of CV127 soybean from the F_8 generation (lanes 4, 8 and 12) was digested with NcoI (1-4), SpeI (5-8) and XbaI (9-12) restriction enzymes. Blots were hybridized with probe 5' UTR (panel A), probe AHAS (panel B) and probe 3' UTR (panel C). The first and last lanes (labeled M) contain a λ/HindIII ladder; band sizes are indicated in kilobases. Panel D indicates regions of homology between the Southern hybridization probes and the CV127 soybean insert. The arrow in panel B indicates a 885 bp SpeI fragment containing an additional 376 bp fragment of *csr1-2* present in CV127 at the 3' flanking sequence junction.

Note: The 5' UTR probe and the 3' UTR probe each overlap a XbaI site in pAC321 and therefore hybridize to both XbaI fragments of the plasmid. These two bands are marked with a dot to the right of them in lane 11 (Panels A and C).

D

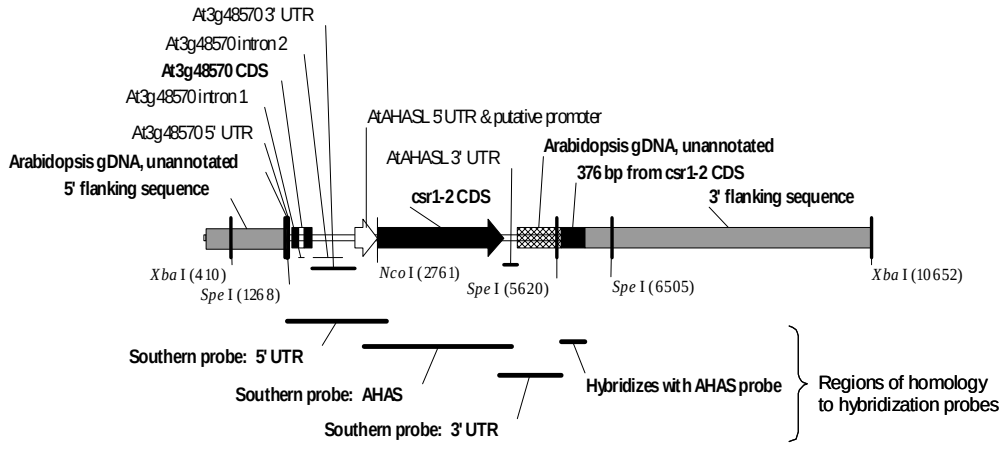


Table V-1. Predicted and Observed Hybridizing Bands on Southern Blots of CV127 Soybean Genomic DNA.

Figure	Probe	Restriction Enzyme	Predicted Fragment Size from CV127 Insert (bp)[a]	Observed CV127 Fragment Size	Predicted Fragment Size from Plasmid pAC321 (bp)	Observed Plasmid Fragment Size
V-2A	**5' UTR**	NcoI	>2760	~4500	8669	~9000
		SpeI	4352	~4400	8292	~8500
		XbaI	10242	~10000	5711[b] 2958	~5500 ~3000
V-2B	**AHAS**	NcoI	>7896	~9000	8669	~9000
		SpeI	4352 885	~4400 ~800[c]	8292	~8500
		XbaI	10242	~10000	5711	~5500
V-2C	**3' UTR**	NcoI	>7896	~9000	8669	~9000
		SpeI	4352	~4400	8292 280 97	~8500
		XbaI	10242	~10000	5711[b] 2958	~5500 ~3000
V-3A	**VP1**	NcoI	none	none	8669	~9000
		SpeI	none	none	8292	~8500
		XbaI	none	none	2958	~3000
V-3B	**VP2**	NcoI	none	none	8669	~9000
		SpeI	none	none	8292	~8500
		XbaI	none	none	2958	~3000

[a]The predicted fragment size is estimated based on the cloned insert and flanking sequences in CV127.
[b]The 5' UTR probe and the 3' UTR probe each overlap a XbaI site and therefore hybridize to both XbaI fragments of the plasmid.
[c]Sequence analysis of the CV127 insert indicates that a small portion of the *csr1-2* coding region was duplicated immediately upstream of the 3' transgene integration site, confirming the identity of the 885 bp band observed in these Southern blots.

B. Analysis for Plasmid pAC321 Backbone

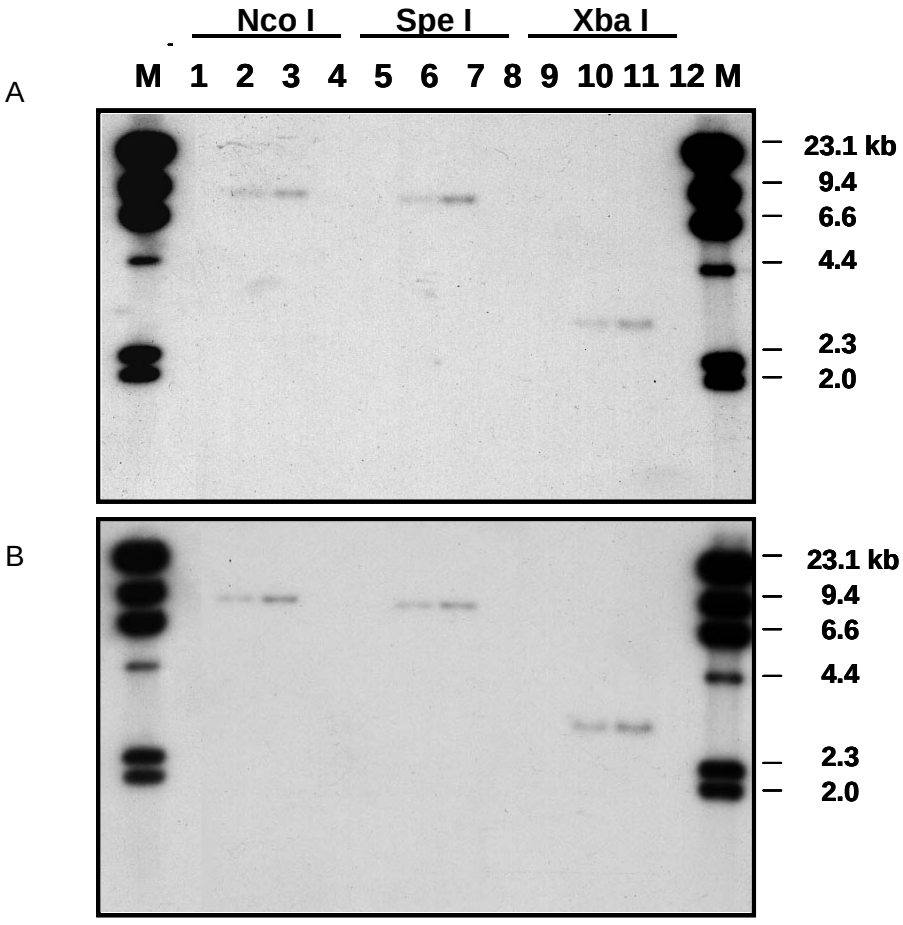
Although the transformation was carried out with the PvuII restriction fragment of pAC321 that included minimal vector backbone DNA, Southern blot studies were conducted to confirm the absence of plasmid pAC321 vector backbone DNA in the CV127 soybean genome. In order to determine whether there was any vector backbone integrated in CV127, the same set of blots used for Southern blot analysis described above (Figure V-2) was hybridized with two vector backbone-specific probes designated VP-1 and VP-2 (Figure V-3, panel C). As expected, no hybridizing bands were detected in lanes containing non-transgenic Conquista genomic DNA. Non-transgenic Conquista genomic DNA spiked with one- or two-genome copy equivalents of transformation plasmid pAC321 showed hybridizing bands of the expected sizes (Figure V-3 panels A and B, and Table V-1). No hybridizing bands were detected in CV127 soybean DNA, clearly indicating that no vector backbone DNA was integrated into the genome of CV127 soybean.

C. Stability of the Insert Across Multiple Generations

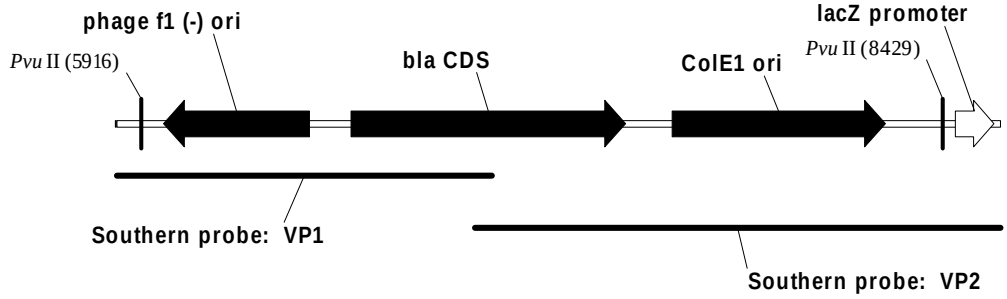
In order to determine the stability of the insert in CV127, DNA samples from four different generations, T_4, F_4, F_8 and F_9, were subjected to Southern blot analysis. For reference, the breeding history of CV127 is presented in Figure III-1. Genomic DNA samples were digested with NcoI and SpeI and probed with either the At*AHAS* 5' UTR, *csr1-2* coding sequence or At*AHAS* 3' UTR probes spanning the entire DNA fragment used for transformation (Figure IV-1). The combination of these restriction enzymes and probes provides a unique fingerprint for the DNA insert in CV127 (Figure V-2). Non-transgenic Conquista genomic DNA was used as a negative control and Conquista spiked with one- and two-genome copy equivalents of pAC321 was used as a positive control. Multiple bands from CV127 T_4 generation DNA digested with either NcoI or SpeI were detected with all three probes, indicating that the T_4 generation contains multiple copies of the *csr1-2* gene cassette (Figure V-4). However, DNA from the F_4, F_8 and F_9 generations all showed the same Southern pattern (Figure V-4) previously observed in the insert and copy number analyses (Figure V-2). This result clearly indicates that the multiple copies of the insert in the T_4 generation segregated in the progeny of the cross between T_4 and Conquista and that only a single copy is retained in the segregant selected. Moreover, these results demonstrate that this single copy is stably integrated in the soybean genome and is inherited across subsequent breeding generations.

Figure V-3. Southern Blot Analysis for the Absence of Vector Backbone Sequence in CV127 Soybean.

Genomic DNA of non-transgenic soybean variety Conquista (lanes 1, 5 and 9); Conquista spiked with 1- (lanes 2, 6 and 10); or 2-genome copy equivalents of pAC321 (lanes 3, 7 and 11); and genomic DNA of CV127 from the F_8 generation (lanes 4, 8 and 12) were digested with NcoI (lanes 1-4), SpeI (lanes 5-8) and XbaI (lanes 9-12) restriction enzymes. The blot was hybridized with probe VP1 (panel A) and probe VP2 (panel B). The first and last lanes (labeled M) contain a λ/HindIII ladder; sizes are indicated in kilobases. Panel C indicates the origin of probes VP1 and VP2 in the sequence of pAC321.

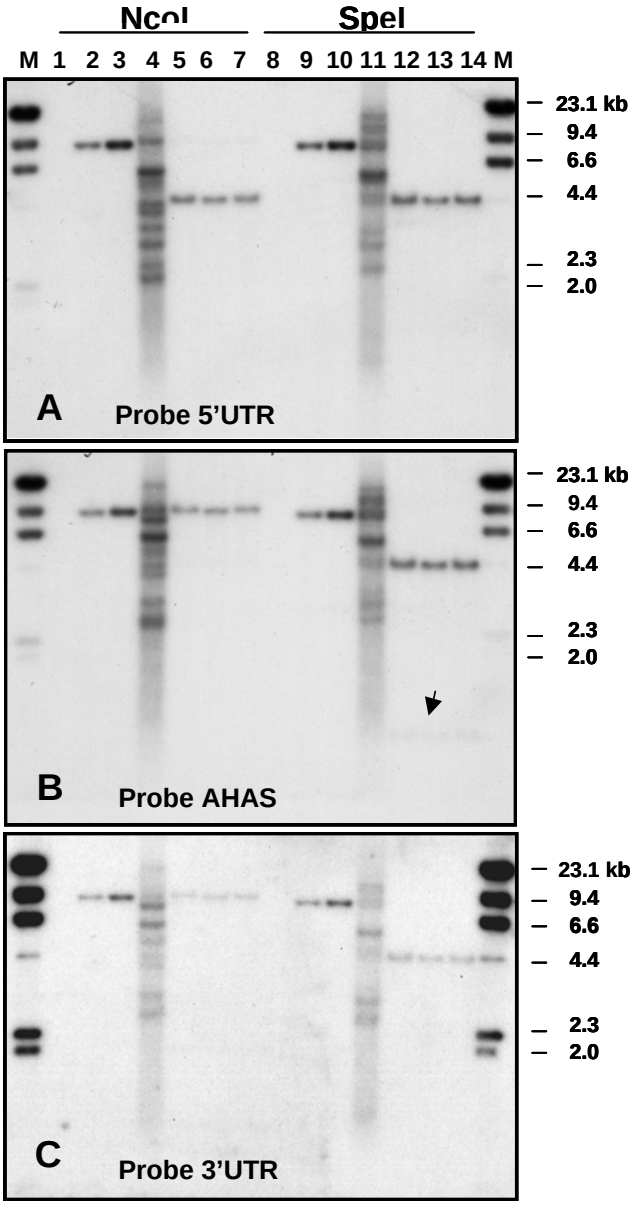


C



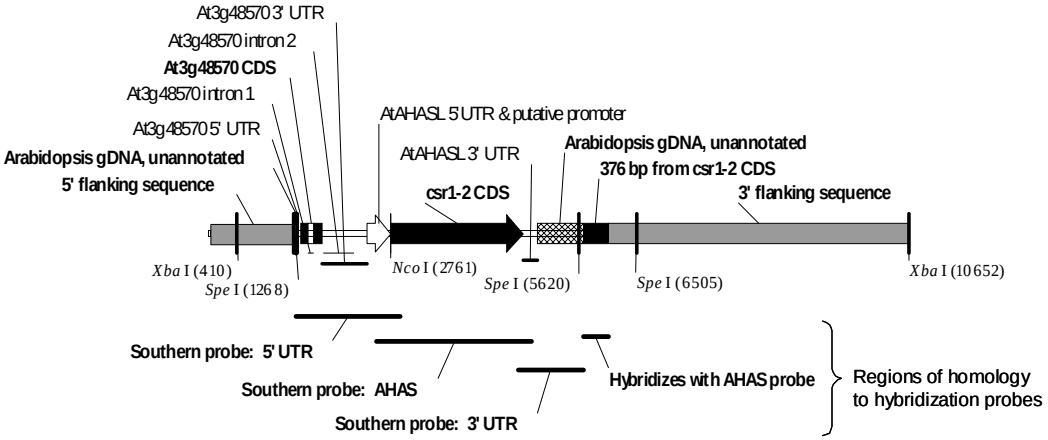
Fragment of pAC321 with probes

Figure V-4. Southern Blot Analysis Demonstrating Intergenerational Stability of the Insert in CV127 Soybean.



Genomic DNA of non-transgenic soybean variety Conquista (lanes 1 and 8); Conquista spiked with 1-copy (lanes 2 and 9), or 2-copy equivalents of pAC321 (lane 3 and 10); and genomic DNA of CV127 of either the T_4 (lanes 4 and 11), F_4 (lanes 5 and 12), F_8 (lanes 6 and 13) or F_9 (lanes 7 and 14) generation was digested with either NcoI (lanes 1- 7) or SpeI (lanes 8- 14) restriction enzymes. The blot was hybridized with either probe 5' UTR (panel A); probe AHAS (panel B); or probe 3' UTR (panel C). The first and last lanes (labeled M) contain a λ/HindIII ladder; sizes are indicated in kilobases. Panel D shows the Southern hybridization probes that were used in this experiment and their relationship to the transgene insertion. The arrow in panel B indicates the 885 bp SpeI fragment containing the 376 bp fragment of *csr1-2* coding sequence inserted near the 3' flanking sequence junction.

D



D. Organization of the Genetic Elements in the Insert of CV127

Although Southern blot analysis demonstrated that the transgene insert in CV127 soybean contains the complete *csr1-2* expression cassette, cloning and sequencing of the insert was performed to confirm insert integrity. The complete sequence of the inserted DNA was obtained by PCR amplification of six overlapping amplicons with Taq DNA polymerase (Figure V-5). The complete soybean CV127 insert sequence is 4758 bp in length and other than the insertion of the 376 bp fragment from *csr1-2* at the 3' integration point, the sequence is identical to the sequence of the transformation fragment except for three point mutations. One of the point mutations is a G to A mutation in the *AHAS* coding sequence, which results in an amino acid change from R_{272} to K_{272}. This is a conservative amino acid substitution and has no impact on the herbicide tolerance or enzymatic properties of the AtAHAS protein. The other two mutations include a G to A mutation and a G to C mutation, both of which are located downstream of the 3'UTR of the *csr1-2* gene and so are genetically silent. These results confirm that the organization of the genetic elements within the DNA insert in CV127 soybean is identical to the organization of the genetic elements in the PvuII linear DNA fragment of pAC321 (Figure IV-1) used to produce CV127 soybean, except for the insertion of the 376 bp fragment from *csr1-2* at the 3' integration point.

The insertion of a 376 bp portion of the *csr1-2* coding sequence near the 3' flanking sequence junction (Figure V-1) created a 501 bp ORF. The possible transcription of this ORF was also investigated by RT-PCR analysis. RT-PCR was carried out with 500 ng of RNA template. CV127 genomic DNA was also used in a positive control reaction with ORF-specific primers (Figure V-7). Primers specific for the soybean endogenous cyclophilin gene were used in positive control reactions to confirm the quality of the template RNA (Figure V-6). The ORF-specific primers amplified a 435 bp fragment from CV127 genomic DNA (Figure V-7). However, no detectable RT-PCR product was observed using total RNA from leaf, root, leaf bud and seed tissue as a template, suggesting that the ORF is not expressed in CV127 soybean (Figure V-6).

Figure V-5. **Diagram of Overlapping PCR Amplicons Used to Demonstrate the Integrity of the Insert in CV127 Soybean**. The insert and flanking sequences are displayed. Six amplicons used for sequencing of the insert that span the length of the insert from the 5' to 3' flanking regions are also indicated.

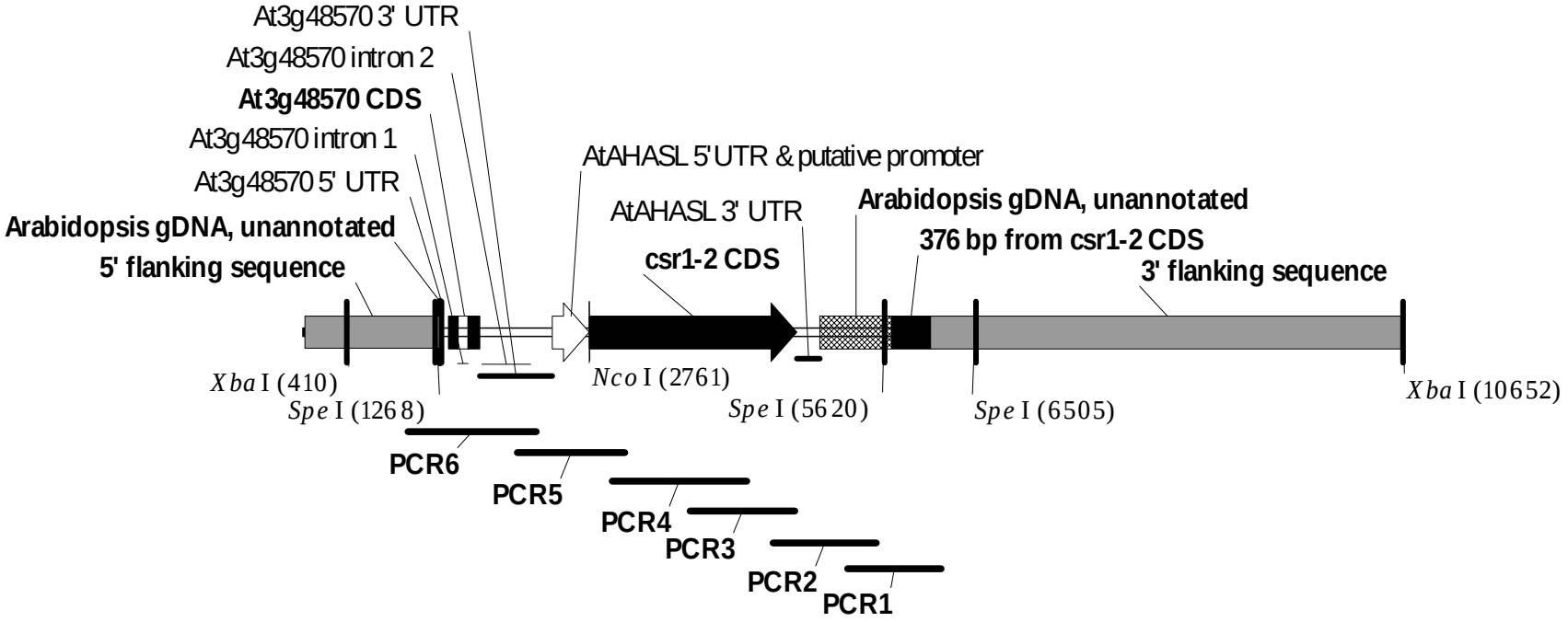
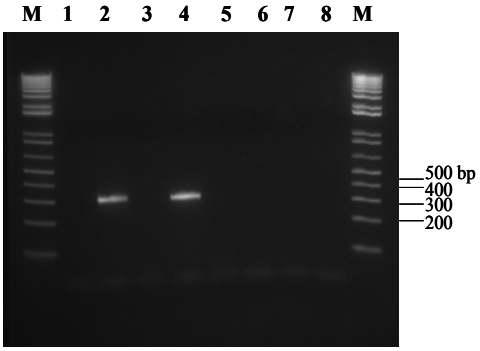
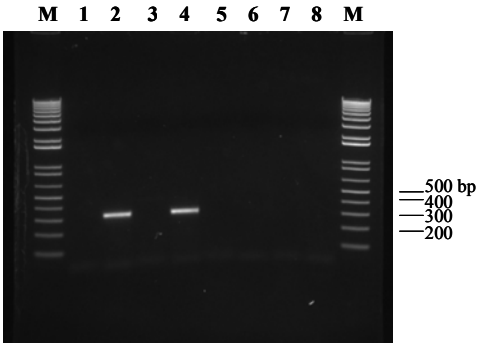


Figure V-6. RT-PCR Analysis of a 501 bp ORF Created by Insertion of a 376 bp Portion of the *csr1-2* Coding Sequence at the 3' Flanking Sequence Junction in CV127 Soybean.
Total RNA was extracted from leaf, root, leaf bud and seed tissue from CV127 soybean and non-transgenic commercial variety Conquista with Qiagen RNeasy Mini Kit and then treated with DNase. RT-PCR was conducted using primers specific for the ORF 501 (anticipated amplicon 435 bp). Primers specific for the soybean endogenous cyclophilin gene, that is constitutively expressed, were used in positive control reactions (anticipated amplicon 315 bp). Panel A: Seed. Panel B: Leaves. Panel C: Leaf buds. Panel D: Roots. Lanes 1, 3, 5 and 7, ORF 501 reactions; lanes 2, 4, 6, and 8, cyclophilin reactions; Lanes 1, 2, 5 and 6 used Conquista RNA template; Lanes 3, 4, 7 and 8 used CV127 RNA as template. Lanes 1 – 4 reactions included reverse transcriptase whereas it was omitted in reactions shown in lanes 5 – 8 to serve as a control. The entire 25 µl reaction was loaded on the gel. Molecular weight markers (1 kb Plus DNA Ladder, Invitrogen) are in the lanes labeled M.

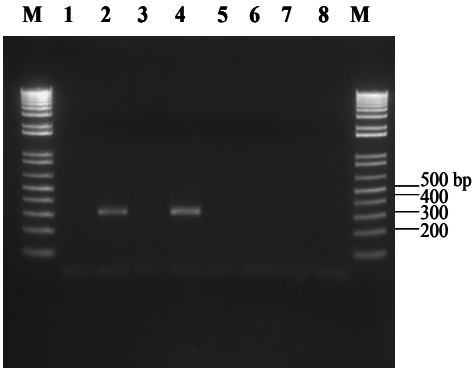
A.



B.



C.



D.

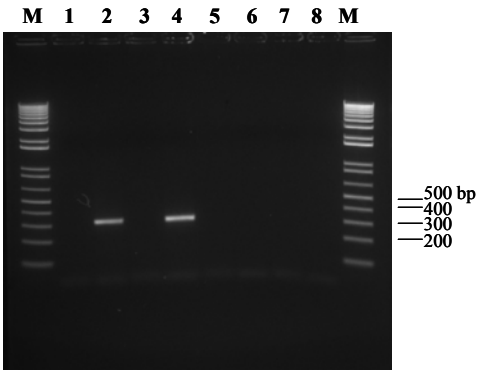
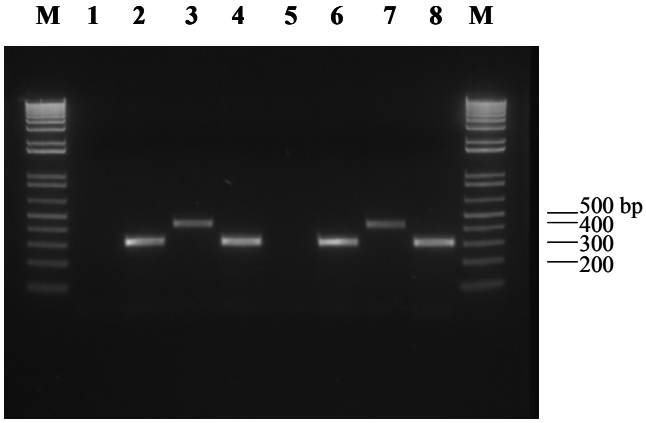
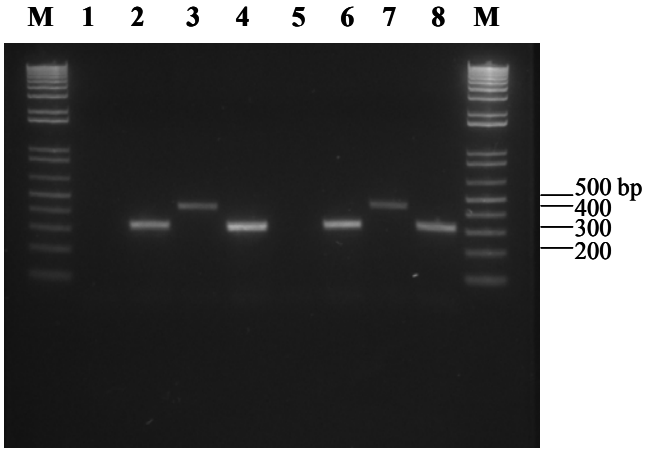


Figure V-7. PCR Analysis of Genomic DNA from CV127 Soybean Confirming the Presence of ORF 501. Genomic DNA from CV127 soybean and Conquista was evaluated for the presence of the ORF 501 and the cyclophilin gene to test the primers for ORF501 and verify the robustness of amplification conditions. PCR primers used were identical to those applied in the RT-PCR experiments in Figure V-6. Panel A: lanes 1 – 4, seed genomic DNA; lanes 5 – 8, leaf genomic DNA. Panel B: lanes 1 – 4, root genomic DNA; lanes 5 – 8, leaf bud genomic DNA. Lanes 1, 3, 5, and 7, ORF 501 PCR; lanes 2, 4, 6, and 8, cyclophilin PCR. Lanes 1, 2, 5 and 6, Conquista genomic DNA, Lanes 3, 4, 7, and 8, CV127 genomic DNA. The entire 25 µl reaction was loaded on the gel. Molecular weight markers (1 kb Plus DNA Ladder) are in the lanes labeled M.

A.



B.



E. *A. thaliana SEC61γ* Subunit Gene

The linear DNA used to produce soybean CV127 contains a 2.5 kb segment that was originally annotated as the *csr1-2* gene promoter and 5' untranslated region (UTR). More-recent sequence analysis has revealed that this segment also contains a previously unannotated Arabidopsis gene encoding the gamma subunit of SEC61 (At*SEC61γ*), a small (69 amino acid) multimeric transport protein of the endoplasmic reticulum that is ubiquitous in all plants and other eukaryotes (Hartmann *et al*., 1994). The CV127 soybean insert sequence contains the majority of the At*SEC61γ* subunit gene including the complete coding sequence. The At*SEC61γ* 5' UTR, as annotated by The Arabidopsis Information Resource, begins 18 nucleotides downstream from the 5' junction of the transgene and soybean genomic DNA flanking the integration site. As such, it is extremely unlikely that the insert contains the complete native promoter for the At*SEC61γ* subunit gene.

The possible transcription of the Arabidopsis At*SEC61γ* subunit gene found in the insert in CV127 soybean was evaluated using RT-PCR. RT-PCR was carried out using DNase-treated total RNA extracted from the F_7 generation of CV127 soybean as a template. Primers specific to two endogenous soybean genes, *Iota* and Gm*SEC61γ,* were used as positive controls to confirm the quality of the template RNA. Total RNA from Arabidopsis leaf and root tissues without DNase treatment was also used as a positive control. Results showed that a very low amount of mRNA specific to the At*SEC61γ* subunit gene in the CV127 F_7 generation was present, indicating that the gene is only weakly transcribed (Figure V-8; for more details on the methods used see Appendix A). The amplified 393 bp At*SEC61γ* subunit DNA band amplified by RT-PCR is the same size as that amplified from Arabidopsis leaves and roots (Figure V-8). The same set of primers also amplified a band of the expected size, 965 bp, from contaminating genomic DNA in Arabidopsis leaf and root samples (Figure V-8).

To confirm the identity of the soybean CV127 At*SEC61γ* subunit gene transcript, the 5' end of the Arabidopsis *SEC61γ* subunit transcript in CV127 soybean was determined by RNA-ligase mediated rapid amplification of 5' complementary DNA ends (RLM-5'-RACE). (For more details on the methods used in the RLM-5'-RACE experiment see Appendix A.) Figure V-9 shows the AtSec61γ RLM-5'-RACE secondary PCR result for CV127. Secondary PCR amplifications using soybean Sec61γ subunit-specific primer served as a positive control to confirm the ability to amplify endogenous soybean transcripts in the RLM-5'-RACE experiment. Due to the lack of a published full-length cDNA sequence, it was difficult to calculate an expected size for the endogenous soybean Sec61γ subunit secondary PCR product. Using an expressed sequence tag (GenBank accession BM731537) which shows 100% identity to both the primary and secondary PCR primers, the endogenous soybean Sec61γ subunit secondary PCR product could be roughly estimated to be 199 bp, taking into account that 30 bp of the PCR product are derived from the GeneRacer RNA Oligo. The soybean positive control lanes in both conventional cultivar Conquista and CV127 show PCR products with similar intensity at the estimated size for the endogenous soybean Sec61γ subunit (Figure V-9). This proportional amplification of the positive control indicates that the band observed with Arabidopsis SEC61γ-specific primers in CV127, but not in conventional Conquista, is not due to non-specific background amplification.

CV127 RLM-5'-RACE secondary PCR products amplified with Arabidopsis SEC61γ subunit-specific primer RS42 are not a single distinct band, indicating multiple transcript sizes (Figure V-9). The presence of PCR products in the corresponding "minus tobacco acid pyrophosphatase (TAP)" control lane indicates that some of these PCR products are derived from partial transcripts. However, because the largest PCR product in this "minus TAP" control lane is clearly smaller than the largest PCR products observed in the corresponding "plus TAP" lane, it is expected that the largest CV127 secondary PCR products amplified with Arabidopsis SEC61γ subunit-specific primers represent full-length transcripts. The largest CV127 RLM-5'-RACE secondary PCR product amplified with Arabidopsis SEC61γ subunit−specific primers is clearly larger than the PCR product amplified from Arabidopsis ecotype Col-0 with Arabidopsis SEC61γ subunit-specific primers suggesting a transcriptional fusion in CV127 (Figure V-9).

The resulting RLM-5'-RACE PCR product was sequenced and compared with the predicted mRNA sequence of the At*SEC61γ* subunit gene. The sequence data obtained indicated that the Arabidopsis *SEC61γ*-subunit transcript in CV127 soybean is a transcriptional fusion with a short segment (89 nucleotides) of adjacent soybean flanking sequence. However, the first "ATG" start codon in the sequence coincides with the predicted translation initiation site of the native Arabidopsis *SEC61γ*-subunit gene. As there are no changes at the nucleotide level, the predicted protein sequence of the Arabidopsis SEC61γ-subunit protein in soybean CV127 does not differ from the native SEC61γ-subunit protein in Arabidopsis. Further experiments were conducted to determine if detectable amounts of the AtSEC61*γ*-subunit protein are produced in CV127 tissues. These experiments demonstrated that there are no detectable amounts of the SEC61γ-subunit protein present in CV127 tissues (LOD = 30 ng/g fresh weight for leaf tissue and 110 ng/g grain tissue). Further details of these experiments are presented in Section VI.E.

Figure V-8. RT-PCR Analysis of Transcription of the At*SEC61* γ Subunit Gene in CV127 Soybean.
Total RNA from CV127 F_7 generation plant leaf tissue was extracted with Qiagen RNeasy Mini Kit and treated with DNase and total RNA from Arabidopsis leaf (At-L) and root (At-R) was extracted with TRIzol reagent without DNase treatment. Primers specific to the soybean proteasomal *Iota* subunit gene and the endogenous soybean *SEC61*γ subunit gene (Gm*SEC61*γ) are used in positive control reactions. Reactions without template RNA using At*SEC61* γ subunit-specific primers and Gm*SEC61*γ subunit-specific primers are used as negative controls. M: 1 kb DNA ladder. The arrow indicates the faint RT-PCR product corresponding to the At*SEC61*γ subunit gene fragment amplified from soybean CV127.

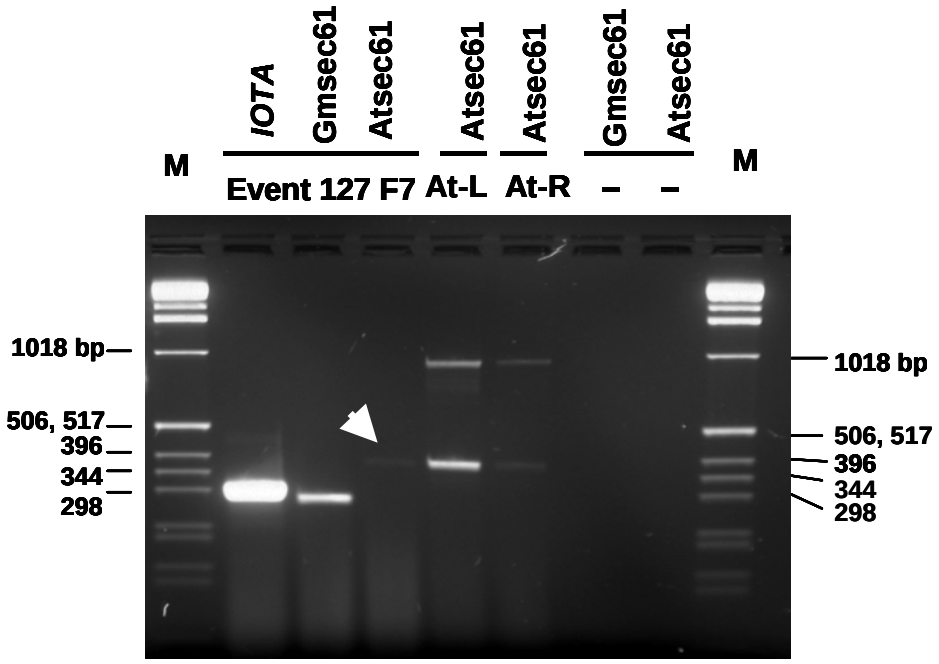
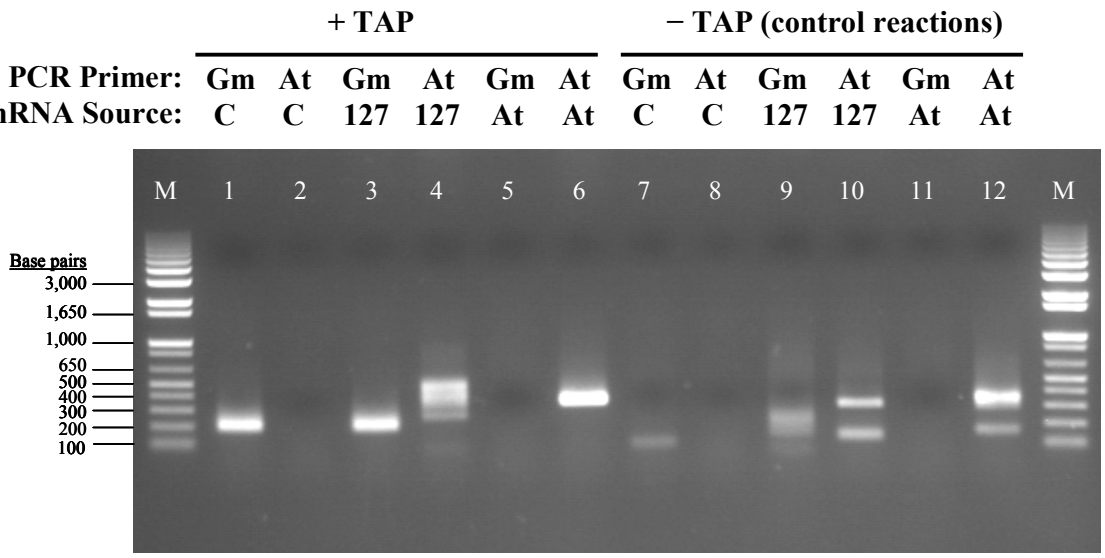


Figure V-9. RLM-5'-RACE Secondary PCR Analysis of the At*SEC61* γ Subunit Gene in CV127 Soybean.

Messenger RNA extracted from CV127 ("127" in figure below), conventional soybean variety Conquista ("C" in figure below), and Arabidopsis ecotype Col-0 ("At" in figure below) leaf tissue was used as a template for RLM-5'-RACE. In the RLM-5'-RACE secondary PCR, the GeneRacer 5' Nested Primer (Invitrogen) was paired with either a primer expected to specifically amplify endogenous soybean *Sec61γ* ("Gm" in figure below) or a primer expected to specifically amplify Arabidopsis *SEC61γ* ("At" in figure below). Lanes 7 through 12 are identical to lanes 1 to 6, respectively, with the exception that lanes 7 through 12 were not treated with tobacco acid pyrophosphatase (TAP) during the RLM-5'-RACE procedure. Samples in lanes 7 through 12 serve as controls: any products observed in these lanes are derived from non-full-length transcripts. Lanes labeled "M" each contain 700 ng of 1 kb Plus DNA Ladder (Invitrogen).



Summary of Expected and Observed Results for RLM-5'-RACE Secondary PCR

Lane in Gel	mRNA Source	Gene-Specific Secondary PCR Primer	Expected Band Size	Expected Result Observed?
1	Conquista leaf	*Sec61γ*, soybean	~199 bp[a]	yes
2	Conquista leaf	*SEC61γ*, Arabidopsis	none	yes
3	CV127 leaf	*Sec61γ*, soybean	~199 bp[a]	yes
4	CV127 leaf	*SEC61γ*, Arabidopsis	?	smeared band
5	Arabidopsis leaf	*Sec61γ*, soybean	none	yes
6	Arabidopsis leaf	*SEC61γ*, Arabidopsis	342 bp[b]	yes

[a]This "expected" band size is estimated: 30 bp are derived from the GeneRacer RNA Oligo and an estimated 169 bp are derived from soybean *Sec61γ*.

[b]A 342 bp band is expected: 30 bp are derived from the GeneRacer RNA Oligo and 312 bp are derived from Arabidopsis *SEC61γ*.

F. Inheritance of the Imidazolinone-Tolerance Trait in CV127.

The inheritance of the imidazolinone herbicide tolerance trait in CV127 soybeans was determined by evaluating the F_3 and F_4 progeny from the cross of CV127 line CV603 (F_8 generation, homozygous for the *csr1-2* gene) with a conventional, imidazolinone-sensitive line with the pedigree Conquista*3 x BRI98-641 (Figure III-1). Individual F_1 seed, hemizygous for the *csr1-2* gene, were planted in a greenhouse and the plants were self-pollinated to produce individual populations of F_2 seed segregating for the *csr1-2* gene (one F_2 population per each F_1 plant). Seed from one F_2 population were planted in the greenhouse and each plant was self-pollinated to produce individual F_3 families (one F_3 family per each F_2 plant).

Seed from each F_2-derived F_3 family ($F_{2:3}$) were planted in a greenhouse and six to eight plants within each family were sprayed with imazapyr herbicide (100 g ai/ha) to identify families that were segregating for the *csr1-2* gene. The number of homozygous, hemizygous, or null plants present within each of two families, 'family 12' and 'family 13', segregating for the *csr1-2* gene was determined by a quantitative PCR assay. The proportion of individuals in families 12 and 13 that were homozygous, hemizygous, or null for the *csr1-2* gene and the results of the chi-square test for the 1:2:1 segregation pattern expected for a single gene are presented in Table V-2. The results of the chi-square test ($P<0.05$) support the single-gene hypothesis and indicate that tolerance to the imidazolinone herbicide is expressed by a single gene that is inherited according to the principles of Mendelian genetics.

Each F_3 plant within families 12 and 13 which was identified as hemizygous based on the PCR assay, was self-pollinated. The F_4 seed from each hemizygous F_3 plant were bulked together, by family, and planted in a greenhouse. After spraying each F_4 plant with imazapyr (100 g ai/ha), each plant was classified 'resistant' (no foliar symptoms) or 'sensitive' (foliar symptoms and/or plant death) to the herbicide. The proportion of individuals in families 12 and 13 that were resistant (homozygous or hemizygous) or sensitive (null) to the imidazolinone herbicide and the results of the chi-square test for the 3:1 segregation pattern expected for a single gene are presented in Table V-3. The results of the chi-square test ($P<0.05$) again support the conclusion that tolerance to an imidazolinone herbicide is due to a single dominant gene that is inherited in a typical Mendelian fashion. Further, the results of these genetic studies are consistent with the conclusions of the molecular characterization studies conducted with CV127 soybean which showed that a single functional *csr1-2* gene is integrated in the genome of CV127.

Table V-2. Chi-Square Analysis Demonstrating Single-Gene Mendelian Inheritance (1:2:1 Segregation) of the *csr1-2* Gene in Two F_3 Families, Each Derived from an Individual Hemizygous F_2 Plant, Based on a Quantitative PCR Assay.

PCR result	Observed number of plants	Expected number of plants	Chi-square value for 1:2:1 hypothesis
Family 12			
Hemizygous	30	28.50	
Homozygous (positive)	18	14.25	$X^2 = 3.00$
Homozygous (negative)	9	14.25	$P = 0.22$
Total	57	57	
Family 13			
Hemizygous	23	26	
Homozygous (positive)	14	13	$X^2 = 0.73$
Homozygous (negative)	15	13	$P = 0.69$
Total	52	52	

Table V-3. Chi-Square Analysis Demonstrating Single-Gene Mendelian Inheritance (3:1 Segregation) of the *csr1-2* Phenotype in Two F_4 Progeny Derived from Hemizygous Plants from Two F_3 Families Based on a Herbicide Tolerance Assay.

Herbicide result	Observed number of plants	Expected number of plants	Chi-square value for 3:1 hypothesis
Family 12			
Tolerant (homozygous or hemizygous)	493	508	$X^2 = 1.89$
Sensitive (null)	185	169	$P = 0.17$
Total	678	678	
Family 13			
Tolerant (homozygous or hemizygous)	330	340	$X^2 = 1.30$
Sensitive (null)	124	113	$P = 0.26$
Total	454	454	

G. Conclusions of the Molecular Characterization

Molecular analyses were performed to characterize the integrated DNA insert in CV127 soybean. Southern blot analyses were used to determine the number of DNA insertions in the soybean genome, the number of copies of the insert, the intactness of the *csr1-2* gene cassette, and to establish the absence of plasmid backbone elements in the genome. The stability of the DNA insert across multiple generations was also demonstrated by Southern blot analysis. Finally, the complete nucleotide sequence of the insert was determined to confirm the organization and integrity of the genetic elements within the insert.

The results of these analyses demonstrated that one intact copy of the *csr1-2* gene cassette was integrated at a single locus in the chromosome. No elements derived from the backbone of the plasmid pAC321 either linked or unlinked to the insert were detected in the genome of CV127. Southern blot analyses of DNA isolated from multiple generations of CV127 soybean demonstrated that the transgene insert is stably integrated in the soybean genome over multiple breeding generations. In addition, determination of the nucleotide sequence of the entire insert confirmed the intactness of the insert and demonstrated that the organization of the genetic elements are identical to their organization in plasmid pAC321. Also, trait inheritance studies across breeding generations confirmed the expected trait segregation ratios, further confirming that the imidazolinone herbicide tolerance trait in CV127 soybean is conferred by a single functional copy of the *csr1-2* gene and that the DNA insert in CV127 soybean is stably integrated in the plant genome across multiple breeding generations.

VI. Characterization of Proteins Expressed in CV127 Soybean

CV127 soybeans were produced by introduction of the imidazolinone-tolerant acetohydroxyacid synthase large subunit gene *csr1-2* from *Arabidopsis thaliana* into the soybean plant genome. The *csr1-2* gene encodes an AtAHAS protein that is tolerant to imidazolinone herbicides due to a single nucleotide mutation that results in a single amino acid substitution in which the serine residue at position 653 of the protein is replaced by asparagine (S653N). This amino acid change in plant AHAS proteins is known to prevent the binding of imidazolinone herbicides and thereby to result in tolerance to these herbicides (Tan *et al.*, 2005).

As part of the food, feed and environmental safety assessment of CV127 soybean, studies were conducted to confirm that the AtAHAS protein produced in CV127 soybeans is equivalent to other AHAS proteins that are found ubiquitously among plant species and have a history of safe use in food and feed products, as well as safety to the environment. These safety assessments of the AtAHAS protein included a mouse acute gavage study and a digestive fate study conducted with purified AtAHAS protein. Because the AtAHAS protein is expressed at extremely low levels in tissues of CV127 soybean plants (refer to Section VI.D. of this petition), it was not technically feasible to extract sufficient quantities of the AtAHAS protein from soybean tissues for the safety assessments of the protein. Therefore, the same AtAHAS coding sequence introduced in the CV127 soybean genome was introduced into *Escherichia coli* for over-expression of the AtAHAS protein. Gram quantities of the AtAHAS protein were purified from the *Escherichia coli* over-expression system for the protein safety assessment studies. Key biochemical and functional parameters of the CV127-produced and *Escherichia coli*-produced AtAHAS proteins were characterized to demonstrate that the plant-produced protein is equivalent to the microbially-produced AtAHAS protein, and therefore justify the use of the microbially-produced protein for safety assessment studies of the AtAHAS protein produced in CV127 soybean.

This section of the petition presents the following characterization studies of the AtAHAS protein expressed in CV127 soybeans:

A. Biochemistry of the AtAHAS protein and imidazolinone herbicide tolerance;
B. Characterization of the AtAHAS protein produced in CV127 soybean and equivalence to the *E. coli*-produced protein used in safety studies;
C. A summary of the food, feed and environmental safety of the AtAHAS protein;
D. Expression levels of the AtAHAS protein in CV127 soybean tissues.

In addition, studies are presented in this section of the petition that address whether the AtSEC61γ subunit protein is expressed in CV127 soybean tissues, and a summary of the food, feed and environmental safety of the AtSEC61γ subunit protein is included.

A. Biochemistry of the AtAHAS Protein and Imidazolinone Herbicide Tolerance

The mechanism of action of imidazolinone herbicides on weeds and non-tolerant plants is by inhibition of the AHAS enzyme and branched-chain amino acid biosynthesis. The AHAS enzyme is ubiquitous in plants and microbes and catalyzes the first step in the biosynthesis of the essential branched-chain amino acids valine, leucine, and isoleucine (Stidham and Singh, 1991). The AHAS enzyme of eukaryotes is composed of a large catalytic subunit (AHAS) and a small regulatory subunit. The enzyme catalyzes the condensation of two molecules of pyruvate to form acetolactate, the precursor of valine and leucine, or the condensation of a molecule of pyruvate with a molecule of 2-ketobutyrate to form 2-aceto-2-hydroxybutyrate, an intermediate in isoleucine biosynthesis (Delfourne *et al*., 1994; Singh and Shaner, 1995; Duggleby and Pang, 2000). AHAS is the key control enzyme within the branched-chain amino acid biosynthetic pathway and is regulated by feedback inhibition by the end-product amino acids, valine, leucine and isoleucine. Regulation is mediated through binding of these amino acids to the AHAS small (regulatory) subunit.

Imidazolinone herbicides readily inhibit the activity of AHAS by binding to the active site of the protein resulting in plant death (Duggleby and Pang, 2000; Tan *et al*., 2005). Molecular modeling of the AHAS–imidazolinone interaction suggests that the herbicide binding pocket is at the entry site for the substrate of the AHAS enzyme, and imidazolinones may inhibit the enzyme by impeding substrate binding to AHAS (Tan *et al*., 2005). Studies have shown that specific single-nucleotide mutations in the *AHAS* genes result in single amino acid substitutions in the AHAS protein, and these amino acid substitutions confer tolerance to imidazolinone herbicides by altering the binding site for these herbicides on the mutant AHAS enzymes (Tan *et al*., 2005). Imidazolinone herbicide-tolerant amino acid substitutions are located in all three domains of the AHAS protein, but protein folding places all the amino acid substitutions that confer herbicide tolerance at the proposed herbicide-binding site of the protein. While these amino acid substitutions confer herbicide tolerance to the AHAS protein, they have no effect on feedback regulation by branched-chain amino acids and the normal biosynthetic function of the enzyme (Newhouse *et al*., 1992).

CV127 soybean contains the *csr1-2* gene from *Arabidopsis thaliana* that encodes an imidazolinone-tolerant AHAS enzyme (Sathasivan *et al*., 1990). The *csr1-2* gene encodes a single polypeptide of 670 amino acids that includes the *A. thaliana* native chloroplast transit peptide (CTP) which is predicted to consist of 85 amino acids (Mazur *et al*., 1987). During transport into the chloroplast, it is predicted that the chloroplast transit peptide is removed to produce the mature AtAHAS enzyme that is approximately 64,000 molecular weight and consists of 585 amino acids (Mazur *et al*., 1987). The amino acid residues of AtAHAS are counted from the N-terminus of the 670 amino acid full-length protein containing the chloroplast transit peptide. The *csr1-2* gene is expressed from its native *A. thaliana* promoter and it also contains the native 3'-untranslated region (UTR) bearing the polyadenylation signal.

The AtAHAS protein encoded by the *csr1-2* gene in CV127 soybean is functionally identical to the native AtAHAS, except for its tolerance to imidazolinone herbicides. The mutation in the *csr1-2* gene responsible for imidazolinone herbicide tolerance is a single nucleotide change of

guanine to adenine, which results in a codon change from AGT to AAT and a single amino acid substitution of serine to asparagine at position 653 of the AtAHAS protein. This amino acid change in plant AHAS proteins is known to confer imidazolinone herbicide tolerance, but have no effect on feedback regulation by branched-chain amino acids or normal biosynthetic function of the enzyme (Newhouse *et al*., 1992; Tan *et al*., 2005). In addition, a second mutation was discovered in the *csr1-2* gene integrated in the genome of CV127 soybean. This mutation, in which an arginine residue at position 272 is replaced by lysine, does not impact the enzymatic function of the AHAS enzyme or herbicide tolerance properties. A study was conducted to compare the AtAHAS subunit protein expressed in CV127 that contains both the S653N and R272K mutations with the AtAHAS encoded by the *csr1-2* gene that contains only the S653N mutation. In this study it was demonstrated that both AtAHAS enzymes had equivalent levels of catalytic activity and tolerance to imidazolinone herbicides. Feedback regulation of AHAS activity by the branched-chain amino acids is effected through the AHAS small subunit. The AtAHAS enzyme encoded by the *csr1-2* gene in CV127 is expected to interact with the endogenous soybean AHAS small subunit protein (AHASS) to achieve this regulation. Therefore, the feedback regulation of the AtAHAS encoded by the *csr1-2* gene in CV127 was expected to be identical to that of the endogenous soybean AHAS. This was confirmed in enzyme activity studies with the CV127-produced AtAHAS protein (section VI.B.). Also, this conclusion was supported by results of grain compositional analyses that showed levels of branched-chain amino acids in CV127 soybean are comparable to levels in the control soybean (refer to section VII. of this petition), indicating that regulation of this biosynthetic pathway is equivalent between CV127 soybean and its conventional comparators.

Several imidazolinone herbicide-tolerant crops that produce AHAS enzymes with the same serine to asparagine substitution at residue 653 have been commercialized and cultivated for many years without any adverse environmental or health effects. These crops have been marketed under the **Clearfield®** brand name and include (*Zea mays* L.), rice (*Oryza sativa* L.), bread wheat (*Triticum aestivum* L.), and oilseed rape (*Brassica napus* L.). Therefore, there has been a long history of safe production of crops containing an imidazolinone herbicide-tolerant AHAS protein with the same S653N amino acid substitution as that in the AtAHAS encoded by the *csr1-2* gene in CV127 soybeans. These crops have been used to produce food and feed products that have proven to be as nutritious and as safe as similar products produced from conventional crops, and have posed no different environmental impact than the corresponding conventional crop.

B. Characterization of the AtAHAS Protein Produced in CV127 Soybean and Equivalence to the *E. coli*-Produced Protein Used in Safety Studies

As described above in this section of the petition, it was not technically feasible to purify gram quantities of the AtAHAS protein from CV127 soybean to conduct protein safety assessment studies. Therefore, the same coding sequence for the predicted mature AtAHAS protein in CV127 soybean was introduced into *Escherichia coli* for over-expression of the AtAHAS protein, and the microbially-produced protein was used for the protein safety studies, including digestive fate and an acute mouse gavage study. Therefore, the evaluation of the AtAHAS protein produced in CV127 soybean is presented in this section and the results are compared to those of a similar evaluation of the protein produced in the *Escherichia coli* expression system in order to establish equivalence between these AtAHAS proteins from different sources, and justify use of the protein safety data generated from the *Escherichia coli*-produced protein to confirm the safety of the AtAHAS produced in CV127 soybean.

The AtAHAS produced in the *Escherichia coli* expression system lacks the predicted 85 amino acid N-terminal chloroplast transit peptide (CTP) sequence that targets the protein *in planta* to the chloroplast (Mazur *et al*., 1987). The *Escherichia coli*-produced AtAHAS protein contains the S653N amino acid substitution present in the AtAHAS protein encoded by the *A. thaliana csr1-2* gene, as well as the R272K amino acid substitution that was discovered in the *csr1-2* gene in CV127 soybean. AHAS protein was purified from young leaf tissue of CV127 soybean plants and the biochemical characteristics of the protein were assessed and compared to that of the *E. coli*-produced AtAHAS protein. The biochemical characteristics that were assessed included: 1) molecular weight, 2) immunoreactivity with AHAS-specific antibody, 3) enzymatic activity and the inhibition of the activity by imidazolinone herbicide and feedback inhibition by branched-chain amino acids, 4) glycosylation, and 5) determination of the amino acid sequence of several peptide fragments derived from purified AHAS protein preparations. The results from these evaluations show that the CV127 soybean-produced AtAHAS protein is equivalent to the AtAHAS protein produced in *Escherichia coli*. A summary of the findings of these analyses are presented below and the materials and methods and a detailed description of the studies are presented in Appendix B of this petition.

The molecular weight and immunoreactivity of the AHAS produced in CV127 (AtAHAS and endogenous soybean AHAS) were shown to be similar to that of the endogenous AHAS from the nontransgenic isoline control soybean and the reference AtAHAS produced in the *Escherichia coli* over-expression system. The microbially-produced AtAHAS and the plant-produced AHAS proteins demonstrated the expected enzymatic activity with the substrate pyruvate, confirming the same functional activity of the AHAS proteins from both microbial and plant sources. The specific activity of the AHAS produced in CV127 soybean was very similar to the endogenous soybean AHAS and was higher than the AHAS in AtAHAS produced in *Escherichia coli*. AHAS is a very unstable protein and readily loses activity upon purification (Chang and Duggleby, 1997). Therefore, it was not unexpected that the AHAS activity in the relatively impure state purified from plant tissues was higher than that retained in the much more pure material produced in *Escherichia coli*. Both CV127-produced and microbially-produced AtAHAS proteins showed tolerance to inhibition by the imidazolinone herbicide imazethapyr

compared to the control plant-produced protein. An intermediate level of inhibition of the CV127-produced AHAS activity was observed, and this was as expected due to the mixture of sensitive (endogenous) and tolerant (transgenic) AHAS proteins present in CV127. Feedback inhibition by branched-chain amino acids, leucine and valine, was very similar for both the AHAS proteins in CV127 and the nontransgenic isoline control soybean. No feedback inhibition was observed for the microbially-produced AtAHAS as expected. Acetohydroxyacid synthase in *Escherichia coli* is composed of two subunits. However, previous studies have demonstrated that when the *A. thaliana* acetohydroxyacid synthase catalytic (large) subunit (AtAHAS) is expressed in *E. coli*, the *E. coli* small (regulatory) subunit is incapable of interacting with the *Arabidopsis thaliana* catalytic or "large" subunit to confer the feedback inhibition observed in *Arabidopsis thaliana* seedlings (Singh *et al*., 1992; Chang and Duggleby, 1997). This result also confirms that the endogenous soybean AHASS must interact with the AtAHAS in CV127 soybean to effect feedback regulation by the branched-chain amino acids. There was no evidence of glycosylation associated with either the AtAHAS in CV127 soybean leaves or the *Escherichia coli*-produced protein.

The amino acid sequence of the AtAHAS protein produced in CV127 soybean was compared with that produced in the *Escherichia coli* expression system. Attempts to obtain N-terminal amino acid sequence of the plant-produced protein using Edman degradation methods were unsuccessful. Therefore, SDS-PAGE followed by liquid chromatography coupled with tandem mass spectroscopy (LC/MS/MS) was used to obtain amino acid sequence data. Peptides derived from trypsin-treated AtAHAS protein prepared from CV127 soybean were isolated and their amino acid sequence determined by liquid LC/MS/MS. The amino acid sequences of peptides derived from CV127 soybean were compared to the deduced amino acid sequence of the AtAHAS protein encoded by the *csr1-2* gene in CV127 soybean. The amino acid sequence obtained covered approximately 23% of the entire predicted AtAHAS amino acid sequence of the CV127-produced protein. All amino acid sequence data obtained from the AtAHAS of CV127 soybeans was identical to the corresponding amino acid sequence of the *Escherichia coli*-produced AtAHAS with the exception of a region near the N-terminus that was predicted to contain the CTP. Based on a sequence comparison of the deduced amino acid sequence of plant and microbial AHAS proteins, Mazur *et al.* (1987) predicted that the CTP of the AtAHAS is cleaved at the C-terminal side of residue 85. However, because AHAS proteins are present at very low levels in plant tissues, the amino acid sequence of the mature AtAHAS has not been determined and the precise location of the cleavage site for the CTP is unknown. Amino acid sequence analysis of the AtAHAS protein produced in CV127 showed a peptide fragment corresponding to amino acid residues 52 to 73. Therefore, the CTP cleavage site of the AtAHAS protein produced in CV127 is most likely not at the predicted site at the C-terminal side of residue 85, but is located at least at the C-terminal side of residue 51.

Even though the data in this study suggest that the mature AtAHAS protein produced in CV127 consists of the predicted mature protein (585 amino acids) plus additional amino acids in the CTP predicted by Mazur *et al*. (1987), the microbially-produced AtAHAS protein is considered equivalent to the CV127 protein for the following reasons. The plant-produced and *E. coli*-produced proteins should show structural, functional, and biochemical equivalence (Codex, 2003). The CV127-produced AtAHAS and *E. coli*-produced AtAHAS demonstrated the same expected enzymatic activity with the substrate pyruvate, thus confirming that AtAHAS from

both sources possessed the same functional activity. Both are resistant to inhibition by the imidazolinone herbicide imazethapyr (Figure B-2), a characteristic produced by the serine to asparagine amino acid substitution at residue 653 (relative to the *Arabidopsis thaliana* sequence). CV127 leaf extracts contain a mixture of both AtAHAS (R272K, S653N) and endogenous GmAHAS and exhibited similar sensitivity to feedback inhibition by the metabolic pathway products, branched–chain amino acids, as leaf extracts from the isoline control soybeans (Figure B-3). Due to the absence of the acetohydroxyacid synthase small (regulatory) subunit in the *E. coli* expression system, the *E. coli*-produced *Arabidopsis thaliana* acetohydroxyacid synthase large (catalytic) subunit did not display feedback inhibition by leucine and valine. This observation is not surprising given that the feedback inhibition is mediated by the regulatory (small) subunit. *In planta*, it is believed that the endogenous *Glycine max* acetohydroxyacid synthase regulatory (small) subunit is capable of interacting with the AtAHAS large (catalytic) subunit to impart the branched-chain amino acid feedback inhibition characteristic to both the AtAHAS and GmAHAS catalytic subunits, thus preserving normal biochemical control mechanisms for a crucial anabolic pathway in CV127 soybean plants.

In addition, the microbially-produced AtAHAS protein and CV127-produced AHAS proteins had similar immunoreactivity to antibodies specific to the AHAS protein based upon results of both western blot and ELISA analyses. Both the microbially-produced AtAHAS protein and the CV127-produced protein showed no evidence of glycosylation. Also, both proteins shared the same amino acid sequence of the predicted mature protein of 585 amino acids, based upon amino acid sequence analysis of overlapping peptide fragments. Finally, *in vitro* digestibility and lability studies also supported the equivalence of the CV127-produced AtAHAS and *E. coli*-produced AtAHAS proteins. Because some food allergens show resistance to digestion by the gastric enzyme pepsin, the CV127-produced AtAHAS and *E. coli*-produced AtAHAS were tested for susceptibility to digestion by pepsin in simulated mammalian gastric fluid (Figure H-1). Both the *E. coli*-produced AtAHAS as well as AtAHAS from grain and leaf extracts of CV127 were rapidly degraded in simulated mammalian gastric fluid. Thus, AtAHAS from CV127 was not shown to display this characteristic of allergenic proteins and was equivalent to the *E. coli*-produced AtAHAS in this respect.

Furthermore, other studies confirm the food, feed and environmental safety of the AtAHAS protein expressed in CV127 soybean.

History of safe use

The acetohydroxyacid synthase large subunit protein is ubiquitous in the plant world. As such, AHAS has a long history of safe use in the diet of humans and animals. While AtAHAS (R272K, S653N), as expressed in CV127, and the microbially-produced AtAHAS sequences do differ at the N-terminal end, numerous other dicotyledon AHAS sequences also show significant sequence divergence at the N-terminal end in the region corresponding to the transit peptide. Although *Arabidopsis thaliana* is not a commonly-consumed crop, the AtAHAS sequence shows extremely high protein sequence identity to AHAS from commonly-consumed crop species: 90% identity to *Brassica napus* AHASL1A (accession number P27819), 79% identity to *Glycine max* AHASL2 (JGI locus Glyma13g31470.1), 77% identity to *Helianthus annuus* AHASL1 (AAT07322), 77% identity to *Phaseolus vulgaris* AHASL (ACV84152), and 77% identity to *Solanum tuberosum* AHASL (ADI56521.1). Moreover, the model plant *Arabidopsis thaliana* is

closely related to *Brassica oleracea*, a species which includes the widely consumed cultivars broccoli, cabbage, brussel sprouts, cauliflower, collard greens, and kohlrabi (Zhang and Wessler, 2004). Thus, while *Arabidopsis thaliana* itself does not have a history of safe use, it shares a common ancestor with a relative with a record of safe consumption.

The AtAHAS (R272K, S653N) protein expressed in CV127 soybean differs from the AtAHAS (P197S) protein expressed in sulfonylurea herbicide-tolerant *Linum usitatissimum* L. (flax) CDC Triffid (FP967) by only three amino acids (McHughen, 1989; Nakamura *et al*., 2010). As is the case in CV127, the AtAHAS (P197S) coding sequence in flax line FP967 is under the control of the native *Arabidopsis thaliana* AHAS large subunit promoter and would be expected to display an expression pattern similar to that of AtAHAS in CV127. The environmental safety of the flax line FP967 was reviewed by the USDA and approved for unconfined environmental release in the United States 12 years ago (University of Saskatchewan, 1999).

Bioinformatics analyses

The full-length deduced amino acid sequence of AtAHAS (R272K, S653N), as expressed in CV127 prior to cleavage of the transit peptide, was subjected to bioinformatics analyses to assess its allergenicity and toxicity potential. The CV127 full-length AtAHAS deduced amino acid sequence, including the entire transit peptide, did not show significant homology to a known protein toxin as defined in the United States Code of Federal Regulations (40 CFR Part 725.421). The CV127 full-length AtAHAS deduced amino acid sequence did not show greater than 35% identity over 80 amino acids to a potential allergen and did not share a sequence of eight or more consecutive identical amino acids with a potential allergen. Thus, the bioinformatics analyses of the full-length AtAHAS (R272K, S653N), as expressed in CV127 soybean prior to cleavage of the transit peptide, do not provide any indication of a potential allergenicity or toxicity concern due to sequence homology to known protein toxins or known allergens.

Expression level and dietary intake

AtAHAS in CV127 utilizes its native plant promoter which expresses low levels of AtAHAS, consistent with typical native AHAS expression patterns. The resulting low level of dietary exposure is a key consideration since acute toxicity is related to dosage.

The level of exposure to a given protein is also a factor in potential allergenicity since the majority of major food allergens are expressed at a high level, greater than 1% of total protein (Lehrer *et al.*, 2002; Metcalfe *et al.*, 1996). AtAHAS protein levels in CV127 grain were shown to be detectable, but not quantifiable, in enzyme-linked immunosorbent assays (ELISAs) using AHAS-specific antibodies (section VI.D.). The AtAHAS protein is very unstable and would be expected to rapidly degrade under the extreme conditions present during standard soybean grain processing, further reducing the already-low levels of AtAHAS in CV127 in human food and animal feed.

Additional safety assessment studies

A 42-day broiler feeding study was conducted to compare the performance of poultry fed soybean meal from CV127 soybeans and broilers fed meal from conventional soybean grain (Table VII-11). This study demonstrated that there was no evidence of a significant difference in the performance of the broilers fed a feed containing soybean meal from CV127 soybean

compared to those fed a feed containing soybean meal from the grain of conventional soybeans. Thus, the nutritional equivalence of CV127 and its isoline control were confirmed and no adverse effects were observed in broilers fed meal containing CV127 soybeans.

Therefore, results of these studies justify the use of the microbially-produced AtAHAS protein as an appropriate substitute for the AtAHAS produced in CV127 in safety assessment studies of the AtAHAS protein (see Appendices B and H for details of these studies).

C. A Summary of the Food, Feed and Environmental Safety of the AtAHAS Protein

The data and information relevant to a safety assessment of the AtAHAS protein produced in CV127 soybean are summarized and reviewed in this section. More detailed information relating to the safety of this protein is reported in other sections of this petition. A comprehensive food and feed safety and nutritional assessment of CV127 soybean has been submitted to the FDA and it includes the following conclusions:

1. The source organism of the *csr1-2* gene that encodes the AtAHAS protein in CV127 soybean, *Arabidopsis thaliana*, is not a known human or animal pathogen and is not known to cause allergic reactions in humans. Similarly, *A. thaliana* is not known to produce toxic substances.

2. The AtAHAS protein expressed in CV127 soybean is structurally and biologically closely related to the AHAS proteins in all plants. For example, there is 79% amino acid identity between the AtAHAS and endogenous soybean AHASL2 (JGI locus Glyma13g31470.1) proteins. Similar levels of amino acid identity and homology are observed between AtAHAS and the AHAS proteins from corn and wheat. Therefore, the AtAHAS protein expressed in CV127 soybean is highly homologous to the AHAS proteins present in these crops with a history of safe production and use in food and feed products.

3. Several imidazolinone-tolerant crops that produce AHAS enzymes with the same serine to asparagine substitution at residue 653 that is present in CV127 soybeans have been commercialized and cultivated for many years without any adverse environmental or health effects. These crops have been marketed under the **Clearfield®** brand name and include maize (*Zea mays* L.), rice (*Oryza sativa* L.), bread wheat (*Triticum aestivum* L.), and oilseed rape (*Brassica napus* L.).

4. An acute oral toxicity study with mice was conducted with purified AtAHAS protein and the results demonstrated no adverse effects in mice with a No Observable Effect Level (NOEL) of equal to or greater than 2620 mg AtAHAS protein/kg body weight, the highest dose level tested.

5. Bioinformatic analyses showed no biologically relevant amino acid sequence homology between the AtAHAS protein expressed in CV127 soybean and known toxins. In addition, analyses were conducted to confirm that the AtAHAS amino acid sequence did not share potentially immunologically relevant amino acid sequence segments or structure with known allergens. Results of these analyses showed that the amino acid sequence of the AtAHAS

protein expressed in CV127 soybean did not share either 35% or greater identity over 80 amino acids or a sequence of eight or more consecutive identical amino acids with any potential allergens. Furthermore, the AtAHAS protein is rapidly degraded in simulated digestive fluids and is rapidly inactivated by temperatures above 60°C, which are characteristics typical of other dietary proteins with a history of safe use in food and feed products.

6. The AtAHAS protein is present at extremely low levels in CV127 soybean grain, approximately less than 1.5 x 10^{-6} % on a dry weight basis (refer to section VI.D. of this petition). Based on this level of protein expression in the grain, as well as average U.S. consumption of soybean food products, it was estimated that the average U.S. dietary exposure to the AtAHAS protein expressed in CV127 soybean would be no greater than $0.5x10^{-10}$ g/kg body weight/day. Compared to the NOEL of >2620 mg/kg body weight, the exposure level represents a human dietary safety factor of greater than 10^{10} for the AtAHAS protein in CV127 soybeans.

7. The AtAHAS produced in CV127 soybean is an enzyme, all enzymes are proteins, and all proteins have a primary structure that consists of their amino acid sequence. Since proteins are regarded as nutrients and are degraded in the vertebrate digestive system to their constituent amino acids, the main basis for toxicity of a protein is due to its biological mode of action. The mode of action of AHAS enzymes is well known and there is no scientific basis to suggest that its activity would result in toxicity.

Therefore, results of these studies show that the AtAHAS protein expressed in CV127 soybean does not possess any attributes of known food allergens, is not toxic to mammals, and supports the environmental as well as food and feed safety of the AtAHAS protein expressed in CV127 soybean.

D. Levels of the AtAHAS Protein in CV127 Soybean

To assess the level of production (or expression) of the AtAHAS enzyme in CV127 soybean that is encoded by the *csr1-2* gene, the amount of AHAS protein was determined in tissues of CV127 soybean and the isoline control that were grown in Brazil in two different growing seasons. Seven field trials in the 2006/2007 growing season and six in the 2007 growing season were conducted in geographically distinct locations that were representative of commercial soybean production areas in Brazil. The field sites were located in or near the locations listed as follows:

Field Trial Locations for the AHAS Expression Study		
Location	Trial Season 1 Oct 2006-Mar 2007	Trial Season 2 Feb –July 2007
Santo Antônio de Posse – SP	√	
Ponta Grossa – PR/South	√	
Londrina – PR/North	√	
Uberaba – MG	√	√
Brasília – DF	√	√
Santo Antônio de Goiás – GO	√	√
Sete Lagoas-MG	√	√
Teresina - PI		√
Vilhena - RO		√

A detailed description of these field trials is presented in Section VIII.C. The results of the AHAS protein expression study are summarized here and the materials and methods used in the study are presented in Appendix C.

Each trial location had two replications each of CV127 soybean and the isoline control soybean for this study. All plots of the CV127 soybean were sprayed with an imidazolinone herbicide for weed control, whereas all plots of the isoline control were treated with a conventional herbicide. In each of the two field trial seasons, leaf and grain samples were collected at all field trial sites at the V2 and R8 growth stages, respectively. In addition, six whole plants per plot, including roots, were collected at two of the trial sites at three different developmental stages, including the V2 (plants 15-20 cm tall with three nodes and two unfolded leaflets), R2 (plants in full bloom), and R8 (full maturity) stages. Three whole plants were maintained as such, and the remaining three whole plants were dissected into plant parts, including leaves, stems, roots, flowers and pods, depending on the stage of plant development. Because of a late planting date coupled with the southerly location of the Ponta Grossa field site in the 2006/2007 growing season, plants of all treatments failed to reach full maturity and grain was not harvested from treatments at this field site. Therefore, there is no AHAS expression data for grain from either CV127 or the isoline control at this field location.

Expression levels of AHAS enzyme in different tissue samples were determined by enzyme-linked immunosorbent assay (ELISA) using AHAS-specific antibodies. Due to the high level of

amino acid homology between the AtAHAS and the endogenous soybean AHAS enzymes, the AHAS-specific antibodies used in the ELISA assay were not capable of distinguishing between the AtAHAS and endogenous soybean AHAS proteins, and the total AHAS levels of soybean tissues were measured in this study. Therefore, it was expected that tissues from CV127 soybean that are expressing both the AtAHAS protein and the endogenous soybean AHAS proteins would have higher levels of total AHAS protein compared to the isoline control that only expresses the endogenous soybean AHAS proteins. The difference between the AHAS amount in CV127 plants and the isoline control is attributed to the expression of the AtAHAS protein. The limit of quantification (LOQ) and limit of detection (LOD) for AHAS by the ELISA assay were determined experimentally for each tissue type in each experiment and these are listed in Table VI-1. The limit of detection (LOD) is defined as the lowest amount of AHAS that can be detected with suitable confidence by the assay, but not necessarily reliably quantified. The LOD is determined by taking the results of at least 24 separately-determined "blank" assays and calculating two times the standard deviation. The limit of quantitation (LOQ) is the lowest amount of AHAS that can be accurately quantified by the assay with a defined degree of confidence. The ELISA used in the analyses of AHAS protein levels in plant tissues yields a sigmoidal response curve to different concentrations of the AHAS protein. The assay is accurate only in the linear portion of this curve. Values which are above zero, but below the linear portion of the standard response curve are considered to be non-quantifiable since the accuracy of the standard curve in this region is low. Therefore, these values may be considered to be detectable (if above the LOD) but not quantifiable (below the LOQ).

Amount of AHAS enzyme in CV127 soybean and isoline control tissues. AHAS protein levels were generally higher at all field locations in CV127 soybean than in the isoline control, which is consistent with the fact that the ELISA assay detects both the AtAHAS protein encoded by the *csr1-2* gene and the endogenous soybean AHAS enzymes. AHAS enzymatic activity is known to be highest in growing tissues where the need for branched-chain and other amino acids is greatest due to the higher level of *de novo* protein synthesis (Stidham and Singh, 1991). The AHAS protein expression results from both trial seasons reflect this since the highest levels of AHAS were detected in leaves and whole plants at the V2 growth stage (Tables VI-2 and VI-3). During the 2006/2007 season AHAS protein levels in leaves of CV127 soybean plants from all sites at the V2 stage ranged from 53 to 128 ng AHAS/g tissue and 335 to 714 ng AHAS/g tissue on a fresh and dry weight basis, respectively (Tables VI-2 and VI-3). AHAS levels of 18 to 59 ng AHAS/g fresh tissue and <80 to 254 ng AHAS/g dry weight were determined for the leaves of CV127 soybean plants at the V2 stage during the 2007 season (Tables VI-2 and VI-3). In the isoline control leaf tissues at the V2 stage, the AHAS protein levels on a fresh weight basis at all sites ranged from below the limit of quantification (LOQ = 13 ng/g) to 16 ng/g during the 2006/2007 season and were equal to or below the LOQ (14 ng/g) at all locations during the 2007 season (Tables VI-2 and VI-3).

Expression levels of the AHAS protein decreased with age of the plant and AHAS protein levels were either barely detectable or below the limit of detection of the assay in roots, older leaves, reproductive tissues and grain, although season one CV127 tissues at Santo Antônio de Posse did have higher AHAS levels on a dry-weight basis than were observed at other sites and seasons at this same growth stage. Plant tissues collected from CV127 and isoline control plants at two locations in the R2 and R8 growth stages during the 2006/2007 season had low levels of AHAS

protein ranging from below the LOQ (13 ng/g fresh weight) to 34 ng/g fresh weight (Table VI-2). Similar results were determined during the 2007 season with AHAS levels below or near the LOQ. It is noteworthy that AHAS levels in all grain samples from CV127 soybean and the isoline control from both growing seasons were at or below the LOQ (Tables VI-2 and VI-3). Even though the expression levels of the AtAHAS protein in CV127 soybean are extremely low, especially at later stages of plant growth and development, these levels were sufficient to confer herbicide tolerance to CV127 soybean plants.

Table VI-1. The Calculated Limit of Detection (LOD) and Limit of Quantification (LOQ) for AHAS Protein by ELISA.
Data are presented for different tissues derived from soybeans during the field trials conducted in Brazil during the 2006/2007 (Season 1) and 2007 (Season 2) growing seasons.

LOQ (ng AHAS/g Fresh Wt.)		Season 1		Season 2	
		13		14	
Tissue	Growth Stage	LOQ (ng AHAS/g Dry Wt.)		LOD (ng AHAS/g Dry Wt.)	
		Season 1	Season 2	Season 1	Season 2
Whole Plants	V2	68	78	16	17
	R2	65	66	15	14
	R8	15	28	3	6
Leaves	V2	70	76	16	16
	R2	62	59	14	13
Roots	V2	41	51	9	11
	R2	38	41	9	9
	R8	17	37	4	8
Flowers	R2	77	83	18	18
Pods	R8	15	17	3	4
Grain	R8	15	15	3	3

Table VI-2. AHAS Protein Levels (± Std. Dev.) on a Fresh- and Dry-Weight Basis in Different Tissues and Growth Stages of CV127 Soybean and the Isoline Control.

Plants were cultivated at Londrina (Lond), Santo Antônio de Posse (SAP), Brasília (Bras), and Santo Antônio de Goiás (SAG) during the 2006/2007 and 2007 growing seasons (Season 1 and Season 2, respectively).

Growth Stage	Soybean Line	Tissue	Mean ng AHAS/g Fresh Wt (± Std Dev)				Mean ng AHAS/g Dry Wt[1] (± Std Dev)			
			Season 1		Season 2		Season 1		Season 2	
		Location	Lond	SAP	Bras	SAG	Lond	SAP	Bras	SAG
V2	CV127	Whole Plant	6 ± 10	40 ± 6	<14	<14	314 ± 59	214 ± 37	<78	<78
		Leaves	103 ± 24	128 ± 47	27 ± 9	18 ± 4	511 ± 116	714 ± 284	133 ± 51	113 ± 31
		Roots	<13[2]	<13	<14	<14	<41	<41	<51	<51
		1st Trifoliate	55 ± 5	60 ± 12	22 ± 8	21 ± 4	278 ± 27	300 ± 47	111 ± 40	126 ± 23
	Isoline Control	Whole Plant	<13	<13	<14	<14	<68	<68	<78	<78
		Leaves	15 ± 3	14 ± 1	<14	<14	78 ± 17	73 ± 8	<76	<76
		Roots	<13	<13	<14	<14	<41	<41	<51	<51
		1st Trifoliate	<13	<13	<14	<14	<70	<70	<76	<76
R2	CV127	Whole Plant	ND[3]	34 ± 6	<14	<14	ND	160 ± 33	<66	<66
		Leaves	<13	24 ± 4	<14	<14	<62	106 ± 19	<59	<59
		Roots	<13	17 ± 4	<14	<14	<38	50 ± 13	<41	<41
		Flowers	<13	22 ± 9	<14	-[4]	<77	125 ± 45	<83	-
	Isoline Control	Whole Plant	ND	<13	<14	<14	ND	<65	<66	<66
		Leaves	ND	<13	<14	<14	ND	<62	<59	<59
		Roots	<13	<13	<14	<14	<38	<38	<41	<41
		Flowers	<13	<13	<14	-	<77	<77	<83	-

Table VI-2. continued.

Growth Stage	Soybean Line	Tissue	Mean ng AHAS/g Fresh Wt (± Std Dev)				Mean ng AHAS/g Dry Wt[1] (± Std Dev)			
			Season 1		Season 2		Season 1		Season 2	
		Location	Lond	SAP	Bras	SAG	Lond	SAP	Bras	SAG
R8	CV127	Whole Plant	<13	<13	<14	<14	<15	<15	<28	<28
		Pods	24 ± 1	26 ± 2	<14	<14	26 ± 1	30 ± 2	<17	<17
		Roots	<13	<13	15 ± 5	17 ± 8	<17	<17	42 ± 13	48 ± 27
		Grain	<13	<13	<14	<14	<15	<15	<15	<15
	Isoline Control	Whole Plant	<13	<13	<14	<14	<15	<15	<28	<28
		Pods	<13	<13	<14	<14	<15	<15	<17	<17
		Roots	ND	ND	15 ± 3	<14	ND	ND	41 ± 8	<37
		Grain	<13	<13	<14	<14	<15	<15	<15	<15

[1]The limit of quantification (LOQ) on a dry-weight basis varied for each tissue type and growth stage based on the moisture content of the fresh tissues. Values below the LOQ are reported as less than the LOQ for that tissue and growth stage. LOQ values for different tissues are shown in Table VI-1.
[2]Sample had detectable but non-quantifiable amounts of AHAS. That is, value was below the limit of quantification (13 or 14 ng AHAS/g fresh weight in the 2006/2007 and 2007 seasons, respectively), but detectable.
[3]ND = AHAS was considered non-detectable because the result generated by ELISA was below the limit of detection (LOD) of the method. LOD values for different tissues are shown in Table VI-1.
[4]Not tested due to insufficient amount of flower tissues at this site.

Table VI-3. AHAS Protein Levels (± Std. Dev.) on a Fresh- and Dry-Weight Basis in Leaves (V2 stage) and Grain (R8 stage) of CV127 Soybean and the Isoline Control.
Plants were cultivated at field locations in Brazil during the 2006/2007 (Season 1) and 2007 (Season 2) growing seasons.

Growth Stage / Tissue	Location	Soybean Line	Mean ng AHAS/g Fresh Wt (± Std Dev)		Mean ng AHAS/g Dry Wt (± Std Dev)	
			Season 1	Season 2	Season 1	Season 2
V2 / Leaves	Uberaba	CV127	80 ±12	59 ± 57	427 ± 73	254 ± 227
		Isoline	16 ± 0	<14	86 ± 9	<76
	Sete Lagoas	CV127	53 ± 31	40 ± 23	335 ± 189	220 ± 141
		Isoline	<13[1]	<14	<70	<76[2]
	Sto. Ant. de Goiás	CV127	61 ± 13	-[3]	337 ± 53	-
		Isoline	<13	-	<70	-
	Brasília	CV127	61 ± 42	-	363 ± 257	-
		Isoline	<13	-	<70	-
	Ponta Grossa	CV127	92 ± 29	-	478 ± 136	-
		Isoline	<13	-	<70	-
	Teresina	CV127	-	<14	-	<80[4]
		Isoline	-	<14	-	<76
	Vilhena	CV127	-	39 ± 10	-	207 ± 56
		Isoline	-	14	-	<83[4]
R8 / Grain	Uberaba	CV127	<13	<14	<15	<15
		Isoline	<13	<14	<15	<15
	Sete Lagoas	CV127	13 ± 0	<14	<15	<15
		Isoline	<13	<14	<15	<15
	Sto. Ant. de Goiás	CV127	<13	-	<15	-
		Isoline	<13	-	<15	-
	Brasília	CV127	<13	-	<15	-
		Isoline	<13	-	<15	-
	Teresina	CV127	-	<14	-	<15
		Isoline	-	<14	-	<15
	Vilhena	CV127	-	<14	-	<15
		Isoline	-	<14	-	<15

[1] Sample had detectable but non-quantifiable amounts of AHAS. That is, AHAS levels were below the limit of quantification (13 or 14 ng AHAS/g fresh weight in the 2006/2007 and 2007 seasons, respectively), but detectable.
[2] Values below the LOQ are reported as less than the LOQ for that tissue and growth stage. LOQ values for different tissues are shown in Table VI-1.
[3] Sample was not tested at this location.
[4] Value shown is the mean of the values determined for the two replicates at the location. One of these values was determined to be <LOQ. Thus, the mean presented here is the average of the LOQ and value which exceeded the LOQ.

E. Levels of the AtSEC61γ Subunit Protein in CV127 Soybean

As described in section V.E of this petition, the complete coding sequence of the *A. thaliana* gene encoding the *Arabidopsis* SEC61γ (AtSEC61γ) subunit protein resides in the insert of CV127 soybean near the 5' junction with genomic soybean DNA. Although the At*SEC61γ* gene in CV127 soybean appears to lack the majority of its native promoter, experiments were performed to evaluate its possible expression. These demonstrated that a very low amount of mRNA is produced from this gene that, if translated, would encode the native AtSEC61γ–subunit protein. Since the transcript was highly amplified by RT-PCR to maximize detection capability, and only a faint band corresponding to the At*SEC61γ* subunit transcript was detected, it was presumed that either no AtSEC61γ subunit protein is produced in CV127, or if produced the protein would be present at extremely low levels in the plant, acknowledging that transcript and protein levels may not be correlated.

To determine if AtSEC61γ subunit protein is expressed in CV127 soybean tissues, western blot analysis of microsomal membrane protein fractions prepared from leaf and grain samples of CV127 was conducted (methods for these studies are presented in Appendix C of this petition). Identity of the microsomal membrane fraction was confirmed by measuring the activity of cytochrome c reductase, a marker enzyme for the endoplasmic reticulum (found in the microsomal membrane fraction), (Figure VI-2). Protein samples were derived from leaf and grain of CV127 soybean plants as well as leaf and grain tissues from the parental soybean variety Conquista. Both CV127 soybean and control (Conquista) plants were grown under greenhouse conditions. Arabidopsis leaf and seed tissue were also harvested from greenhouse-grown plants. The AtSEC61γ subunit protein used as a standard in these studies was produced and purified from an *Escherichia coli* expression system. An ECL Plus Western Blotting Detection method was used for protein detection on the western blot (Figure VI-1). The rabbit anti-AtSEC61γ polyclonal antibody can detect approximately 0.4 ng of SEC61γ subunit protein by the western blot development method utilized.

The *E. coli*-produced SEC61γ subunit protein was readily detected by western blot analysis in the standard protein lanes at a molecular weight of approximately 7000 (Figure VI-1). Furthermore, expression of the endogenous AtSEC61γ subunit protein in Arabidopsis was detected in microsomal membrane protein preparations from seed but was not detected in Arabidopsis leaf tissue (Figure VI-1). This result showed that the AtSEC61γ subunit protein can be detected in tissues of the plant species which served as the donor of the At*SEC61γ* subunit coding sequence introduced into the genome of CV127 soybean; thus, the Arabidopsis seed tissue served as a positive control for this study. In contrast, western blot analysis detected no AtSEC61γ subunit protein in leaf or grain microsomal membrane protein preparations of CV127 (Figure VI-1, panels A and B). Because of the high amino acid sequence homology of SEC61γ subunit proteins across species (e.g., 86% homology between soybean and Arabidopsis) it was expected that the antibody would detect the endogenous soybean SEC61γ subunit protein; however, no protein band corresponding to the SEC61γ subunit protein was detected in tissue extracts of the control soybean Conquista. Therefore, AtSEC61γ subunit protein was not detectable at levels greater than 30 ng/g leaf issue or 110 ng/g grain tissue or greater than 30 and 110 ppb for leaf and grain of CV127, respectively. This result is not unexpected given the

extremely low levels of the transcript corresponding to the At*SEC61γ* subunit gene detected in CV127.

These results demonstrate that no detectable amounts of the AtSEC61γ subunit protein are produced in either leaf tissue or grain of CV127 soybean. However, in the unlikely event that the AtSEC61γ subunit protein is present in tissues of CV127 at extremely low levels, below the limit of detection of the assay, the following summary confirms the food, feed and environmental safety of the AtSEC61γ subunit protein.

1. The source organism of the AtSEC61γ subunit protein, *Arabidopsis thaliana*, is not a known human or animal pathogen and is not known to cause allergic reactions in humans. Similarly, *A. thaliana* is not known to produce toxic substances.

2. The SEC61γ subunit is part of the protein translocation complex associated with the endoplasmic reticulum and is ubiquitous and highly conserved in eukaryotes as well as being structurally related to analogous proteins in prokaryotes (Hartmann *et al*., 1994). There is a high degree of amino acid sequence homology (86%) between the soybean SEC61γ subunit and that from Arabidopsis (see Figure VI-3 for the amino acid alignment). The amino acid sequence of the Arabidopsis SEC61γ subunit protein is 86% identical and 93% similar to the corresponding protein in soybean. Therefore the AtSEC61γ subunit protein is highly homologous to the same protein produced in a crop with a history of safe use in food and feed products, as well as safety to the environment.

3. Bioinformatic analyses showed no biologically relevant amino acid sequence homology between the AtSEC61γ subunit protein and known toxins. In addition, bioinformatic analyses were conducted to confirm that the AtSEC61γ subunit protein amino acid sequence did not share potentially immunologically relevant amino acid sequence segments or structure with known allergens. Results of these analyses showed that the amino acid sequence of the AtSEC61γ subunit protein did not share either 35% or greater identity over 80 amino acids or a sequence of eight or more consecutive identical amino acids with any potential allergens. Furthermore, the AtSEC61γ subunit protein is rapidly degraded in simulated digestive fluids which is a characteristic typical of other dietary proteins with a history of safe use in food and feed products.

Therefore, results of these studies show that the AtSEC61γ subunit protein does not possess the attributes of known food allergens, is not homologous to known protein toxins, and supports the environmental as well as food and feed safety of the AtSEC61γ subunit protein.

Figure VI-1. SEC61γ Subunit Western Blot Analysis of Leaf and Grain Microsomal Membrane Protein Preparations from CV127 and Control (Conquista) Soybeans

Microsomal membrane proteins were prepared from leaf and grain samples and subjected to electrophoresis on an 8 – 16% polyacrylamide gel. Western blot analysis was conducted using rabbit anti-AtSEC61γ subunit polyclonal antibodies and developed using a chemiluminescent substrate. The molecular weight of AtSEC61γ subunit protein is approximately 7000. All samples except for the AtSEC61γ subunit protein standard are from microsomal membrane protein preparations. Panel A. Lanes 1, and 5, purified SEC 61γ subunit protein standard, 2.4 and 1.2 μg, respectively; lane 2, 20 μg protein from Arabidopsis leaf microsomal membrane protein preparation; lane 3, 20 μg protein from Conquista soybean leaf microsomal membrane protein preparation; lane 4, 20 μg protein from CV127 leaf microsomal membrane protein preparation; lane 6, 15 μg protein from Arabidopsis seed microsomal membrane protein preparation; lane 7, 15 μg protein from Conquista soybean seed microsomal membrane protein preparation and lane 8, 15 μg from CV127 soybean seed microsomal membrane protein preparation. Panel B. Longer exposure of same samples (different blot) from Panel A, lanes 5 – 8. Molecular weight standards are shown to the left of Panel A.

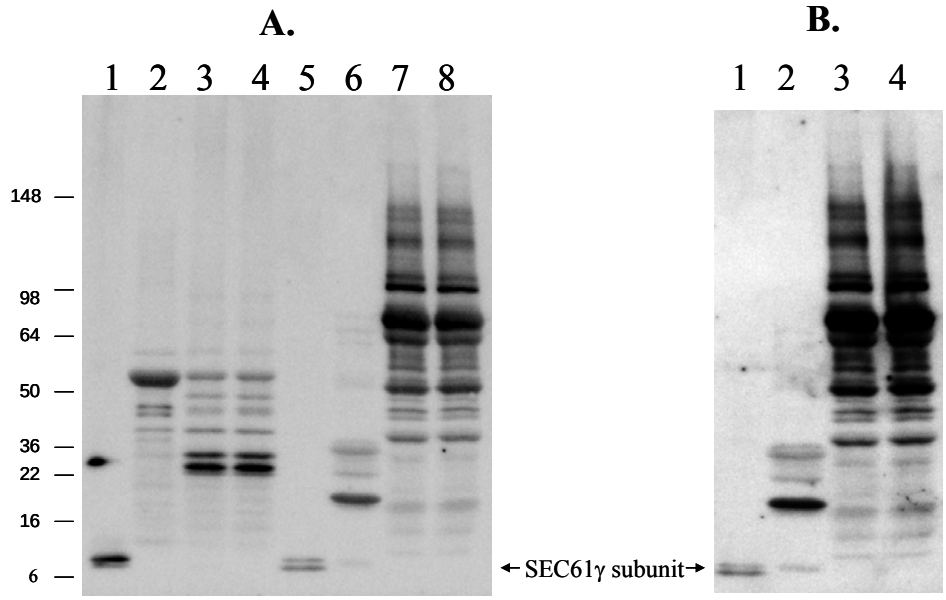
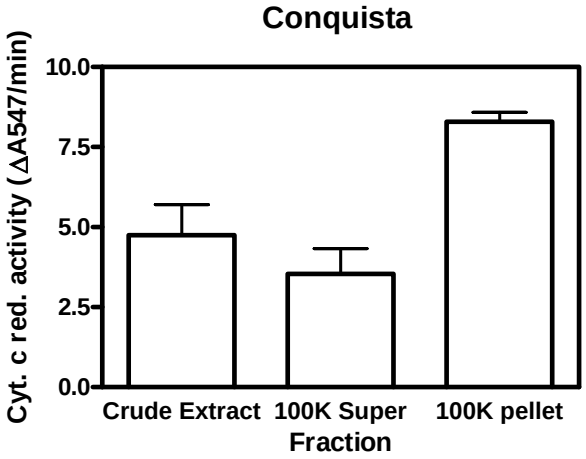


Figure VI-2. Cytochrome c Reductase Activity in Various Fractions Prepared from Leaves of CV127 and Control (Conquista) Soybeans

The crude extract (supernatant after low speed centrifugation, 12,000 x g) and the microsomal membrane fraction (pellet after high speed centrifugation, 100,000 x g) as well as the high-speed centrifugation supernatant were assayed for the endoplasmic reticulum enzyme marker, cytochrome c reductase.



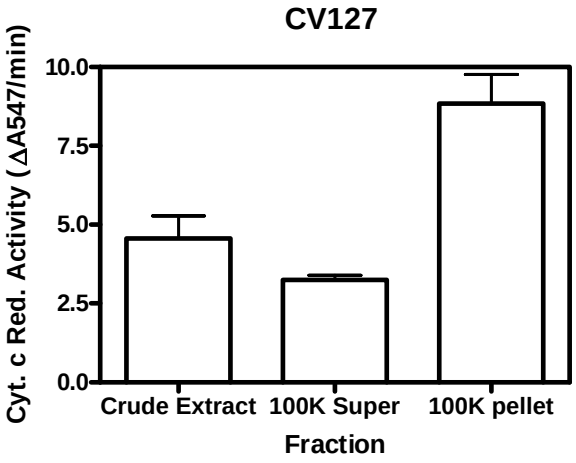


Figure VI-3. Alignment of the Deduced Amino Acid Sequences of the SEC61γ Subunit Proteins from Soybean (Gmax) and *Arabidopsis thaliana* (Athal).
The amino acid sequence for soybean SEC61γ (*G. max* gene Glyma02g42660) was obtained from the Joint Genome Institute Glyma 1.0 annotation available through Phytozome (release version 7.0), http://www.phytozome.net/soybean. The amino acid sequence for *A. thaliana* SEC61γ was deduced from the sequence of the gene encoding the AtSEC61γ subunit protein in CV127 soybeans. Identical amino acids are shaded in grey, conservative replacements are presented as white text in a black background, and non-conservative amino acid differences are unshaded.

```
                10        20        30        40        50
        .........|.........|.........|.........|.........|
Gmax    MDAIDSVFDPLREFAKDSVRLVKRCHKPDRKEFSKVAVRTAIGFVVMGFV
Athal   MEAIDSAIDPLRDFAKSSVRLVQRCHKPDRKEFTKVAVRTAIGFVVMGFV

                60        70
        .........|.........|
Gmax    GFFVKIIFIPINNIIVGSG
Athal   GFFVKLVFIPINNIIVGSS
```

VII. Compositional and Nutritional Assessments of CV127 Soybean

Soybean has many uses in human and animal nutrition. The grain is typically processed into two commodity products, oil and meal. The major food uses for soybean include the production of soybean oil that is utilized in margarines, shortenings, and salad and cooking oils. Soybeans are also used in various food products that include tofu, soya sauce, and simulated milk and meat products. Also, the isolated soybean protein is used in the production of infant formula and other food products as a source of amino acids. Soybean meal is used as a source of protein for animal feed. Other components of the soybean plant, including the forage, are also fed to a limited extent to animals, primarily to cattle. Therefore, the purpose of this section of the petition was to demonstrate that the grain and forage of CV127 is substantially equivalent in composition to grain and forage from the isoline control and other conventional soybean varieties, and that CV127 grain is appropriate for use in human food and animal feed.

Compositional analyses were conducted on grain and forage to confirm that the nutrient and antinutrient levels in the grain and the proximate and fiber levels in forage derived from CV127 soybean are comparable to those from the nontransgenic, isoline control soybean that is closely related to CV127 soybean but that lacks the *csr1-2* gene. Two additional conventional soybean varieties that are typical of soybean varieties commonly cultivated in Brazil, Monsoy 8001 and Coodetec 217, were also included in these analyses to establish a range of natural variability for each analyte for soybeans grown in Brazil. Furthermore, results were compared to analytical data for soybean grain in the International Life Sciences Institute Crop Composition Database (ILSI, 2008), with comparisons to globally-derived values as well as data produced in Brazil, where available. A summary of the results of these comparisons are presented in this section of the petition, and detailed results for grain and forage analyses at each field trial location are

presented in Appendix D. Results showed that CV127 soybean is compositionally and nutritionally equivalent to conventional soybean varieties that are currently cultivated in Brazil as well as globally.

Grain samples of CV127 soybean, the isoline control, and the two conventional soybean varieties were harvested from soybeans grown in four replicated plots at each of six field trial locations in Brazil during the 2006/2007 season and from four field trial locations in the 2007 short season. Forage tissues were harvested at the R2 growth stage from four replicated plots at each of six field trial locations in Brazil during the 2007/2008 growing season (all field trial locations for the production of grain and forage for compositional analyses are presented in Appendix D). Since CV127 soybeans are intended to be cultivated using imidazolinone herbicides for weed control, the grain and forage composition data for CV127 soybeans that is presented in this petition are derived from plants treated with imidazolinone herbicide (70g ai/ha). In the case of grain compositional analyses, an additional treatment of CV127 treated with conventional herbicide (same herbicide application as applied to controls) was included in the study. However, because this treatment will not be used in the commercial production of CV127 soybean, the means and ranges of analyte values across field trial locations for this treatment are not presented in this section of the petition. Data for this treatment at each field trial location are presented in Appendix D. The samples that were obtained from the isoline control and the two conventional soybeans varieties were derived from plants that were treated with bentazon and acifluorfen-sodium (commercial name Volt®) at the rate of 1.0 liters/ha for the control of weeds. The field sites used in this study were located in regions of Brazil where soybeans are typically cultivated and were representative of locations where soybeans are cultivated commercially. The cultivation practices and a description of the protocol for the field trials that produced the grain used in this composition study are presented in Appendix F.

Components analyzed for grain included proximates (protein, fat, ash, carbohydrates, calories, total dietary fiber [TDF], and moisture), crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acids, fatty acids (C14-C24), minerals (calcium, iron, phosphorus, magnesium, and potassium), vitamins (α-, β-, γ-, δ-, and total tocopherols, vitamins E and B_1, and folic acid), isoflavones (daidzin, malonyl-daidzin, daidzein, glycitin, malonyl glycitin, glycitein, genistin, malonyl genistin, and genistein), phospholipids (phosphatidyl ethanolamine, phosphatidic acid, phosphatidyl inositol, and phosphatidyl choline), and antinutrients (phytic acid, raffinose and stachyose, lectins, urease and trypsin inhibitor). Components analyzed in forage samples included proximates (protein, fat, ash, moisture, carbohydrates and calories), crude fiber, ADF and NDF. Compositional analyses were conducted at the Instituto de Tecnologia de Alimentos (ITAL), Campinas, Brazil on grain and forage samples, and complete methods of analyses are presented in Appendix D.

In addition to the grain and forage compositional analyses, the nutritional equivalence of CV127 soybean to the isoline control and conventional soybean varieties was confirmed in a poultry feeding study. A 42-day feeding trial with broiler chickens was conducted to compare the performance of the animals fed with soybean meal from CV127 soybeans to those fed soybean meal from conventional soybean grain. This study demonstrated that there was no evidence of a significant difference in the performance of the animals fed a feed containing soybean meal from CV127 soybean compared to those fed a feed containing soybean meal from the grain of

conventional soybeans, thereby showing that meal derived from CV127 grain is as wholesome as meal derived from grain of the isoline control or conventional soybean varieties for animal feed.

Collectively, results of the above studies as well as the poultry feeding study demonstrate that the grain and forage produced by CV127 soybean are compositionally equivalent to, and as nutritious as, grain and forage produced by the isoline control and other conventional soybean varieties.

The generations of CV127 and comparators used in each of these studies described above are shown below.

Comparative Analyses Performed, Generations Used for Analyses, and Comparators Used as Controls (Refer to Figure III-1 for Identity of CV127 and Isoline Control Generations).

Analysis	CV127 Soybean Generation Used	Control Used
Compositional analysis (Grain) 2006/2007	F5 (CV127 line 127)	Isoline control (F5 null) Monsoy 8001 Coodetec 217
Compositional analysis (Grain) 2007	F6 (CV127 line 127)	Isoline control (F6 null) Monsoy 8001 Coodetec 217
Compositional analysis (Forage) 2007/2008	F7 (CV127 line 127)	Isoline control (F7 null) Monsoy 8001 Coodetec 217
Poultry feeding study 2006/2007	F9 (CV127 line 603)	Conquista Monsoy 8001 Coodetec 217

A. Grain Composition

Field trials for grain production and compositional analyses were conducted under standard agronomic practices in a complete randomized block design with four replicate blocks per location. For the CV127 and isoline control treatments, grain was harvested separately from each replicate block at the conclusion of the growing season and approximately 2 kg of each replicate grain sample was separated for compositional analyses. For the two conventional soybean varieties, the same grain harvest and separation procedures were followed, but approximately 500 g of grain was further sub-sampled from each 2 kg replicate grain sample, and the four 500 g replicate samples for each conventional soybean variety from each field trial location were pooled to make a single sample from each location for compositional analyses. Therefore, statistical analyses of the compositional data were only conducted for the CV127 and isoline control treatments, and data from the two conventional soybean varieties were used for comparative purposes to establish a range of natural variability for each analyte for soybeans grown in Brazil.

Analysis of variance was carried out on the grain composition data using SAS Version 9.1 (SAS Institute Inc., Cary, North Carolina) following two procedures, the General Linear Model and the Mixed Model. With the exception of moisture content, all data were expressed on a dry-weight

basis for statistical analyses. Differences were assessed across location and by location. The model for across-location analyses is the following:

y = variety + location + variety x location + block(location) + e
Random effects: location, variety x location, block(location).
where y is the response variable (any analyte measured)

The model for separate analyses by location is the following:

y = variety + block + e
where e is the response error

For grain composition, contrasts were carried out to compare CV127 soybean with the isoline control. For forage composition, contrasts were carried out to compare the isoline control as well as the conventional soybean varieties to CV127. Values were considered statistically significantly different at the $p < 0.05$ level.

Proximates. Grain samples from the 2006/2007 (season 1) and 2007 (season 2) field trial seasons were analyzed for moisture, ash, protein, crude fat, total dietary fiber, carbohydrate and calorie content. The carbohydrate composition and calorie values were calculated. The means and ranges of values for proximates in CV127 soybean, the isoline control, and the two conventional soybean varieties in two growing seasons and across all locations in comparison with the means and ranges published in the ILSI Crop Composition Database (ILSI, 2008) are shown in Table VII-1. These data show that in both seasons there were no statistically significant differences in the ash, total dietary fiber, or carbohydrate content between CV127 and the isoline control soybeans. In addition, in season 2 there was no significant difference for calories or crude fat between grain from the isoline control and CV127 soybeans. Slight grain moisture differences were observed between CV127 and the isoline control in both seasons, but the differences were inconsistent, since CV127 grain moisture content was lower than the isoline control in season 1, but higher than the isoline control in season 2. Therefore, these differences were not considered to be biologically meaningful. Furthermore, the means and ranges of grain moisture levels in CV127 were comparable to the mean and range levels in the two conventional soybean varieties cultivated in the same trials, and were within the range of moisture values reported for soybeans globally as well as for soybeans produced in Brazil. In season 1, grain from CV127 soybean was statistically significantly higher for crude fat and caloric content and statistically significantly lower for protein content compared to grain from the isoline control soybeans. In season 2, there was no statistically significant difference in grain crude fat levels between the two treatments, but the protein content of grain from CV127 soybean was statistically significantly higher than that for grain from the isoline control. However, all means and ranges of values for these nutrients in CV127 grain from both seasons were either within or comparable to the range of values for the conventional soybean varieties cultivated in the same trials as well as to the range reported for soybeans globally and for soybeans produced in Brazil. Overall, these results show that proximate levels (moisture, ash, protein, crude fat, total dietary fiber, carbohydrates and calories) in CV127 grain are equivalent to levels in the isoline control grain and comparable to and in the same range as grain proximate content of other conventional soybean varieties with a history of safe food and feed uses as well as safety to the environment.

Fiber composition. Grain samples were analyzed for crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF). The means and ranges of values for CF, ADF and NDF in CV127 soybean, the isoline control and two conventional soybean control treatments in two field trial seasons and across all locations in comparison with the means and ranges published in the ILSI Crop Composition Database (ILSI, 2008) are shown in Table VII-2. In seasons 1 and 2, there were no statistically significant differences in crude fiber values between the isoline control and CV127 soybeans, and no statistically significant differences in grain ADF values in season 2 between CV127 soybeans and the isoline control. However, the ADF values in season 1 and NDF values in both seasons in CV127 grain were statistically significantly different (higher) than those in grain from the isoline control (Table VII-2). The CF, ADF and NDF means and ranges of values obtained for CV127 in the two seasons were either within or comparable to the means and ranges of values of the conventional soybean varieties cultivated in the same trials as well as to the ranges of values reported globally for soybeans and for soybeans produced in Brazil (ILSI, 2008). These results indicate that grain fiber content of CV127 soybean is equivalent to the grain fiber content of the isoline control and comparable to and in the same range as the grain fiber content of other conventional soybean varieties with a history of safe food and feed uses as well as safety to the environment.

Amino Acids. Grain samples from the two field trial seasons were analyzed for amino acid content. The means and ranges of values for all amino acids in CV127 soybean, the isoline control and the two conventional soybean varieties in two field trial seasons and across all locations in comparison with the means and ranges published in the ILSI Crop Composition Database (ILSI, 2008) are shown in Table VII-3. For grain obtained from season 1, there were no statistically significant differences detected in amino acid content of the grain between CV127 and the isoline control soybeans, except for small but statistically significant differences in values for cysteine and methionine between the two treatments. In the grain from season 2, there were no statistically significant differences in amino acid content between CV127 and the isoline control soybeans, except for alanine, cysteine, histidine, methionine, and tyrosine where statistically significant differences were observed between the two treatments. The means and ranges of values for the amino acid content of CV127 grain from both seasons were either within or comparable to the range of values of the conventional soybean varieties cultivated in the same trials as well as to the ranges of values reported globally for soybeans and for soybeans produced in Brazil. These results demonstrate that the amino acid content of CV127 soybean grain is equivalent to the content of the isoline control grain and is comparable to, and in the same range as, grain amino acid content of conventional soybean varieties with a history of safe food and feed uses as well as safety to the environment.

Results of the amino acid analyses in grain also confirm that the amino acid mutation in the AtAHAS protein responsible for conferring imidazolinone herbicide tolerance in CV127 soybean has no impact on the feedback regulation of this enzyme by branched-chain amino acids. The AHAS enzyme catalyzes the first common step in branched-chain amino acid biosynthesis (leucine, isoleucine and valine). The data presented in this section show there were no statistically significant differences in levels of these amino acids between grain of CV127 and the isoline control. Therefore, these data confirm that the mutation in the AtAHAS enzyme has

no effect on the feedback regulation of the enzyme by the branched-chain amino acids or the biosynthetic function of the enzyme.

Fatty Acids. Grain samples from the two field trial seasons were analyzed for fatty acid content. The eight most prevalent fatty acids in the grain from both seasons included myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, and behenic acids. In addition, low levels of eicosenoic acid were detected in grain of the different soybean treatments from season 1, but were not detected in the grain from season 2. The means and ranges of values for fatty acids in grain from CV127, the isoline control soybeans and two conventional soybean varieties in two field trial seasons and across all locations in comparison with the means and ranges for fatty acids in soybean grain published in the ILSI Crop Composition Database (ILSI, 2008) are shown in Table VII-4. In grain from season 1 there were no statistically significant differences in levels of myristic, stearic, arachidic, eicosenoic, and behenic acids between the grain from CV127 and the isoline control soybeans (Table VII-4). Two fatty acids (palmitic and oleic) were statistically significantly higher and two others (linoleic and linolenic) were statistically significantly lower in grain from CV127 soybean compared to the isoline control soybean in season 1. In grain from season 2, there were no statistically significant differences in the levels of myristic, palmitic, arachidic and behenic acids between the grain from CV127 and the isoline control soybeans (Table VII-4). Statistically significant differences in levels of stearic, oleic, linoleic, and linolenic fatty acids were observed between grain of CV127 and the isoline control in season 2. However, for all fatty acids, the means and ranges of fatty acid values for CV127 soybean grain were either within or comparable to the range of values of the conventional soybean varieties cultivated in the same trials as well as to the ranges of values reported globally for soybeans and for soybeans produced in Brazil (Table VII-4). These results demonstrate that the fatty acid content of CV127 grain is equivalent to the content in isoline control grain and is comparable to and in the same range as the grain fatty acid content of other conventional soybean varieties with a history of safe food and feed use as well as safety to the environment.

Minerals. Soybeans are considered a significant source of potassium and magnesium and a source of bioavailable iron in the animal feed diet (Baker, 2000). Soybeans are also a source of phosphorus and calcium in the animal feed diet; however, the bioavailability of these minerals is typically limited by chelation by phytate. Therefore, these five minerals (calcium, iron, magnesium, phosphorous and potassium) were quantified in soybean grain harvested from the different treatments from two growing seasons. The means and ranges of values for minerals in grain from CV127, the isoline control soybeans and two conventional soybean varieties in two field trial seasons and across all locations in comparison with the means and ranges for minerals in soybean grain published in the ILSI Crop Composition Database (ILSI, 2008) are shown in Table VII-5. In season 1, the mean values for calcium and phosphorus in grain from CV127 soybean were not statistically significantly different from those of the isoline control, while levels of iron (lower), magnesium (higher), and potassium (lower) were statistically significantly different from the isoline control. All mineral mean values determined for grain from CV127 soybean in season 2 were statistically significantly different from the mean values obtained for grain from the isoline control soybean, except for grain iron content. However, for each of the five minerals measured, the mean and range of mineral values for CV127 soybean grain were either within or comparable to the range of values of the conventional soybean varieties cultivated in the same trials as well as to the range of values reported globally for soybeans

(Table VII-5). These results demonstrate that the mineral content in grain produced from CV127 soybean is comparable to, and in the same range as, the mineral content of the isoline control as well as other conventional soybean varieties with a history of safe food and feed use as well as safety to the environment.

Vitamins. The tocopherols (α, β, γ, δ and total tocopherols) together with vitamins E, B1, folic acid, niacin, and riboflavin were analyzed in the soybean grain samples obtained from trials conducted in two field trial seasons. Levels of niacin and riboflavin in the grain samples were below the level of detection for the assays and so these analytes are not included in the data table. The means and ranges of values for vitamins in grain from CV127, the isoline control soybeans and two conventional soybean varieties in two field trial seasons and across all locations in comparison with the means and ranges for vitamins in soybean grain published in the ILSI Crop Composition Database (ILSI, 2008) are shown in Table VII-6. The mean vitamin content of CV127 grain produced in season 1 was found to be statistically significantly lower than that in the grain of the isoline control for folic acid, γ–tocopherol and vitamin B1 and significantly higher for α-, β-, and δ-tocopherol, and vitamin E. The mean values for total tocopherols in the grain of CV127 and the isoline control soybeans from season 1 were not statistically significantly different. There were no statistically significant differences in the mean vitamin content of grain from CV127 soybean in season 2 compared to those from the isoline control except for α- and γ–tocopherols. However, for each of the different vitamins measured, the means and ranges of vitamin values for CV127 soybean grain were either within or comparable to the range of values of the conventional soybean varieties cultivated in the same field trials. Global ranges for typical vitamin values in soybean grain are only available for folic acid, vitamin B1 and α-tocopherol. The mean values of folic acid and α-tocopherol in grain from CV127 soybeans in both seasons were within the ranges reported globally for soybeans in the ILSI Crop Composition Database. In the case of vitamin B1, the grain from CV127 and the isoline control soybeans in both seasons had higher values compared to the typical global range of values for this vitamin, and this may be a characteristic of soybean germplasm adapted for Brazilian growing conditions. Overall, these results demonstrate that vitamin levels in grain produced by CV127 soybean are comparable to and in the same range as vitamin levels in the isoline control as well as other conventional soybean varieties with a long history of safe food and feed use as well as safety to the environment.

Isoflavones. Grain samples from two field trial seasons were analyzed for their isoflavone composition, including daidzin, malonyl daidzin, daidzein, glycitin, malonyl glycitin, glycitein, genistin, malonyl genistin, and genistein. The mean and range of values for the total isoflavones (total daidzein, total genistein, and total glycitein) in grain from CV127, the isoline control soybeans and two conventional soybean varieties in two field trial seasons and across all locations in comparison with the mean and range of values for isoflavones in soybean grain published in the ILSI Crop Composition Database (ILSI, 2008) are shown in Table VII-7. There was no statistically significant difference in the mean values for total glycitein between the grain from CV127 and the isoline control soybeans in season 1, but levels of glycitein were statistically significantly lower in grain of CV127 compared to levels in the grain of the isoline control in season 2. Levels of total daidzein and genistein were statistically significantly lower in grain of CV127 compared to levels in the isoline control in both field trial seasons. However, for each of the isoflavones, the mean and range of isoflavone values for CV127 soybean grain

were either within or comparable to the range of values of the conventional soybean varieties cultivated in the same field trials as well as to the range of values reported globally for soybeans and for soybeans produced in Brazil (Table VII-7). These results demonstrate that the isoflavone content in grain produced from CV127 soybean is comparable to, and in the same range as, the isoflavone content of the isoline control as well as conventional soybean varieties with a history of safe food and feed use as well as safety to the environment.

Phospholipids. Phospholipids are common contaminants during soybean oil processing, often referred to as gums in the solvent-extracted oil. If not removed, the gum material typically settles out and can cause significant losses in oil refining. Therefore, phospholipid levels were analyzed in grain samples and the mean and range of values for phosphatidyl ethanolamine, phosphatidic acid, phosphatidyl inositol and phosphatidyl choline in grain from CV127, the isoline control soybeans and two conventional soybean varieties in two field trial seasons and across all locations are shown in Table VII-8. Data were not available for these analytes in the ILSI Crop Composition Database. In season 1, the mean values for all measured phospholipids in grain from CV127 soybean were statistically significantly lower compared to the corresponding values from the grain of the isoline control. Conversely, in season 2, there were no statistically significant differences in the mean phospholipid values of grain from CV127 and the isoline control soybeans, except for levels of phosphatidic acid levels that were slightly but statistically significantly higher in grain of CV127 compared to levels in grain of the isoline control. Therefore, the differences observed from the first field trial season were not considered biologically meaningful since they were not consistently observed. In general, the values for the phospholipids obtained from grain of CV127 soybean were comparable to the values obtained from grain produced by the isoline control at all locations. Since phospholipid content of soybeans is not included in the ILSI Crop Composition Database, comparison of these values to this database was not possible. However, comparisons to the means and ranges of values for the two conventional soybean varieties that were included in this study demonstrated that levels of phospholipids in grain from CV127 soybean were comparable to those in grain produced by conventional soybean varieties that are commonly cultivated in Brazil and that have a long history of safe food and feed use and safety to the environment.

Antinutrients. Grain samples were analyzed for antinutrient content, and the mean and range of values for phytic acid, raffinose, stachyose, lectins, urease and trypsin inhibitor in grain from CV127, the isoline control and two conventional soybean varieties in two field trial seasons and across all locations in comparison with the mean and range of values for antinutrients in soybean grain published in the ILSI Crop Composition Database (ILSI, 2008) are shown in Table VII-9. There were no statistically significant differences in the levels of phytic acid between grain from CV127 and the isoline control soybeans in either field trial season, and the mean and range of phytic acid values for CV127 grain were either within or comparable to the range of values for the conventional soybean varieties included in this study. However, the mean and range of phytic acid values for CV127, the isoline control and the conventional soybean varieties values were lower than the range that is typical for soybeans cultivated globally (Table VII-9), and this most likely reflects a germplasm characteristic of soybean varieties adapted for cultivation in Brazil.

In the case of raffinose, the mean value for grain from CV127 soybeans produced in season 1 was slightly, but statistically significantly higher than the grain content of the isoline control, whereas in season 2 there was no statistically significant difference in grain raffinose levels between CV127 and the isoline control. Therefore the difference in grain raffinose levels observed between the two treatments in season 1 was not considered biologically meaningful since it was not observed consistently. Although the mean values for raffinose in both CV127 and the isoline control soybeans in grain from both seasons were higher than the range for raffinose in soybeans cultivated globally (ILSI Crop Composition Database), the mean and range of raffinose levels in both CV127 and the isoline control were comparable to the mean and range of raffinose values obtained for the two conventional soybean varieties included in the field trials (Table VII-9). Therefore the difference in raffinose levels in grain of CV127, the isoline control and the conventional soybean varieties from the range of raffinose levels that are typical for soybeans cultivated globally, most likely reflects a characteristic of soybean varieties adapted for cultivation in Brazil.

The mean values for stachyose content in grain of CV127 soybeans in both field trial seasons were slightly, but statistically significantly lower than the mean values for the grain from the isoline control. Although the mean and range of values for stachyose in grain from both CV127 and the isoline control soybeans in both seasons were higher than the range for stachyose in soybeans cultivated globally, these means and ranges of values were comparable to the mean and range of stachyose values obtained for the two conventional soybean varieties included in the field trials (Table VII-9). Again, this suggests that higher stachyose levels observed in grain of CV127, the isoline control and the conventional soybean varieties compared to levels recorded for soybeans cultivated globally, is a characteristic of soybean varieties adapted for cultivation in Brazil.

The mean values for lectins in season 1, and urease and trypsin inhibitor in both seasons for CV127 were not statistically significantly different from levels of the same analytes in the isoline control soybeans. The mean value for lectin levels in grain from CV127 soybeans from season 2 was statistically significantly lower than the values obtained from the grain of the isoline control. The mean and range of values for lectins in CV127 were either within or comparable to the range of values of the conventional soybean varieties cultivated in the same field trials as well as to the range of values reported globally for soybeans and for soybeans produced in Brazil in the ILSI Crop Composition Database (Table VII-9). In the case of trypsin inhibitor, the mean and range of values in grain of both CV127 and the isoline control soybeans were comparable to the mean and range of trypsin inhibitor values obtained for the two conventional soybean varieties, but values were below the range reported globally (Table VII-9).

Urease activity is typically used as an indicator of reduction of trypsin inhibitor activity in meal as a result of heat treatment during soybean processing. This has been a reliable method because trypsin inhibitor is readily reduced by heat as is urease activity. Urease is not harmful to non-ruminant animals but can cause ammonia toxicity in cattle fed urea-supplemented diets (Dr. G. Weigel, personal communication). The means and ranges of urease values in grain of both CV127 and the isoline control soybeans were comparable to the means and ranges of urease values obtained for the two conventional soybean varieties. Overall, comparing antinutrient levels in CV127 soybeans to levels in the isoline control and the conventional Brazilian

commercial varieties, these results show that antinutrient levels in grain produced by CV127 soybeans are comparable to and in the same range as antinutrient levels in the isoline control as well as conventional soybean varieties cultivated in Brazil and/or globally that have a long history of safe food and feed uses and safety to the environment.

Conclusions. The results of the compositional analyses conducted with grain produced in Brazil in two different growing seasons demonstrate that the introduction of the *csr1-2* gene into the soybean genome does not impact the nutritional composition of grain produced by CV127 soybeans. In summary, results of these analyses demonstrate that grain from CV127 soybeans is compositionally equivalent to, and as nutritious as, grain from the isoline control well as other conventional soybean varieties typically cultivated in Brazil. Therefore, grain derived from CV127 soybean is appropriate for use in human food and animal feed products. Minor differences in amounts of individual nutritional constituents that were detected between the grain of CV127 soybean and that of the isoline control soybean are likely due to the natural genetic heterogeneity that exists between these two. Also, analyte differences from the global composition database most likely reflect characteristics of soybean germplasm bred and developed for production under tropical growing conditions in Brazil. The complete results of these composition analyses are presented in Appendix D.

Table VII-1. Grain Proximate Composition.
Comparison of the proximate composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte (unit)	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
	N = 24		N = 12	N = 15		N = 8	N = 80 – 323	N = 69
	Mean (range)							
Moisture (%)	10.1 a† (9.2 – 10.9)	9.4 b (8.8 – 9.9)	9.8 (9.4-10.5)	7.6 b (7.1 – 8.2)	7.9 a (7.6 – 8.2)	7.7 (7.0-8.2)	10.1 (4.7 – 34.4)	9.8 (7.6 – 11.2)
Ash (g/100 g DW)	5.0 a (4.6 – 5.3)	4.9 a (4.5 – 5.4)	4.9 (4.5-5.3)	5.2 a (4.8 – 5.7)	5.1 a (4.7 – 5.6)	5.2 (4.9-5.5)	5.32 (3.89 – 6.99)	5.00 (4.58 – 5.47)
Protein (g/100 g DW)	40.3 a (38.1 – 42.2)	39.4 b (37.3 – 41.9)	37.6 (36.4-39.6)	39.2 b (36.4 – 41.6)	39.7 a (36.7 – 41.6)	39.2 (36.8-42.0)	39.47 (33.19 – 45.48)	40.15 (37.19 – 44.85)
Crude Fat (g/100 g DW)	21.7 b (20.0 – 23.3)	22.7 a (20.1 – 24.2)	22.8 (20.2-24.8)	20.2 b (17.7 – 23.8)	20.5 ab (18.9 – 24.1)	20.6 (16.9-25.1)	16.68 (8.10 – 23.56)	18.85 (14.44 – 23.56)
Total Dietary Fiber (g/100 g DW)	24.59 a (21.93 – 26.90)	24.70 a (22.13 – 28.11)	25.43 (22.29-28.03)	24.54 a (18.94 – 28.01)	24.66 a (21.37 – 28.43)	25.0 (21.9-27.3)	NA*	NA
Carbohydrate[1] (g/100 g DW)	33.1 a (31.6 – 34.4)	32.9 a (31.9 – 34.9)	34.7 (27.39-42.03)	35.4 a (25.2 – 44.9)	34.7 a (24.2 – 43.3)	35.0 (26.7-39.8)	38.2 (29.6 – 50.2)	36.0 (29.6 – 41.6)
Calories (kcal/100 g DW)	390 b (379 – 402)	395 a (384-405)	393 (377-400)	382 a (362 – 403)	383 a (365 – 413)	382 (374-395)	NA	NA

†Values in the same season and row followed by the same letter are not significantly different at P<0.05.
*NA = not available
[1]Carbohydrates including total dietary fiber

Table VII-2. Grain Fiber Composition.
Comparison of the fiber composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte (g/100 g DW)	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
	N = 24		N = 12	N = 15		N = 8	N = 80 – 323	N = 69
	Mean (range)							
Crude Fiber	8.5 a[†] (6.9 – 11.0)	8.1 a (6.4 – 11.3)	8.2 (6.7-9.3)	7.9 a (6.7 – 10.6)	8.2 a (6.2 – 14.7)	8.2 (7.2-12.1)	7.81 (4.12 – 13.87)	8.46 (6.42 – 10.93)
ADF	11.39 b (8.84 – 14.95)	13.76 a (10.96 – 19.00)	11.76 (9.32-14.43)	10.25 ab (8.53 – 12.05)	10.49 a (7.35 – 13.40)	11.11 (8.89-12.59)	11.97 (7.81 – 18.61)	11.34 (7.81 – 16.39)
NDF	14.98 b (12.04 – 17.32)	17.52 a (14.06 – 20.01)	14.85 (10.63-16.92)	14.08 a (11.32 – 15.93)	15.13 b (12.53 – 18.17)	14.11 (12.26-16.18)	12.33 (8.53 – 21.25)	12.39 (8.53 – 21.25)

[†]Values in the same season and row followed by the same letter are not significantly different at $P<0.05$.

Table VII-3. Grain Amino Acid Composition.
Comparison of the amino acid composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte (g/100 g DW)	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
	N = 24		N = 12	N = 15		N = 8	N = 80 – 323	N = 69
	Mean (range)							
Alanine	1.64 a† (1.45 – 1.92	1.63 a (1.27 – 1.76)	1.56 (1.39-1.68)	1.51 b (1.29 – 1.71)	1.62 a (1.38 – 1.86)	1.58 (1.41-1.79)	1.72 (1.51 – 2.10)	1.73 (1.62 – 1.85)
Arginine	3.03 a (2.64 – 3.09)	3.01 a (2.36 – 3.33)	2.71 (2.34-2.97)	2.84 a (2.37 – 3.34)	2.84 a (2.53 – 3.15)	2.95 (2.49-3.45)	2.84 (2.29 – 3.40)	2.93 (2.59 – 3.28)
Aspartate	4.64 a (4.02 – 5.37)	4.61 a (3.66 – 5.17)	4.32 (3.94-4.72)	4.17 a (3.50 – 4.66)	4.29 a (3.85 – 4.65)	4.45 (3.70-5.06)	4.49 (3.81 – 5.12)	4.59 (4.23 – 5.12)
Cysteine	0.54 a (0.51 – 0.58)	0.52 b (0.48 – 0.56)	0.52 (0.49 – 0.56)	0.51 a (0.43 – 0.55)	0.52 b (0.43 – 0.56)	0.52 (0.44 – 0.59)	0.59 (0.37 – 0.81)	0.57 (0.50 – 0.81)
Glutamate	7.61 a (6.59 – 8.92)	7.54 a (5.83 – 8.45)	6.98 (6.16-7.68)	6.74 a (5.72 – 7.41)	6.78 a (6.17 – 7.46)	7.18 (5.90-8.55)	7.09 (5.84 – 8.20)	7.29 (6.58 – 8.09)
Glycine	1.65 a (1.45 – 1.87)	1.65 a (1.32 – 1.81)	1.56 (1.41-1.71)	1.50 a (1.28 – 1.68)	1.50 a (1.34 – 1.83)	1.56 (1.35-1.72)	1.69 (1.46 – 2.00)	1.70 (1.56 – 1.82)
Histidine	0.87 a (0.78 – 1.03)	0.85 a (0.67 – 0.97)	0.79 (0.72-0.86)	1.11 b (0.80 – 1.32)	1.37 a (0.91 – 2.13)	1.10 (0.73-1.34)	1.04 (0.88 – 1.18)	1.06 (0.98 – 1.18)
Isoleucine	1.61 a (1.42 – 1.94)	1.61 a (1.24 – 1.75)	1.54 (1.38-1.71)	1.46 a (1.22 – 1.62)	1.43 a (1.08 – 1.97)	1.50 (1.35-1.65)	1.81 (1.54 – 2.08)	1.85 (1.59 – 2.04)
Leucine	2.89 a (2.51 – 3.40)	2.87 a (2.24 – 3.16)	2.80 (2.45-3.65)	2.56 a (2.19 – 2.86)	2.61 a (2.44 – 2.85)	2.73 (2.39-3.15)	3.04 (2.59 – 3.62)	3.07 (2.81 – 3.38)
Lysine	2.48 a (2.12 – 2.85)	2.46 a (1.93 – 2.68)	2.33 (2.08-2.54)	2.24 a (1.97 – 2.47)	2.27 a (1.99 – 2.62)	2.32 (2.03-2.58)	2.56 (2.29 – 2.84)	2.58 (2.42 – 2.82)

Table VII-3. continued.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
(g/100 g DW)	N = 24		N = 12	N = 15		N = 8	N = 80 – 323	N = 69
	Mean (range)							
Methionine	0.63 a (0.60 – 0.67)	0.62 b (0.59 – 0.68)	0.60 (0.55 – 0.65)	0.60 b (0.53 – 0.66)	0.61 a (0.51 – 0.66)	0.60* (0.53 – 0.69)	0.55 (0.43 – 0.68)	0.55 (0.50 – 0.68)
Phenylalanine	1.99 a (1.77 – 2.37)	1.98 a (1.52 – 2.19)	1.87 (1.64-2.09)	1.76 a (1.52 – 1.94)	1.78 a (1.65 – 1.90)	1.87 (1.64-2.12)	1.98 (1.63 – 2.35)	2.06 (1.82 – 2.24)
Proline	1.98 a (1.74 – 2.32)	1.91 a (1.49 – 2.12)	1.82 (1.58-2.03)	1.74 a (1.50 – 1.95)	1.73 a (1.11 – 1.89)	1.86 (1.60-2.05)	2.00 (1.69 – 2.28)	2.06 (1.86 – 2.28)
Serine	2.09 a (1.83 – 2.39)	2.07 a (1.63 – 2.29)	1.96 (1.78-2.16)	1.82 a (1.54 – 2.05)	1.83 a (1.65 – 1.97)	1.92 (1.67-2.21)	2.02 (1.11 – 2.48)	2.17 (1.96 – 2.48)
Threonine	1.56 a (1.34 – 1.80)	1.55 a (1.25 – 1.72)	1.48 (1.36-1.55)	1.37 a (1.21 – 1.50)	1.37 a (1.17 – 1.67)	1.40 (1.23-1.55)	1.47 (1.14 – 1.86)	1.40 (1.28 – 1.52)
Tryptophan	0.73 a (0.59 – 0.84)	0.76 a (0.60 – 1.03)	0.77 (0.57-1.16)	0.64 a (0.53 – 0.74)	0.67 a (0.51 – 0.84)	0.65 (0.56-0.73)	0.43 (0.36 – 0.50)	0.44 (0.38 – 0.49)
Tyrosine	1.34 a (1.17 – 1.55)	1.31 a (1.06 – 1.42)	1.27 (1.12-1.39)	1.18 a (0.98 – 1.33)	1.14 b (1.02 – 1.38)	1.25 (1.14-1.45)	1.32 (1.02 – 1.61)	1.38 (1.27 – 1.56)
Valine	1.66 a (1.36 – 1.98)	1.64 a (1.26 – 1.79)	1.58 (1.40-1.67)	1.56 a (1.35 – 1.83)	1.62 a (1.41 – 1.79)	1.62 (1.31-1.87)	1.91 (1.60 – 2.20)	1.91 (1.63 – 2.08)

†Values in the same season and row followed by the same letter are not statistically significantly different at P<0.05.
*N = 7

Table VII-4. Grain Fatty Acid Content.
Comparison of the fatty acid composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte (unit)	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
(%Total FA)	N = 24		N = 12	N = 15		N = 8	N = 80 – 323	N = 69
	Mean (range)							
Myristic 14:0	<0.09 a† (nd‡ – 0.10)	<0.09 a (nd – 0.10)	0.10 (0.9-0.11) N=7	0.11 a N=5 (0.09 – 0.12)	0.11 a N=7 (0.10 – 0.11)	0.09 N=4 (0.06 – 0.12)	NA*	NA
Palmitic 16:0	9.77 b (9.12 – 10.44)	9.98 a (9.35 – 10.59)	10.17 (9.08-11.16)	10.32 a (9.67 – 10.82)	10.18 a (9.75 – 10.64)	9.77 (8.31 – 10.67)	11.12 (9.55 – 15.77)	11.27 (10.28 – 12.73)
Stearic 18:0	3.39 a (2.84 – 3.87)	3.30 a (2.77 – 3.61)	3.46 (2.97-3.92)	4.04 b (3.14 – 5.05)	4.21 a (3.50 – 4.80)	3.99 (3.04 – 5.01)	4.01 (2.70 – 5.88)	3.95 (2.70 – 5.52)
Oleic 18:1	20.07 a (18.47 – 21.02)	22.07 b (20.10 – 25.76)	20.06 (18.54-21.38)	24.38 b (21.72 – 33.15)	27.79 a (22.65 – 43.63)	23.39 (20.13 – 28.06)	20.7 (14.3 – 32.2)	22.6 (18.7 – 28.9)
Linoleic 18:2	45.87 a (44.01 – 47.68)	45.00 b (42.60 – 46.62)	53.54 (52.36-54.40)	48.86 a (42.54 – 50.83)	45.65 b (31.97 – 49.57)	50.10 (46.78 – 52.80)	53.3 (42.3 – 58.8)	52.6 (48.2 – 55.5)
Linolenic 18:3	5.65 a (5.05 – 6.10)	5.10 b (4.59 – 5.72)	7.23 (6.31-8.15)	6.62 a (4.20 – 8.11)	6.32 b (3.42 – 8.12)	7.06 (4.76 – 8.52)	8.34 (3.00 – 12.52)	7.06 (5.92 – 8.18)
Arachidic 20:0	0.37 a (0.32 – 0.44)	0.34 a (0.26 – 0.39)	0.31 (0.26-0.40)	0.39 a (0.32 – 0.49)	0.42 a (0.27 – 0.55)	0.33 (0.26 – 0.48)	0.32 (0.16 – 0.48)	0.37 (0.28 – 0.48)
Eicosenoic 20:1	0.13 a (0.09 – 0.19)	0.13 a (0.08 – 0.18)	0.14 (0.09-0.20)	nd	nd	nd	0.20 (0.14 – 0.35)	0.22 (0.17 – 0.28)
Behenic 22:0	0.46 a (0.37 – 0.52)	0.43 a (0.39 – 0.53)	0.39 (0.37-0.50)	0.51 a (0.40 – 0.75)	0.50 a (0.38 – 0.80)	0.44 (0.37 – 0.51)	0.40 (0.28 – 0.60)	0.45 (0.37 – 0.57)

†Values in the same season and row followed by the same letter are not significantly different at P<0.05.
‡nd = not detected, *NA = not available

Table VII-5. Grain Mineral Composition.
Comparison of the mineral composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte (unit)	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
(mg/100 g DW)	N = 24		N = 12	N = 15		N = 8	N = 80 – 323	
	Mean (range)							
Calcium	268 a† (221 – 330)	266 a (214 – 318)	254 (205-313)	246 b (172 – 359)	286 a (198 – 484)	234 (167-329)	217 (117 – 307)	NA
Iron	8.50 a (6.01 – 10.43)	7.75 b (5.79 – 10.48)	8.57 (6.54-10.35)	9.16 a (7.98 – 10.90)	9.15 a (7.80 – 11.40)	10.16 (7.93-14.13)	7.81 (5.54 – 10.95)	NA
Magnesium	246 b (204 – 266)	266 a (227 – 304)	269 (225-308)	238 b (211 – 266)	287 a (268 – 309)	255 (223-293)	264 (219 – 313)	NA
Phosphorus	687 a (541 – 834)	667 a (546 – 760)	670 (527-875)	768 a (655 – 845)	732 b (580 – 871)	745 (614-808)	715 (507 – 935)	NA
Potassium	1928 a (1782 – 2071)	1881 b (1703 – 2069)	1961 (1772-2103)	1744 b (1593 – 2061)	1864 a (1599 – 2021)	1758 (1595-2033)	2061 (1868 – 2316)	NA

†Values in the same season and row followed by the same letter are not significantly different at P<0.05.
*NA = not available

Table VII-6. Grain Vitamin Composition.
Comparison of the vitamin composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte (unit)	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
	N = 24		N = 12	N = 15		N = 8	N = 80–323	N = 69
	Mean (range)							
Folic Acid (µg/100 g DW)	330 a† (216 – 456)	270 b (183 – 338)	291 (205-403)	365 a (249 – 462)	374 a (320 – 464)	323 (261-438)	360 (240 – 470)	NA*
α-tocopherol (mg/100 g DW)	3.04 b (2.33 – 4.44)	3.49 a (2.63 – 4.69)	3.22 (2.45-4.61)	3.21 b (1.74 – 8.17)	3.67 a (1.96 – 9.23)	3.54 (2.07-7.51)	1.91 (0.19 – 6.17)	3.44 (1.36 – 6.17)
β-tocopherol (mg/100 g DW)	0.60 b (0.20 – 1.01)	0.90 a (0.58 – 1.22)	0.70 (0.12-1.08)	0.84 a (0.54 – 1.15)	0.96 a (0.68 – 1.37)	0.88 (0.53-1.34)	NA	NA
γ-tocopherol (mg/100 g DW)	16.51 a (11.62 – 20.83)	15.81 b (12.31- 18.84)	16.28 (12.68-20.88)	16.41 a (13.79 – 21.63)	14.96 b (12.28 – 18.46)	15.68 (11.65-22.27)	NA	NA
δ-tocopherol (mg/100 g DW)	6.18 b (4.41 -7.53)	6.56 a (4.9 – 7.99)	6.09 (5.31-7.42)	7.65 a (4.52 – 9.72)	8.15 a (3.31 – 11.35)	6.99 (5.16-8.94)	NA	NA
Total tocopherol (mg/100 g DW)	26.19 a (19.26 – 31.54)	26.75 a (21.95 – 31.12)	26.32 (21.68-31.03)	28.12 a (25.02 – 34.43)	27.41 a (23.38 – 31.40)	27.09 (21.56-33.23)	NA	NA
Vitamin B1 (mg/100 g DW)	0.65 a (0.44 – 0.86)	0.52 b (0.34 – 0.78)	0.58 (0.42-0.71)	0.52 a (0.34 – 0.80)	0.55 a (0.28 – 0.75)	0.48 (0.31-0.71)	0.20 (0.10 – 0.25)	NA
Vitamin E (mg/100 g DW)	5.51 b (4.11 – 7.21)	5.91 a (4.68 – 7.16)	5.70 (4.95 - 7.05)	5.75 a (3.94 – 10.79)	6.05 a (4.35 – 11.21)	5.98 (4.64-9.92)	NA	NA

†Values in the same season and row followed by the same letter are not significantly different at P<0.05.
*NA = not available

Table VII-7. Grain Isoflavone Composition.
Comparison of the isoflavone composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
(mg/100 g DW)	N = 24		N = 12	N = 15		N = 8	N = 80 – 323	N = 69
	Means (range)							
Total Daidzein	72.2 a† (48.4 – 106.6)	52.4 b (41.4 – 72.4)	81.3 (66.2-96.2)	114.6 a (19.7 – 237.8)	79.0 b (14.7 – 161.0)	100.2 (30.8-186.4)	86.3 (6.0 – 245.4)	51.0 (6.0 – 112.9)
Total Genistein	101.7 a (57.1 – 153.4)	83.4 b (60.2 – 121.0)	134.5 (102.8-166.6)	144.5 a (26.1 – 270.1)	114.6 b (11.8 – 246.8)	144.6 (54.3-255.1)	97.9 (14.4 – 283.7)	65.2 (14.4 – 135.7)
Total Glycitein	22.3 a (14.9 – 31.4)	21.7 a (14.6 – 30.2)	49.2 (36.3-75.4)	19.0 a (7.3 – 30.2)	16.5 b (7.4 – 23.9)	35.3 (27.9-43.0)	16.1 (1.5 – 31.0)	13.3 (1.5 – 26.4)

†Values in the same season and row followed by the same letter are not significantly different at $P<0.05$.

Table VII-8. Grain Phospholipid Composition.
Comparison of the phospholipid composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)		
Analyte	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds
(mg/g fat)	N = 24		N = 12	N = 15		N = 8
	Means (range)					
Phosphatidyl ethanolamine	101.9 a† (89.7 – 127.9)	90.9 b (57.9 – 108.9)	98.2 (79.4-113.7)	109.4 a (65.7 – 156.8)	106.0 a (52.1 – 139.5)	100.5 (51.1-133.7)
Phosphatidic acid	4.0 a (1.8 – 6.9)	2.9 b (1.0 – 4.7)	4.1 (2.1-7.5)	2.1 b (0.7 – 6.7)	2.6 a (0.5 – 9.9)	3.0 (0.8-7.2)
Phosphatidyl inositol	11.8 a (10.1 – 14.2)	9.6 b (6.1 – 11.2)	12.3 (11.4-13.5)	10.4 a (8.6 – 14.8)	9.7 a (8.0 – 11.0)	10.8 (8.1-12.4)
Phosphatidyl choline	29.3 a (24.9 – 38.5)	26.9 b (15.9 – 32.3)	30.7 (25.6-38.2)	32.9 a (20.6 – 45.0)	32.5 a (17.5 – 40.2)	33.4 (17.0-42.0)

†Values in the same season and row followed by the same letter are not significantly different at $P<0.05$.

Table VII-9. Grain Antinutrient Composition.
Comparison of the antinutrient composition of grain derived from CV127 soybean with the isoline control and two conventional soybean varieties grown in two seasons as well as with global and Brazilian ranges of values from the ILSI Crop Composition Database. The mean values for the conventional soybean varieties, Monsoy 8001 and Coodetec 217, were combined to provide a single mean value (Conv Stds) for each analyte.

	Season 1 (2006/2007)			Season 2 (2007)				
Analyte (unit)	Isoline	CV127	Conv Stds	Isoline	CV127	Conv Stds	Global	Brazil
	N = 24			N = 15		N = 8	N = 80 – 323	N = 69
	Means (range)							
Phytic Acid (mg/g DW)	2.95 a† (1.43 – 6.09)	2.54 a (0.71 – 5.27)	2.89 (1.47-7.39)	3.36 a (1.89 – 6.00)	3.81 a (2.63 – 4.77)	4.06 (2.37-5.44)	11.21 (6.34 – 19.60)	NA*
Raffinose (g/100 g DW)	1.1 b (0.9 – 1.5)	1.3 a (1.0 – 1.7)	1.1 (0.8-1.6)	1.30 a (1.00 – 1.50)	1.2 a (0.9 – 1.7)	1.3 (1.1-1.4)	0.355 (0.212 – 0.661)	NA
Stachyose (g/100 g DW)	3.7 a (3.0 – 4.2)	3.6 b (3.1 – 4.1)	3.8 (3.1-4.6)	4.0 a (3.0 – 4.6)	3.6 b (2.4 – 4.1)	4.1 (3.1-4.8)	2.19 (1.21 – 3.50)	NA
Lectins HU‡/mg DW	2.24 a (1.35 – 3.35)	2.20 a (1.27 – 3.49)	2.03 (1.38-3.53)	1.70 a (0.85 – 3.43)	0.92 b (0.11 – 3.43)	1.65 (0.67-2.67)	1.718 (0.105 – 9.038)	0.815 (0.299 – 1.892)
Urease (ΔpH)	1.93 a (1.27 – 2.12)	1.91 a (0.90 – 2.21)	1.93 (1.43-2.16)	1.53 a (0.28 – 2.06)	1.58 a (0.49 – 2.03)	1.63 (0.27-2.02)	NA	NA
Trypsin Inhibitor (TIU/mg DW)	12.29 a (6.03 – 16.4)	12.01 a (9.14 – 15.13)	11.45 (5.03-19.64)	13.16 a (8.48 – 17.97)	13.80 a (7.82 – 18.03)	14.02 (9.84-16.76)	48.33 (19.59 – 118.68)	NA

†Values in the same season and row followed by the same letter are not significantly different at P<0.05.
‡HU = Hemagglutinating units
*NA = not available

B. Forage Composition

Soybean has many uses in animal and human nutrition. The main soybean product fed to animals is the defatted/toasted soybean meal (OECD, 2001). However, other components of the soybean plant, including the forage, are also fed to a limited extent to animals, primarily to cattle. Soybean forage is typically harvested between the time the plants reach the sixth node stage to the beginning of pod formation. Therefore, the purpose of the current study was to demonstrate that the forage of CV127 soybeans, treated with an imidazolinone herbicide, is substantially equivalent in composition to forage from the isoline control and other conventional soybean varieties, and that CV127 forage is appropriate for use in animal feed. The forage was produced from plants grown in replicated field trials (three replicate plots per treatment at each location) at six locations in Brazil during the summer of 2007/2008 (field trial locations included Santo Antônio de Goiás, GO; Uberaba, MG; Sete Lagoas, MG; Londrina, PR; Brasília, DF; and Santo Antônio de Posse, SP and are listed in Appendix D). The components analyzed included: proximates (moisture, fat, ash, protein, carbohydrates and calories) and fiber (crude fiber, acid detergent fiber and neutral detergent fiber). The results of analyses for CV127 were compared to the isoline control and to two conventional soybean varieties, Monsoy 8001 and Coodetec 217, grown in the same field trials.

At each field trial location, the above-ground portion of three plants from three replicate plots for each soybean treatment was harvested when the plants were at the R2 growth stage. The forage samples were shipped on wet ice to the Instituto de Tecnologia de Alimentos (ITAL), Campinas, Brazil where they were processed and analyzed for proximate and fiber composition. The three plants from each replicate plot were pooled together prior to analyses, and analyses were conducted for each replicate treatment at each field site. The methods used for this study are presented in Appendix D. Also, the forage composition data from each individual field site are presented in Appendix D of this petition. Results are reported on a dry-weight basis. Statistical analysis was conducted using the dry-weight data. In this study, statistical analyses of the compositional data included all treatments: CV127 + imi, isoline control, and the two conventional soybean varieties. Statistical analysis methods were as described for compositional analyses of the grain, except that for forage composition, contrasts were carried out to compare the isoline control as well as the conventional soybean varieties to CV127 soybean. Values were considered statistically significantly different at the $P < 0.05$ level.

The proximate and fiber composition results calculated and statistically analyzed across all locations for forage samples of CV127 soybean treated with imidazolinone herbicide are compared in Table VII-10 to those of the isoline control and the two conventional varieties treated with conventional herbicide. Proximate analyses included moisture, ash, fat, protein, carbohydrates, and calories. The fiber constituents included crude fiber, acid detergent fiber (ADF) and neutral detergent fiber (NDF). There were no statistically significant differences in levels of moisture, ash, fat, protein, carbohydrates, calories, crude fiber, ADF or NDF in forage samples produced from CV127 soybean compared to levels of these analytes in the isoline control soybean. In addition, comparison of the forage composition of CV127 soybean and the isoline control with that of the two conventional soybean varieties demonstrated that there were no statistically significant differences among these treatments for fat, calories, ADF or NDF.

Where small differences in analyte values were observed between CV127 and the conventional soybean varieties, this was attributed to germplasm differences, since the same differences were observed between the isoline control and the conventional varieties.

The results of this study confirm that forage derived from CV127 soybeans contains the same level of nutrients as the isoline control and similar levels as conventional soybean varieties that are currently cultivated. Therefore, forage derived from CV127 soybeans is appropriate for use in animal feed. These results further support the conclusion that CV127 soybean is compositionally equivalent to and as nutritious as conventional varieties with a long history of safe use in animal feed.

Table VII-10. Proximate and Fiber Composition of Soybean Forage.
Forage composition of CV127 soybean was compared to composition of the isoline control and two conventional soybean varieties (Conv Std 1 and Conv Std 2) across six locations in Brazil in the 2007/2008 Season.

Analyte/Unit	Isoline	CV127	Conv Std 1	Conv Std 2
	N=18**			
Proximates	Mean (range)			
Moisture g/100 g FW	81.4 ab* (79.0 – 84.3)	81.1 b (78.4 – 85.3)	82.3 a (78.4 – 85.3)	81.4 a (73.1 – 84.2)
Ash g/100 g DW	8.5 c (7.1 – 10.3)	8.6 bc (7.0 – 10.7)	9.0 a (6.4 – 11.2)	9.0 ab (6.8 – 10.5)
Fat g/100 g DW	2.5 a (1.7 – 3.5)	2.6 a (1.6 – 3.4)	2.6 a (1.9 – 3.5)	2.5 a (1.7 – 4.1)
Protein g/100 g DW	17.7 b (15.1 – 19.5)	17.3 b (15.3 – 19.5)	19.0 a (15.9 – 23.1)	19.1a (17.0 – 22.4)
Carbohydrates g/100 g DW	71.0 a (66.9 – 73.1)	71.6 a (66.7 – 75.6)	69.5 b (62.4 – 74.9)	69.6 b (64.8 – 73.5)
Calories kcal/100 g DW	378 a (367 – 386)	379 a (371 – 387)	377 a (368 – 385)	378 a (372 – 387)
Fiber				
Crude Fiber g/100 g DW	29.8 a (27.5 – 33.2)	29.8 a (27.9 – 32.7)	28.8 b (25.1 – 32.3)	29.6 a (27.0 – 33.1)
ADF g/100 g DW	36.57 a (31.79 – 42.81)	36.41 a (33.39 – 42.16)	35.68 a (28.94 – 41.82)	36.00 a (30.43 – 44.58)
NDF g/100 g DW	45.28 a (39.71 – 50.90)	45.36 a (40.07 – 52.85)	44.45 a (39.33 – 50.67)	44.68 a (38.93 – 52.28)

*Numbers followed by the same letter are not statistically significantly different at $P < 0.05$.
**N=17 for Conv Std 1 and Conv Std 2 for ADF and NDF

C. Poultry Feeding Study

Soybeans are used primarily to produce oil and high protein soybean meal. Soybean meal is the predominant protein component of animal feeds with 97% of soybean meal being used in animal feeds and accounting for nearly 65% of the world's protein in animal feed. In order to assess the wholesomeness of soybean meal from CV127 soybeans and to compare it with that of soybean meals from conventional soybean varieties, a 42-day feeding study with rapidly growing broiler chickens was conducted. The broiler chicken consumes high amounts of feed per unit of body weight and is a well-recognized model animal for assessing the wholesomeness of feed ingredients (ILSI, 2003).

In order to produce the grain for this feeding study, CV127 soybeans and three conventional soybean varieties, including Conquista, Monsoy 8001, and Coodetec 217, were grown at a field trial location near Santo Antônio de Posse, Brazil during the 2006/2007 growing season. Conquista is the conventional variety that was originally transformed with the *csr1-2* gene encoding an imidazolinone-tolerant AtAHAS enzyme and it is closely related genetically to CV127 soybean. The soybeans were cultivated according to standard agricultural practices for soybean production in Brazil, and cultivation practices were the same as used in the 2006/2007 agronomy field trials described in Appendix F. The generation of CV127 soybean used as a grain source in this feeding study was designated as the F_9 generation of imidazolinone-tolerant CV127 Line 603 (refer to Figure III-1). The CV127 plants were treated with 70 g imazapyr/ha, while the conventional soybean varieties Conquista, Monsoy 8001, and Coodetec 217 were treated with the conventional soybean herbicide Volt®.

Grain from each treatment was harvested from the field trial and was processed separately into toasted soybean meal. The soybean grain processing was conducted by the Instituto de Tecnologia de Alimentos (ITAL) in Campinas, Brazil, using a pilot-scale processing method**.** First, the soybeans were cleaned of impurities such as dust, stones, branches and weed seeds using manual separation and sieves. The soybeans were flaked in an expeller type press with a 40 kg/h processing capacity. Approximately 5% of the oil was removed during this preparation. The flaked soybean material was extracted in a batch-type extractor, using indirect steam to heat the n-hexane solvent to 45-50ºC. In the production of the defatted and toasted soybean meal, the extractors were heated with indirect steam for 20 minutes in order to evaporate the solvent in the meal. Subsequently, direct steam (30 psi) was applied for 30 minutes through an orifice plate with a 2.5 mm aperture. The residual solvent was removed by treating with direct steam for 30 minutes under vacuum (250 mm Hg). To finish the process, the meal was subjected to a pressure of 0.2 kg/cm^2 for 10 minutes. The meal moisture was reduced to less than 12% (w/w) in a flash dryer with a temperature of 200-250 ºC for 54 seconds.

The identity of test and control soybean grain used to produce the meal as well as the soybean meal was confirmed using an event-specific PCR for the detection of the *csr1-2* gene in CV127. These analyses were conducted by GeneScan, Brazil. Of the four grain and four related soybean meal samples, only the CV127 soybean grain and soybean meal tested positive by PCR, thereby verifying the identity of these samples and demonstrating that there was no cross-contamination of the conventional soybean grain or meal with grain or meal from CV127 soybean. In addition, the grain samples and the corresponding soybean meal samples were tested for the presence of

important mycotoxins, including aflatoxins B1, B2, G1, and G2, zearalenone, and ochratoxin A by the Instituto de Tecnologia de Alimentos (ITAL). None of these mycotoxins were detected in any of the soybean grain or meal samples. The grain samples were also tested by Bioensaios (São Paulo, Brazil) for the presence of residues of pesticides that were used in the cultivation of the soybeans. For all pesticides tested, residues were either not detectable or were well below the levels of concern for broiler performance studies.

The poultry feeding experiments were conducted by the Embrapa Suínos e Aves (Embrapa Swine and Poultry) at their animal feeding facilities in Concórdia, Brazil. Preliminary experiments were conducted with feeds containing the soybean meals from CV127 soybean and the three conventional soybean varieties to determine the apparent metabolizable energy corrected for nitrogen and the true digestibility values of the amino acids. The results of these experiments were used to produce nutritionally-balanced feeds for the feeding study intended to assess the wholesomeness of soybean meal from CV127 soybeans. Five hundred and seventy-six broiler chickens of the lineage AgRoss 508, half male and half female, were used in the study. The birds were organized randomly in blocks by body weight with 12 replications with 12 broilers (six males and six females) per replication for a total of 144 broilers per treatment. Feeds were formulated for each of the four different treatments that included the soybean meal and all feeds were formulated to be isoenergetic and isoproteic. The experiment was divided into four phases, the initial (one to ten days of age), growth (11 to 28 days), and two final stages (29 to 35 and 36 to 42 days, respectively), and the feed was balanced for each phase to meet the changing nutritional requirements of the animals. The feeds contained varying amounts of soybean meal that were approximately 40% of the feed in the initial stage and declined to approximately 30% in the final stage. During the study the initial weight, body weight, weight gain, feed intake, and feed conversion were assessed at the end of each experimental stage (at 10, 28, 35, and 42 days of age). Statistical analysis of the resulting data was performed using the Dunnett Test (SAS software, 2003) to compare the results from the animals fed feed containing soybean meal from CV127 soybean with those results from the animals fed feed containing the soybean meals from the conventional soybean varieties.

Analysis of the resulting data demonstrated that there was no interaction between the different feed treatments and the sex of the animals, so the statistical analysis was conducted without segregation of the sexes. The results demonstrated that there were no statistically significant differences ($P>0.05$) in body weight, weight gain, feed intake or feed conversion between animals fed feed containing soybean meal from CV127 soybeans and those fed feeds containing soybean meal from the conventional varieties Conquista and Monsoy 8001 (Table VII-11). Animals fed diets containing soybean meal from Coodetec 217 soybeans had statistically significantly lower body weights and weight gain in all growth stages compared to animals fed diets containing soybean meal from CV127 soybean. Based on the results of this study, it can be concluded that soybean meal derived from CV127 soybean is nutritionally comparable to soybean meals derived from conventional soybean varieties that are cultivated commercially. This study also confirms that meal produced from CV127 grain is appropriate for use in animal feed.

Conclusions:

Collectively, results of compositional analyses of whole grain and forage as well as a poultry feeding study demonstrate that the grain and forage produced by CV127 soybean are compositionally equivalent to, and as nutritious as, grain and forage produced by the isoline control and other conventional soybean varieties with a history of safe use in food and feed products, as well as safety to the environment.

Table VII-11. Performance of Broilers Fed CV127, Conquista, Monsoy 8001 or Coodetec 217 Soybean Meal.
Measurements included body weight (BW), weight gain (WG), feed intake (FI), and feed conversion (FC) with their respective average standard errors for broiler chickens during the periods studied.

Performance	Treatments			
	T1	T2	T3	T4
	CV127	CONQUISTA	MONSOY 8001	COODETEC 217
Initial weight (g)	44.44 ± 0.05	44.40 ± 0.05	44.40 ± 0.05	44.43 ± 0.05
Period from 1 to 10 days of age				
BW (g)	289 ± 4	295 ± 4	292 ± 4	273 ± 4 *
WG (g)	244 ± 4	251 ± 4	247 ± 4	228 ± 4 *
FI (g)	272 ± 3	273 ± 3	271 ± 3	258 ± 3*
FC	1.11 ± 0.01	1.09 ± 0.01	1.10 ± 0.01	1.13 ± 0.01
Period from 1 to 28 days of age				
BW (g)	1480 ± 10	1503 ± 10	1507 ± 10	1443 ± 10 *
WG (g)	1436 ± 10	1459 ± 10	1463 ± 10	1398 ± 10 *
FI (g)	1955 ± 15	1983 ± 15	1970 ± 15	1915 ± 15
FC	1.36 ± 0.01	1.36 ± 0.01	1.35 ± 0.01	1.37 ± 0.01
Period from 1 to 35 days of age				
BW (g)	2068 ± 18	2106 ± 18	2101 ± 18	2004 ± 18 *
WG (g)	2024 ± 18	2062 ± 18	2057 ± 18	1959 ± 18 *
FI (g)	3023 ± 18	3055 ± 18	3037 ± 18	2975 ± 18
FC	1.50 ± 0.01	1.49 ± 0.01	1.48 ± 0.01	1.52 ± 0.01
Period from 1 to 42 days of age				
BW (g)	2620 ± 15	2644 ± 15	2666 ± 15	2567 ± 15 *
WG (g)	2576 ± 15	2600 ± 15	2621 ± 15	2522 ± 15 *
FI (g)	4183 ± 22	4187 ± 22	4210 ± 22	4125 ± 22
FC	1.63 ± 0.01	1.62 ± 0.01	1.61 ± 0.01	1.64 ± 0.01

*Averages with an asterisk are significantly different ($P<0.05$) as compared with the CV127 soybean meal treatment.

VIII. Agronomic, Phenotypic and Ecological Evaluations of CV127

The environmental safety of CV127 was shown to be comparable to an isoline control and other conventional soybean varieties through the evaluation of various agronomic, phenotypic, phenologic, and ecological interaction characteristics of CV127 compared to an isoline control as well as other conventional soybean plants. The studies were conducted either as part of multi-location field trials or under laboratory or greenhouse conditions. Field trials with CV127 soybeans were conducted at seven different locations in Brazil during the 2006/2007 growing season and at six trial locations in Brazil during the 2007 growing season. In all field trials, the CV127 soybeans were compared to a near-isogenic, null segregant soybean (referred to as the isoline control) that does not contain the *csr1-2* gene cassette and to two other conventional soybean varieties commonly cultivated in Brazil. The field trial locations were representative of the different regions within Brazil where soybean is commercially cultivated and each trial location consisted of four replications of the test, isoline control and two conventional soybean varieties that were organized in a randomized complete block design. Details on field trial locations, trial management practices, experimental methods and results are presented in detail in Appendix F.

There are many similarities in agronomic practices used in soybean production between the United States and Brazil, including Maturity Groups of soybean cultivars as well as weed, insect and disease control practices. There are some similarities in the climatic conditions between the regions in the southeastern United States where soybeans of maturity group VIII are cultivated and Brazil, such as the average temperatures during the growing season (see section IX and Table IX-4), and there are climatic differences, including rainfall (Table IX-5). A more detailed comparison of the agronomic practices used in the cultivation of soybeans and of the environmental and climatic conditions in the United States and Brazil is presented in section IX.C.4 of this petition. Therefore, in the event that CV127 soybeans were to be introduced into the United States environment, the data generated in the field trials conducted in Brazil to support the environmental as well as food and feed safety of CV127 soybeans are equally applicable to the environmental, food and feed safety assessment of CV127 for the United States environment.

CV127 soybean was compared to the isoline control and two other conventional soybean varieties with respect to key vegetative and reproductive development characteristics associated with the competitiveness and survival of plant species. In field trials, characteristics including seed germination rate, seedling vigor, days to reach key developmental stages, plant height, and grain yield, as well as susceptibility to and interactions with diseases and insects were evaluated. In addition, the impact of cultivating CV127 soybeans on factors relevant to the nitrogen-fixing symbiosis with *Bradyrhizobium japonicum* were assessed and compared between CV127, the isoline control and conventional soybean varieties. Furthermore, laboratory- and greenhouse-based studies on seed germination as well as pollen number and pollen germination were also conducted and comparisons of these characteristics made between CV127 soybeans and the isoline control and, for the seed germination studies, the two other conventional soybean varieties.

Results of these studies demonstrated that, except for herbicide tolerance, soybean CV127 is agronomically, phenotypically, and phenologically equivalent to the isoline control and other conventional soybean varieties. Further, these studies showed no different biological effect of CV127 compared to the isoline control soybeans with respect to interaction with *Bradyrhizobium japonicum* and nitrogen fixation capacity or interaction with various diseases or insects. Therefore, these results reinforce the conclusion that the cultivation of CV127 soybeans poses no different plant pest or weediness potential and will have no different environmental impact than the cultivation of conventional soybean varieties.

The treatments included in the seed germination, dormancy and quality studies, as well as in the field trials used for phenotypic, agronomic, and ecological evaluations are listed below:

Treatment	Genotype	Herbicide application
Treatment 1 (T1)	CV127	Imazapyr (70 g ai/ha)
Treatment 2 (T2)	CV127	Volt® (570 g ai/ha)
Treatment 3 (T3)	Isoline control	Volt® (570 g ai/ha)
Treatment 4 (T4)	Monsoy 8001	Volt® (570 g ai/ha)
Treatment 5 (T5)	Coodetec 217 (CD 217)	Volt® (570 g ai/ha)

The methods as well as results for all treatments are included in Appendices E and F to this petition. However, because the objectives of the evaluations presented in this section of the petition were to compare the intrinsic agronomic, phenotypic and ecological characteristics of CV127 to those of the isoline control and the conventional soybean varieties in the absence of potential effects of imidazolinone herbicide application to the plant, Treatment 1 is not included in the summary data presented in this section, but is included in the detailed data presented in Appendices E and F. Statistical analyses of the data included all of the above five treatments.

The generations of CV127 and controls used in these studies are shown below.

Comparative Analyses Performed, Generations Used for Analyses, and Comparators Used as Controls.

Analysis	**CV127 Soybean Generation Used**	**Comparator Used**
Seed Quality, Germination and Dormancy: Seed Derived from 2007 Field Trials	F_7 (CV127 line 127)	Isoline Control (F_7 null) Monsoy 8001 Coodetec 217
Pollen Number and Germination: Greenhouse-Grown, Londrina, Brazil, 2007.	F_5 (CV127 line 127)	Isoline Control (F_5 null)
Agronomic, Phenotypic and Ecological Evaluation: Field Trials 2006/07	F_5 (CV127 line 127)	Isoline Control (F_5 null) Monsoy 8001 Coodetec 217
Agronomic, Phenotypic and Ecological Evaluation: Field Trials 2007	F_6 (CV127 line 127)	Isoline Control (F_6 null) Monsoy 8001 Coodetec 217

A. Seed Germination, Dormancy and Quality Characteristics

Assessments of seed germination, dormancy and quality were compared between CV127 soybean, the isoline control and two conventional soybean varieties. Seed germination and quality are important agronomic characteristics used in comparisons of different varieties within a crop species, and seed dormancy or "hard seed" in plants is an important characteristic often associated with plants that are weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003). An assessment of seed dormancy is often used to assess the weediness potential of different plant species (Baker, 1974). Although soybeans do not typically display seed dormancy characteristics (OECD, 2000), the potential for seed dormancy in CV127 soybean was assessed and compared to that of the isoline control as well as two conventional soybean varieties (Monsoy 8001, Coodetec 217 [CD 217]).

Data are presented in this section for comparisons made between CV127 seed produced from plants cultivated using conventional herbicide (570 g Volt® ai/ha) application for weed control, and seed of the isoline control and the two conventional soybean varieties produced with the same conventional herbicide application. Seed samples from all treatments were harvested from field trials conducted in six different locations (listed in Table VIII-5) in Brazil in the 2007 short growing season. Two hundred seed per experimental unit (four sub-samples of 50 seed derived from each replicate treatment at each field trial location) were tested for germination and dormancy, following standard procedures (AOSA, 2007), and for other seed quality parameters by tetrazolium staining (França Neto *et al*., 1998). Prior to the initiation of the tests, the identities of the test (CV127), control (isoline control), and reference (Monsoy 8001 and Coodetec 217) seed materials were verified by testing for the presence or absence of the *csr1-2* transgene by event-specific PCR analyses; chain-of-custody documentation for the identity of the control and reference materials was maintained.

To assess seed germination, each sub-sample of 50 seed was placed in rolled, moist paper towels and incubated at 100% humidity in an incubator at 20/30ºC with the lower temperature maintained for 16 hours and the higher temperature for eight hours. Germination was assessed at five and eight days after planting. Seed that failed to germinate in this test were evaluated for developmental abnormalities and hard or firm seed coat characteristics to determine the cause of lack of germination. Hard seed typically remained unimbibed after the test, whereas firm seed imbibed water and were swollen but did not germinate. The hard or firm seed coat characteristic is related to seed dormancy in soybean. Any seed that did not germinate and did not fall into the "abnormal", "hard" or "firm" seed coat categories were designated as "dead" seed. Hard seed was determined at the final reading of the germination test and the percentage of hard seed was added to the germination percentage, based on the fact that hard-seeded soybean is considered viable (Potts *et al*., 1978).

Tetrazolium staining for determining seed viability and quality was performed with two sub-samples of 50 seed each from all treatments from each field trial location. Initially, seed were kept overnight in a moist germination paper towel at 25ºC for 16 h in an environment with a relative humidity of 100%. After conditioning, the seed were placed in plastic cups, and covered with 0.075% tetrazolium solution. The cups were incubated in an oven at 40ºC for 150 minutes.

After staining, the seed were rinsed with tap water several times to stop the staining reaction. Seed were kept submerged in water to avoid dehydration. Individual seed were examined with a magnifying lens (6X) under fluorescent light. Seed were dissected longitudinally through the midsection of the embryonic axis. After the seed was sectioned, the seed halves were separated and the seed coat was removed to expose the outer surface of the cotyledons. The inner and outer surfaces of the cotyledons were observed to identify seed defects that are revealed by the staining procedure. Damage was attributed to either mechanical, weathering or stink bug causes, and was recorded for each sample. Special care was applied during the evaluation of the radicle-hypocotyl axis and the vascular region on the cotyledons, which are structures of major importance for the viability of the seed. After the seed was sectioned, its internal surfaces were inspected and percent viability was recorded for each sample. Live cotyledons are normally white, due to the lack of diffusion of the tetrazolium solution to the inner tissues of the cotyledons. Data from the germination and tetrazolium-staining tests were statistically analyzed by Tukey's test (Steel and Torrie, 1980) to compare means of sources of variation determined by analysis of variance (ANOVA) to have a significant effect on each of the measured traits. In order to compare treatments cultivated similarly, only the data from the treatments treated with the conventional herbicide are presented. In the tetrazolium staining test, only the most serious seed damage (i.e. ratings between 6 and 8) in the tetrazolium test results was included in the statistical analysis and reported in Table VIII-1. A complete description of the experimental methods and results of this study are presented in Appendix E.

The results of the tetrazolium and germination tests demonstrated that there were no statistically significant differences between CV127 soybean and the isoline control or the conventional soybean varieties across field trial locations in seed damage caused by either mechanical means, weathering or stink bugs. In addition there were no statistically significant differences between the treatments across field trial locations for seed viability, seed germination, or occurrence of abnormal, dead, hard and firm seed (Table VIII-1). Seed damage levels were relatively low across all treatments. Percent viability and germination within each treatment were comparable, with the lowest levels associated with conventional variety Coodetec 217 and the highest values found for CV127. The lack of germination of some seed in all treatments was primarily attributed to seed abnormalities, since firm and hard seed percentages were zero or nearly zero, respectively, and CV127 soybean did not differ from the isoline or conventional control varieties in these characteristics. Together, these results demonstrate that seed germination, dormancy and other seed quality parameters of CV127 soybean are not different from those of conventional soybean varieties. Furthermore, these results support the conclusion that CV127 soybean does not pose any greater weediness potential or any different potential environmental impact than conventional soybean varieties.

Table VIII-1. Means per Treatment Across Locations for Seed Quality, Germination and Dormancy Traits of CV127, the Isoline Control and Two Conventional Soybean Varieties.

	Traits Assessed [1]								
	Tetrazolium Test[2]				Germination Test				
	Dmec (6-8)	Dweath (6-8)	Dstink (6-8)	Viab	Germ	Abnor	Dead	Hard	Firm
CV127	3.17 ab[3]	2.65 a	3.09 a	91.00 a	88.48 a	8.22 a	3.30 a	0.04 a	0.00 a
Isoline	4.91 a	2.48 a	1.83 a	90.78 a	87.43 a	9.13 a	3.43 a	0.72 a	0.00 a
Monsoy 8001	2.71 ab	2.79 a	4.63 a	89.83 a	85.13 a	9.48 a	5.40 a	0.04 a	0.00 a
CD 217	2.63 ab	7.33 a	3.29 a	86.75 a	81.71 a	10.90 a	7.40 a	0.90 a	0.00 a

[1]Dmec: mechanical damage (%); Dweath: weathering damage (%); Dstink: damage by stink bugs (%); Viab: seed viability (%); Germ: seed germination (%); Abnor: abnormal seedling (%); Dead: dead seed (%); Firm: firm seed (%); Hard: hard seed (%).

[2] Seed receiving a rating of 6 to 8 in the assessment are considered nonviable (Franca Neto *et al*., 1998). The cause of the nonviability of these seed was further investigated and the results are reported here.

[3]Means in columns followed by the same letter do not differ statistically significantly at the 5% probability level (Tukey's test).

B. Pollen Number and Germination Characteristics

Soybean is a self-pollinating plant species that is commercially propagated by seed. Artificial hybridization techniques are used for breeding purposes. The stigma of the soybean flower is receptive to pollen about 24 hours prior to anthesis and remains receptive for 48 hours thereafter. The anthers in the bud directly pollinate the stigma of the same flower and as a result, soybeans exhibit a high degree of self-fertilization and cross-pollination is usually less than one percent (Caviness, 1966). The purpose of the current study was to demonstrate that CV127 soybean produces pollen with similar characteristics to the pollen produced by a near-isogenic control soybean. Flowers from CV127 soybean and the isoline control plants that were grown in the greenhouse were collected and used to determine the number of pollen grains in each flower. In addition, the pollen was incubated under conditions to stimulate germination and pollen germination was assessed. The results of this study demonstrate that there is no difference in pollen number or pollen germination between the imidazolinone-tolerant CV127 soybean or its isogenic, non-transgenic counterpart (isoline control).

Plants of CV127 soybean (F_5 generation of CV127, line 127, refer to Figure III-1) and the corresponding isoline control were grown in pots in a greenhouse in Londrina, PR, Brazil. Plants were watered daily and grown under a regime of 28°C day and 20°C night (12 h day/12 h night). Mature anthers were collected from five flowers from each of five different plants one day before anthesis from both CV127 and isoline control plants to determine the number of pollen grains produced per flower. The anthers were collected in the morning using a pair of forceps to remove them from the flowers and the anthers from five flowers from each plant were bulked and were placed immediately into separate Eppendorf tubes containing 0.2 ml distilled water.

Pollen germination was determined by *in vitro* germination using flowers that were collected randomly from five plants of each treatment and the anthers from the five flowers from each plant were bulked and were placed in a sucrose solution (0.45 M) and shaken slightly. The solution containing the pollen grains from each plant was allowed to stand for approximately 3 hours. A grain of pollen was considered germinated when the germination tube length was equal to the grain diameter. Germinated pollen grains were counted in a Neubauer chamber and the number of pollen grains that produced a pollen tube and the mean number of germinated pollen grains were determined (Figure VIII-1). In addition, the size of the pollen tube was measured for 20 pollen tubes of both CV127 soybean and the isoline control that were randomly selected in the Neubauer chamber using a microscope. Statistical analysis of the data was by the t-test (Steel and Torrie, 1980).

Figure VIII-1. Counting the Pollen Grains (A) and Germinated Pollen Grains with Pollen Tubes (B). A Neubauer chamber and 20X magnification were used.

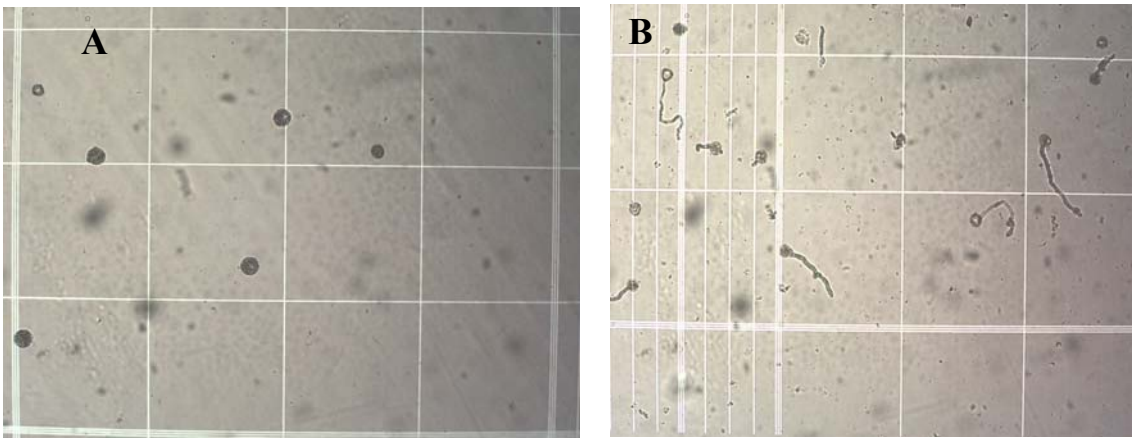


Table VIII-2 shows the quantity of pollen grains in the samples from five plants in each treatment. Results showed no statistically significant difference (at the 5% level) between the number of pollen grains produced by CV127 soybean compared to the isoline control.

Table VIII-2. Pollen Grain Count of Isoline Control Soybean and CV127 Soybean.

Isoline Control	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Average
Pollen per flower*	8200	7933	8000	7933	8400	8093

CV127	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Average
Pollen per flower*	7800	7866	8000	7866	8733	8053

*Mean of 5 flowers

T test:	t value	d.f.[1]	P[2]
	0.2046	8	0.84

[1]d.f.=Degrees of freedom
[2]P=Probability

The number and percentage of germinated pollen grains was determined for each sample of the CV127 and isoline control soybean plants, and results are presented in Table VIII-3. Statistical analysis of the data was by the t-test (Steel and Torrie, 1980). Results showed no evidence of a statistically significant difference at the 5% level between the number of germinated pollen grains produced by CV127 soybean and its isoline control.

The length of the pollen tube was measured for 20 pollen tubes of both CV127 soybean and the isoline control and results are presented in Table VIII-4. Results showed no statistically significant difference at the 5% level between pollen tube length of CV127 soybean compared to the isoline control.

Results of these studies showed no evidence of statistically significant differences in pollen number and pollen germination or tube length between CV127 soybean and the isoline control. Therefore, the production of CV127 soybean by the introduction of the imidazolinone-tolerant acetohydroxyacid synthase large subunit gene (*csr1-2*) into the soybean genome confers herbicide tolerance to the soybean plant but has no effect on pollen production or on the viability of the pollen produced.

Table VIII-3. Number and Percentage of Germinated Pollen Grains of the Isoline Control and CV127 Soybeans using the Neubauer Chamber.

Isoline Control	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Average
Total pollen*	8000	8200	8000	8266	7866	8067
Germinated pollen*	2933	2800	3000	2800	2733	2853
Germination (%)	36.66	34.15	37.50	33.87	34.74	35.37

CV127	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Average
Total pollen*	8133	8266	7866	8066	9200	8307
Germinated pollen*	2933	3000	2600	2666	3400	2920
Germination (%)	36.06	36.29	33.05	33.05	36.96	35.15

*Mean of 5 flowers

T test:	t value	d.f.[1]	P[2]
	0.4437	8	0.6690

[1]d.f.=Degrees of freedom
[2]P=Probability

Table VIII-4. Pollen Tube Length (microns) of Germinating Pollen of the Isoline Control Soybean and CV127 Soybean.

Isoline Control	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Average
Average	101.5	102.5	104.5	103.0	102.0	102.7

CV127	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Average
Average	102.5	104.5	101.0	105.5	102.0	103.1

T test:	t value	d.f[1]	P[2]
	0.4104	8	0.6849

[1]d.f.=Degrees of freedom
[2]P=Probability

C. Agronomic and Phenotypic Evaluations in Replicated Field Trials

Plant growth, development and yield characteristics were assessed under field conditions for CV127 soybean compared to the isoline control and two conventional soybean varieties. The purpose of these evaluations was to confirm that the introduction of the imidazolinone herbicide tolerance trait in CV127 soybean did not affect any phenotypic or agronomic characteristics of CV127 relative to the comparators.

Replicated field trials were conducted in Brazil at 13 field trial locations: seven locations in the 2006/2007 growing season and six locations in the 2007 short growing season. The short season is typically planted in February after the main season in the northern regions of Brazil that have a longer growing season. A similar cultivation practice occurs in the southern United States where soybeans are sometimes planted as a double crop in July following the harvest of winter wheat. According to a survey conducted by the Conservation Technology Information Center, in 2004 through 2008 approximately 7% of the acres planted to soybeans in the U.S. followed this practice (CTIC, 2008). The locations and planting and harvest dates for the field trials are presented in Table VIII-5. Details of field trial management practices, data collection and results are presented in Appendix F.

Table VIII-5. Locations, Planting Dates, and Harvest Dates for each Field Trial Location in Brazil During Two Growing Seasons.

Location – State	2006/2007 Season	2007 Season
Santo Antônio de Posse – SP	Oct. 25 - Mar. 16	
Ponta Grossa – PR	Oct. 10[1]	
Londrina – PR	Oct. 18 - Mar. 14	
Uberaba – MG	Nov. 21 - Mar. 29	Mar. 02 - Jul. 03
Brasília – DF	Nov. 16 - Mar. 29	Mar. 01 - Jul. 09
Santo Antônio de Goiás – GO	Nov. 7 - Mar. 15	Feb. 27 - Jul. 08
Sete Lagoas – MG	Nov. 9 - Mar. 22	Mar. 06 - Jul. 11
Teresina – PI		Mar. 14 - Jul. 04
Vilhena – RO		Mar. 12 - Jul. 10

[1] Poor germination of initial planting on 10 October prompted the need to replant on 31 October, 2006. The trial was destroyed just prior to harvest on 20 April 2007.

In this section of the petition, results from the treatments listed below are presented across locations by year. In all field trial locations, each treatment was replicated four times in a randomized complete block design.

- CV127 soybean (CV127, line 127 F_5 and F_6 generations used in the 2006/2007 and 2007 seasons, respectively; refer to breeding diagram in Figure III-1) with weed control provided by the conventional soybean herbicide Volt® [a combination of bentazon (400 g ai/ha) and acifluorfen (170 g ai/ha)], sprayed at a rate of 570 g ai/ha was included in the trial.

- An isoline control that is a null segregant of CV127 soybean and lacks the *csr1-2* gene cassette but is from the same stage of breeding as the CV127 soybean was included in the trial. Weed control was accomplished using the conventional soybean herbicide Volt® as described above.

- Two conventional soybean varieties were used in these field trials: Monsoy 8001 and Coodetec 217 (CD 217). Both of these varieties are common nontransgenic Brazilian commercial varieties with a similar maturity classification as CV127 soybean (Maturity Groups VIII-IX). Weed control was by application of the conventional soybean herbicide Volt® as described above.

As mentioned earlier, an additional treatment of CV127 soybean grown using an imidazolinone herbicide for weed control was included in the study and the associated statistical analysis, and phenotypic and agronomic evaluations of this treatment are presented in Appendix F. However, to compare only the treatments cultivated in a similar manner, only the data for those treatments treated with the conventional herbicide Volt® are presented in this section of the petition.

Important agronomic characteristics of plants of each treatment, including twelve different characteristics of growth and development were evaluated as presented in Table VIII-6. Evaluation parameters included germination, final plant stand, seedling vigor, plant height, green stem, degree of shattering and lodging, days to full flower, days to full maturity, seed size and grain yield. Statistical analysis of the resulting data from all treatments was conducted by Tukey's test (Steel and Torrie, 1980) to compare means of sources of variation determined by ANOVA to have a significant effect on each measured trait.

Table VIII-6. Agronomic Characteristics Evaluated in the Brazilian Field Trials During the 2006/2007 and 2007 Growing Seasons.

Characteristic Evaluated	Growth Stage at Evaluation	Evaluation Definitions
Germination	V2	Percent emerged plant seedlings relative to the quantity of seed sown.
Seedling Vigor	V2	Overall seedling appearance based on a rating scale: 1=Plant emerged, no trifoliate leaves 2=One trifoliate leaf, unhealthy plant 3=One trifoliate leaf, healthy plant 4=Two trifoliate leaves, unhealthy plant 5= Two trifoliate leaves, healthy plant
Initial Plant Stand	V2	Number of plants per plot at the V2 stage
Days to Flowering	R2	Number of days from planting to full flower
Days to Maturity	R8	Number of days from planting to full plant maturity
Plant Height	R8	Average height of five plants in a plot measured from soil level to the top of the plant
Green Stem	R8	Percent plants in a plot that were green relative to the total number of plants in the plot
Shatter	R8	Percent plants in a plot that show premature dehiscence of pods
Lodging	R8	Average standability (lodging) of plants in a plot according to the scale: 1=All plants standing erect (80-90°) 2=Most plants have a slight lean (60-70°) 3=Most plants have a moderate lean (40-50°) 4=Most plants have a severe lean (20-30°) 5=All plants are prostrate (0-10°)
Final Plant Stand	R8	Number of plants per plot at the R8 stage
Yield	R8	Total weight of all grain harvested (adjusted to 12% moisture)
100-Seed Weight	R8	Weight (g) of 100 seeds

Due to the naturally different environmental characteristics (temperature, rainfall, humidity, day length, etc.) among the trial locations, location had a significant effect on all assessed traits in both the 2006/2007 and the 2007 field trial seasons. For field trials in the 2006/2007 growing season, at Ponta Grossa, the most southerly location (latitude 25° S) and the location for which the Conquista soybean variety is least adapted, plants were taller and the number of days to flower and to maturity were higher than at all other locations during the 2006/2007 season (Table VIII-7). Yield was not recorded from this site due to a necessary late replanting of the study at this site and subsequent uncharacteristic plant growth and late maturity of the CV127, the isoline control, and the conventional soybean varieties included in the study. In the 2006/2007 season, seed size and yield were highest at Londrina (Table VIII-7). In fact, during this season grain yield was above the Brazilian average yield at all locations except Brasília. At Brasília, seedling vigor was slightly less than at other locations and yield appeared to be more adversely affected by a high and late incidence of Asian rust infestation than other locations similarly infected (refer to section VIII.D for disease incidence). In general, within each location in the 2006/2007 season, the performance of CV127 soybean treated with Volt® and the performance of the isoline control were not significantly different for germination, final stand, seedling vigor, plant height, green stem, lodging, or yield (Table VIII-7). Only at some locations were significant differences found between CV127 soybean treated with Volt® and the isoline control for days to flowering (3 of 7 locations) and days to maturity (2 of 7 locations), (Table VIII-7). Shattering was not identified in any plot at any location during either season and so data for this characteristic is not presented.

Some statistically significant differences between the conventional soybean varieties and CV127 soybean treated with Volt® and the isoline control were observed for plant height, lodging, days to full flower, days to maturity and yield. Due to the different genetic backgrounds of CV127 soybean and the isoline control compared to the two conventional soybean varieties, these differences were not unexpected.

In the trials of the 2007 season, plant density was nearly ideal at all locations, except Teresina, where the final stand was lower due to low seed germination (Table VIII-8) caused by excessive rainfall shortly after planting. As expected for the short-season growing conditions, plant height was lower compared to the 2006/2007 season, except at Sete Lagoas, where plants reached a mean stature of 82 cm and the lodging reached the higher index of 3.5 (Table VIII-8). The mean for seed size ranged from 16.4 g at Uberaba to 19 g at Vilhena. At the end of the vegetative cycle, flowering occurred before 40 days after planting at Vilhena and Teresina as a consequence of the lower latitudes of these sites. Correlated to vegetative cycle (days to flowering), the total cycle (days to maturity) was relatively short (less than 115 days) in these two sites and also in Uberaba and Sete Lagoas. The yield was relatively high at Brasilia and Sete Lagoas, medium at Teresina and Uberaba and lower at Santo Antonio de Goiás and Vilhena (Table VIII-8). The wide-ranging performance obtained for grain yield among the different sites is expected and considered normal for the short-season in Brazil. However, the coefficients of variation for all traits were considered low and within an acceptable range.

In general, within locations, the performance of CV127 soybean treated with Volt® and the isoline control in the 2007 trials was not significantly different for germination, final stand, seedling vigor, lodging, days to flower, and days to maturity (Table VIII-8). CV127 soybean

treated with Volt® and the isoline control plants only differed significantly for plant height at Sete Lagoas, percent green stem at Uberaba and Vilhena, seed size at Uberaba, Sete Lagoas and Vilhena and yield at Vilhena (Table VIII-8). Some significant differences between the conventional soybean varieties (Monsoy 8001 and Coodetec 217) and CV127 soybean treated with Volt® and the isoline control were observed for plant height, green stem, seed size, yield and days to flowering and maturity. The conventional soybean varieties matured one to four days earlier in some locations and 100-seed weights were generally less in relation to CV127 soybean and the isoline control. Due to the different genetic backgrounds of CV127 soybean and the isoline control compared to the two conventional soybean varieties, these differences were not unexpected.

Although some statistically significant differences did exist between CV127 soybean and the isoline control for specific phenotypic and agronomic traits at specific locations in the trials conducted during the 2006/2007 and 2007 growing seasons, none of the trait differences were consistent across all locations and years, and therefore were not considered biologically significant. Furthermore, all trait values for CV127 soybeans and the isoline control were within the range for soybeans commercially cultivated in Brazil and elsewhere, including the conventional soybean varieties included in these studies. These results support the conclusion that the cultivation of CV127 soybeans presents no different environmental impact than that presented by the cultivation of conventional soybean varieties.

Table VIII-7. Mean Values for Phenotypic and Agronomic Characteristics of Each Combination of Treatment and Location for all Trials Conducted in the 2006/2007 Season in Brazil.

Location	Treatment	Trait†									
		G	FS	V	PH	GS	L	DF	DM	100SW	Yield
Sto. Antônio de Goiás	CV127	86.8	103.1	5.0	56.9 ab‡	0.0	1.0	43 b	119 a	19.1	3275
	Isoline	92.5	109.4	5.0	57.9 a	0.0	1.0	43 b	119 a	17.1	3131
	M8001	94.3	111.1	5.0	56.5 ab	0.0	1.0	43 b	114 b	14.2	3397
	CD217	91.1	108.4	5.0	53.0 b	0.0	1.0	44 a	119 a	13.1	3007
Brasília	CV127	80.1	94.1	4.0	57.4	0.0	1.0	44 a	126 a	18.0	1553 b
	Isoline	81.9	96.3	4.0	58.0	0.0	1.0	44 a	126 a	15.6	1435 b
	M8001	79.0	92.9	4.0	58.1	0.0	1.0	42 b	118 b	12.8	2622 a
	CD217	82.3	96.9	4.0	57.3	0.0	1.0	43 ab	126 a	12.3	2347 ab
Sete Lagoas	CV127	89.6	99.8	5.0	87.3 ab	2.0 ab	2.3 ab	43 c	112 a	18.3	4028
	Isoline	90.6	108.0	5.0	91.9 a	0.0 b	3.5 a	43 c	112 a	16.8	3829
	M8001	85.5	101.5	5.0	84.2 ab	0.0 b	1.0 b	45 b	112 a	12.2	3830
	CD217	87.6	101.9	5.0	80.7 b	0.3 b	3.0 a	47 a	108 b	12.9	4033
Londrina	CV127	91.5	89.0	5.0	100.3 a	3.5	1.8	46 b	129 b	22.1	5171 a
	Isoline	92.1	90.3	5.0	101.9 a	3.0	1.8	47 a	130 a	19.5	4463 ab
	M8001	89.6	86.4	5.0	91.3 b	4.6	1.0	44 c	125 c	15.0	3799 b
	CD217	90.1	89.0	5.0	83.5 c	2.8	2.3	44 c	125 c	16.9	4143 ab
Uberaba	CV127	89.9	101.0	5.0	75.9 a	0.0	1.0	44 b	111 a	17.2	3404
	Isoline	92.4	105.0	5.0	79.5 a	0.3	1.0	44 b	111 a	16.1	3107
	M8001	92.5	104.3	5.0	61.6 b	0.0	1.0	44 b	111 a	11.9	3303
	CD217	90.9	99.6	5.0	62.4 b	0.0	1.0	47 a	108 b	12.4	3276
Sto. Antônio de Posse	CV127	82.4	79.5	5.0	102.3	4.3	1.8 ab	47 a	130 a	18.1	3202
	Isoline	88.4	88.4	5.0	105.2	1.0	2.3 a	45 b	126 b	16.1	2788
	M8001	87.5	84.9	5.0	98.2	4.0	1.0 b	41 c	123 c	13.0	3182
	CD217	79.8	78.0	5.0	91.2	3.0	2.8 a	41 c	123 c	13.4	3392
Ponta Grossa	CV127	80.1	67.1	5.0	104.0 ab	4.5	2.8 b	49 c	154 b	.	.
	Isoline	80.5	64.6	5.0	107.8 a	3.5	2.8 b	51 b	154 b	.	.
	M8001	86.8	73.6	5.0	103.5 ab	2.3	2.0 bc	53 a	148 c	.	.
	CD217	79.3	66.0	5.0	94.9 bc	0.0	4.0 a	53 a	143 d	.	.

†G = initial germination (%); FS = final plant stand; V = seedling vigor; PH = plant height (cm); GS = green stem (%); L = degree of lodging; DF = days to full flower (vegetative cycle); DM = days to full maturity (total cycle); 100SW = seed size (weight of 100 seeds in grams); and Yield = grain yield (kg/ha).

‡ Means within locations followed by the same letter or not followed by a letter do not differ significantly by the Tukey test at 5% probability.

Table VIII-8. Mean Values for Phenotypic and Agronomic Characteristics of Each Combination of Treatment and Location for all Trials Conducted in the 2007 Season in Brazil.

Location	Variety	Trait†										
		G	IS	FS	V	PH	GS	L	DF	DM	100SW	Yield
Uberaba	CV127	70.25	84.00	76.50	5.00 a	48.80 ab	47.50 b	1.00 a	45.00 b	109.00 a	19.53 a	1863.50 a
	Isoline	73.75	88.75	77.50	5.00 a	54.05 a	25.00 c	1.00 a	45.00 b	109.00 a	17.60 b	2593.50 a
	M8001	78.00	93.50	88.00	5.00 a	46.90 b	22.50 c	1.00 a	46.00 a	106.00 b	14.28 c	2019.25 a
	CD217	76.50	92.00	79.25	5.00 a	38.50 c	1.00 d	1.00 a	46.00 a	106.25 b	14.35 c	2091.75 a
Sete Lagoas	CV127	77.75	93.25	86.00	5.00 a	81.85 bc	35.00 bc	4.00 a	48.50 ab	114.00 a	20.23 a	2446.00 a
	Isoline	77.25	93.00	85.75	5.00 a	91.75 a	23.75 c	4.00 a	49.00 ab	114.00 a	18.15 b	2742.50 a
	M8001	78.00	93.50	90.75	5.00 a	85.55 ab	42.50 b	2.75 a	49.25 ab	112.25 b	16.10 c	2509.50 a
	CD217	78.75	94.75	90.25	5.00 a	74.35 d	5.50 d	3.00 a	49.75 a	112.00 b	14.95 c	2939.50 a
Sto. Ant. de Goiás	CV127	94.50	113.25	113.13	5.00 a	58.00 a	10.00 a	1.00 a	44.00 a	131.00 a	19.18 a	1928.75 a
	Isoline	93.25	111.63	110.38	5.00 a	58.00 a	10.00 a	1.00 a	44.00 a	130.50 a	18.53 a	2179.50 a
	M8001	92.50	110.88	109.50	5.00 a	48.50 b	10.00 a	1.00 a	44.00 a	129.25 b	16.28 b	1985.50 a
	CD217	93.00	111.38	109.88	5.00 a	48.00 b	10.00 a	1.00 a	44.00 a	129.00 b	15.90 b	1631.50 a
Brasília	CV127	90.00	108.00	107.63	5.00 a	59.00 a	5.00 a	1.00 a	49.00 a	128.00 a	19.70 ab	3385.50 a
	Isoline	87.25	104.88	103.50	5.00 a	58.75 a	5.00 a	1.00 a	49.00 a	127.25 a	18.88 ab	2898.25 ab
	M8001	87.75	105.50	104.25	5.00 a	45.75 b	0.00 b	1.00 a	49.00 a	125.00 b	16.60 c	2874.00 ab
	CD217	91.50	109.75	108.38	5.00 a	45.00 b	0.00 b	1.00 a	49.00 a	124.75 b	17.63 bc	2771.75 b
Vilhena	CV127	83.25	86.50	77.00	5.00 a	60.00 a	60.00 b	1.00 a	37.50 ab	99.50 bc	21.58 a	1800.50 b
	Isoline	88.00	92.00	86.25	5.00 a	58.25 a	21.25 c	1.00 a	38.00 ab	98.00 bc	18.58 b	2371.25 a
	M8001	96.50	100.50	93.25	5.00 a	54.25 a	90.00 a	1.00 a	35.25 c	104.75 a	18.20 b	1518.75 b
	CD217	89.25	93.25	87.25	5.00 a	41.25 b	3.25 c	1.00 a	39.00 a	97.00 c	15.25 c	2487.75 a
Teresina	CV127	41.50	49.88	49.50	3.75 a	56.50 a	10.00 a	1.00 a	40.00 a	111.00 a	19.10 a	2278.67 a
	Isoline	48.75	58.25	56.88	4.25 a	55.00 a	10.00 a	1.00 a	40.00 a	111.00 a	17.33 ab	2197.00 a
	M8001	70.25	84.25	82.88	4.25 a	49.75 b	0.00 b	1.00 a	38.00 b	107.00 b	17.05 ab	2045.50 a
	CD217	70.50	84.50	81.00	4.00 a	50.25 b	0.00 b	1.00 a	38.00 b	107.00 b	14.63 b	2446.75 a

†G = initial germination (%); IS= initial stand; FS = final plant stand; V = seedling vigor; PH = plant height (cm); GS = green stem (%); L = degree of lodging; DF = days to full flower (vegetative cycle); DM = days to full maturity (total cycle); 100SW = seed size (weight of 100 seed, grams); Yield = grain yield (kg/ha).
‡ Means followed by the same letter do not differ significantly by the Tukey test at 5% probability.

D. Ecological Evaluations

Assessments of interactions between CV127 soybeans and the environment were conducted at all thirteen field trial locations in Brazil during the 2006/2007 and 2007 growing seasons, as described in section VIII.C. Treatments were as described in section VIII.C. Evaluations included interactions with plant diseases, insect pests and characteristics related to the biological nitrogen fixing symbiosis with *Bradyrhizobium japonicum*. The purpose of these evaluations was to assess whether any differences in environmental interactions exist between imidazolinone-tolerant CV127 soybean and the isoline control and other conventional soybean varieties.

D.1. Disease Susceptibility

Disease susceptibility of CV127 soybeans relative to the isoline control and conventional soybean varieties was determined at different stages of plant growth and development throughout the growing season. Diseases that were assessed included Asian soybean rust (*Phakopsora pachyrhizi*), Downy mildew (*Peronospora manshurica*), Powdery mildew (*Erysiphe diffusa*), and end-of-cycle diseases (DC) caused mainly by *Septoria glycines* (brown spot or Septoriose) and *Cercospora kikuchii* (soybean leaf spot) that can cause serious losses to soybean yield in commercial production in Brazil.

The incidence of all diseases present at the time of assessment was recorded for each plot as the percent of affected leaf area. The dominant diseases causing the infection were identified at each assessment. Assessments were made at or near vegetative stage V4 and reproductive stages R1, R5, and R7. However, no disease infestations were observed at the V4 stage with the exception of powdery mildew in Santo Antônio de Goiás in 2007. Due to the potential in Brazil for fungal diseases to devastate the soybean crop and thereby compromise the goals of this study, all plots were uniformly treated with fungicides in order to maintain plant health. Evaluations of disease incidence in each plot were typically made prior to disease control treatments. Data were analyzed by ANOVA on a per location, per disease, and per time of assessment basis. In those cases where differences among treatments were significant, Tukey's procedure (Steel and Torrie, 1980) was used to make pair-wise comparisons of treatment means.

Other than a high incidence of Asian rust at several locations, the incidence of other diseases was generally low during both growing seasons (Tables VIII-9 and VIII-10). Although a few isolated differences in disease susceptibility were noted among the treatments, these were not consistent across field locations and in general there were no differences in susceptibility between CV127 soybean and the isoline control. Where susceptibility differences were observed, they were generally between varieties with the Conquista genetic background (CV127 soybean and the isoline control) and the two conventional soybean varieties with different genetic backgrounds. Therefore, disease susceptibility of CV127 soybean was found to be no different from that of the isoline control plants. This study demonstrated that the insertion of the *csr1-2* gene into the genome of CV127 soybean did not affect the disease susceptibility of CV127 soybean. A complete description of the disease assessment methodology is included in Appendix F.

Table VIII-9. Disease Assessments for CV127 Soybean, the Isoline Control, and Conventional Soybean Varieties (Monsoy 8001 and CD217) during the 2006/2007 Field Season. Evaluations were conducted at seven field trial locations in Brazil and presented for three growth stages[1] (R1, R5 and R7). Values are mean percent affected leaf area.

Location	Treat-ment	R1				R5				R7			
		PM[†]	DM	DC	AR	PM	DM	DC	AR	PM	DM	DC	AR
Ponta Grossa	CV127	6 a[‡]	2	-[§]	-	-	-	5	6.0 ab	-	-	5	43 ab
	Isoline	7 a	2	-	-	-	-	5	6.0 ab	-	-	5	40 ab
	M8001	16 b	2	-	-	-	-	5	5.3 b	-	-	5	30 b
	CD217	8 a	2	-	-	-	-	5	4.8 b	-	-	5	36 ab
Londrina	CV127	-	1.5	-	2.0 c	2	-	-	51 a	-	-	5	60 a
	Isoline	-	1.8	-	2.0 c	2	-	-	51 a	-	-	5	60 a
	M8001	-	1.8	-	2.8 b	2	-	-	44 b	-	-	5	51 b
	CD217	-	1.8	-	4.0 a	2	-	-	30 c	-	-	5	40 c
Sto. Ant. de Posse	CV127	-	-	-	6.8	-	2.0	-	45 a	-	-	5	58 a
	Isoline	-	-	-	7.3	-	1.5	-	50 a	-	-	5	60 a
	M8001	-	-	-	7.5	-	1.8	-	30 b	-	-	5	40 b
	CD217	-	-	-	6.9	-	2.8	-	43 a	-	-	5	52 a
Uberaba	CV127	-	-	-	-	1.5	-	-	2.0	-	-	-	2.5
	Isoline	-	-	-	-	1.5	-	-	2.0	-	-	-	2.5
	M8001	-	-	-	-	1.5	-	-	2.0	-	-	-	2.5
	CD217	-	-	-	-	1.5	-	-	2.2	-	-	-	2.5
Sete Lagoas	CV127	-	-	-	1.5	-	-	-	2.5	-	-	-	2.5
	Isoline	-	-	-	1.5	-	-	-	2.5	-	-	-	2.5
	M8001	-	-	-	1.5	-	-	-	2.5	-	-	-	2.5
	CD217	-	-	-	1.5	-	-	-	2.5	-	-	-	2.5
Brasilia	CV127	-	-	-	2.0	-	-	0.6 ab	42 a	-	-	0.5	55 a
	Isoline	-	-	-	1.7	-	-	0.4 ab	40 a	-	-	0.1	45 b
	M8001	-	-	-	1.9	-	-	1.1 a	19 b	-	-	0.3	23 c
	CD217	-	-	-	1.8	-	-	0.0 b	19 b	-	-	0.6	23 c
Sto. Ant. de Goiás	CV127	-	-	-	1.0	-	-	0.5	17	-	-	2.9	22
	Isoline	-	-	-	0.9	-	-	0.1	19	-	-	2.4	22
	M8001	-	-	-	0.6	-	-	0.3	18	-	-	3.6	21
	CD217	-	-	-	0.8	-	-	0.6	18	-	-	3.1	22

[1]Disease assessments were also conducted at the V4 growth stage but no diseases were observed at any location.

[†] Diseases: PM = Powdery mildew (*Erysiphe diffusa*); DM = Downy mildew (*Peronospora manshurica*); DC = end of cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*). -[§] = Disease not observed.

[‡] Means followed by the same letter do not differ statistically significantly by the Tukey's test at 5% probability.

Table VIII-10. Disease Assessments for CV127 soybean, the Isoline Control, and Conventional Soybean Varieties (Monsoy 8001 and CD217) during the 2007 Field Season. Evaluations were conducted at six field trial locations in Brazil and presented for growth stages V4, R1, R5 and R7. Values are mean percent affected leaf area.

Location	Treat-ment	V4	R1				R5				R7			
		PM[†]	PM	DM	DC	AR	PM	DM	DC	AR	PM	DM	DC	AR
Vilhena	CV127	-[§]	-	-	-	3.7	-	-	-	8.6	-	-	5.0	20.6
	Isoline	-	-	-	-	3.6	-	-	-	7.6	-	-	4.4	20.2
	M8001	-	-	-	-	3.6	-	-	-	8.0	-	-	5.0	19.8
	CD217	-	-	-	-	2.1	-	-	-	5.2	-	-	5.0	10.5
Teresina	CV127	-	-	-	-	-	-	-	-	-	-	-	-	-
	Isoline	-	-	-	-	-	-	-	-	-	-	-	-	-
	M8001	-	-	-	-	-	-	-	-	-	-	-	-	-
	CD217	-	-	-	-	-	-	-	-	-	-	-	-	-
Uberaba	CV127	-	-	-	-	1.5	-	-	1.1 ab	0.7	-	-	1.4 b	0.6
	Isoline	-	-	-	-	1.5	-	-	0.9 ab	0.6	-	-	1.3 b	0.5
	M8001	-	-	-	-	1.5	-	-	1.2 a	1.0	-	-	1.5 a	0.6
	CD217	-	-	-	-	1.5	-	-	0.8 ab	0.8	-	-	1.5 a	0.5
Sete Lagoas	CV127	-				0.6	0.8 b[‡]	-	-	0.8	-	-	-	1.1
	Isoline	-				0.6	0.7 b	-	-	1.0	-	-	-	1.0
	M8001	-				0.6	1.7 a	-	-	0.7	-	-	-	1.1
	CD217	-				0.5	0.6 b	-	-	0.6	-	-	-	1.0
Brasilia	CV127	-	1.6	-	-	-	-	-	-	0.9	-	-	-	1.6
	Isoline	-	1.3	-	-	-	-	-	-	0.7	-	-	-	1.6
	M8001	-	1.6	-	-	-	-	-	-	0.9	-	-	-	1.3
	CD217	-	1.6	-	-	-	-	-	-	1.4	-	-	-	1.6
Sto. Ant. de Goiás	CV127	5	7.1	-	-	-	-	-	-	2.2	-	-	-	1.9
	Isoline	5	6.6	-	-	-	-	-	-	2.5	-	-	-	0.6
	M8001	5	6.3	-	-	-	-	-	-	2.1	-	-	-	1.3
	CD217	5	6.4	-	-	-	-	-	-	3.1	-	-	-	0.9

[†] Diseases: PM = Powdery mildew (*Erysiphe diffusa*); DM = Downy mildew (*Peronospora manshurica*); DC = end of cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*).

[‡] Means followed by the same letter do not differ statistically significantly by the Tukey's test at 5% probability.

-[§] = Disease not observed.

D.2. Insect Interactions

Populations of several different insect orders are common in commercial soybean production fields in Brazil and include the Coleopterans (Diabrotica and Aracanthus spp.), Lepidopterans (Anticarsia, Agrotis, Pseudoplusia, and Spodoptera spp.), and Hemipterans (Euschistus, Piezodorus, and Nezara spp.). Insect pests in these orders can cause serious losses to soybean yield in commercial production in Brazil. Resistance to insect damage and effects on in-field insect populations of CV127 soybean relative to the isoline control and conventional soybean varieties were assessed at different stages of plant growth and development throughout the growing season during the 2006/2007 and 2007 growing seasons in Brazil. Insects on the plants were collected in the field by placing a shake cloth on the ground between the center two rows of each plot. The plants in the two adjacent rows were bent over the cloth and were shaken vigorously to remove all insects within the canopy. All insects thus collected were counted and identified by order. In addition, insect feeding damage on the leaves, stems and pods was evaluated at different stages of plant development according to the following rating scale. Insect populations as well as insect feeding damage were assessed on all plots at each field site at the V1, R1, R5 and R7 stages of plant development. Data were analyzed by ANOVA on a per location, per insect order, and per time of assessment basis. In those cases where differences among treatments were significant, Tukey's procedure (Steel and Torrie, 1980) was used to make pair-wise comparisons of treatment means.

Rating scale used to evaluate each plot for damage caused by insect feeding.

Insect damage rating	Percent of leaves with insect damage
0	0
1	1 – 10
2	11 – 20
3	21 – 30
4	31 – 40
5	41 – 50
6	51 – 60
7	61 – 70
8	71 – 80
9	81 – 90
10	91+

The numbers of Coleopteran, Lepidopteran, and Hemipteran insects detected on CV127 soybean plants were not significantly different from numbers detected on plants of the isoline control or on the conventional soybean varieties at any of four sampling dates during the 2006/2007 (Table VIII-11) and 2007 (Table VIII-12) growing seasons (except for one instance at the Teresina field site in 2007 for Coleopterans at the R5 growth stage, where levels on the conventional soybean variety Monsoy 8001 were statistically significantly higher compared to the other treatments). Leaf, stem, or pod damage from insect feeding was below 2% for all soybean varieties across all treatments and locations during both growing seasons. This damage was considered minimal and no differences were evident among the different soybean treatments. Therefore the insect damage data was not statistically analyzed nor is it presented. Based upon the results of these

evaluations, in-field insect populations and resistance to insect damage of CV127 soybeans was found to be no different from that of the isoline control soybean or the conventional soybean varieties.

Table VIII-11. The Mean Number of Coleopteran (Col), Lepidopteran (Lep), and Hemipteran (Hem) Insects Collected in Each Plot During Field Trials in Brazil in the 2006/2007 Season. No significant differences were determined for any of the mean values for any treatment across all locations and growth stages.

Location	Treatment	Growth Stage											
		V1			R1			R5			R7		
		Insect Order											
		Col	Lep	Hem	Col	Lep	Hem	Col	Lep	Hem	Col	Lep	Hem
Ponta Grossa	CV127	0.25	0.25	0	2	2	0	5.75	3	2	2.25	0	0.75
	Isoline	0.75	0.25	0	2.25	1.25	0	5.5	2.75	2.5	2.75	0	0.75
	M8001	0.25	0.25	0	3.75	2.25	0	4.75	3.25	2.75	2	0	1.25
	CD217	0.5	0.25	0	3.25	2.25	0	6	3	2.25	2.25	0	1
Londrina	CV127	1	0	0.25	2.25	2.25	0	1	2.25	1.25	0	0.25	2.25
	Isoline	1	0	1	1.75	1.75	0	1.25	1.25	0.25	0.75	0.25	1.5
	M8001	1	0	0.25	2.25	2.25	0	0.75	2.25	1.25	0.25	0.75	1.25
	CD217	1.25	0	0.5	1.75	1.75	0	1.25	1	2.25	0.25	0.25	2.75
Sto. Ant. de Posse	CV127	0.25	0	0.25	0	0	0	2.25	3.75	4	0	4.25	3
	Isoline	0.5	0.5	0.25	0	0	0.5	1.75	4	3.75	0	3.25	2.75
	M8001	0.5	0.5	0.25	0	0	0.5	2.25	4	3.75	0	4.25	3.25
	CD217	0.5	0.5	0.25	0	0	0.25	2.75	4.5	4.25	0	4.25	3.25
Uberaba	CV127	0.25	0	0	0	0	0	0	0.5	0	0	0	0.25
	Isoline	0	0	0	0	0	0	0	0.25	0	0	0.25	0.5
	M8001	0.75	0	0	0	0.25	0	0	0	0.25	0	0	0
	CD217	0.5	0	0	0	0.25	0.25	0	0.75	0.25	0	0.75	0.25
Sete Lagoas	CV127	0.25	0	0	0	0.25	0.25	0	0.25	0	0.5	1	0
	Isoline	0.5	0	0	0	0.25	0	0	0.25	0	0	1.25	0.5
	M8001	0.5	0	0	0	0.5	0	0.25	0	0	0.25	0.75	0.25
	CD217	0.75	0	0	0	0.5	0	0.75	0.25	0	0.75	0.5	0
Sto. Ant. de Goiás	CV127	0.25	0	0	0	0	0	0	0	0.5	0	0	0.5
	Isoline	0.5	0	0	1	0	0	0	0	0.25	0	0	0.25
	M8001	1.25	0	0	0.75	1.25	0	0	0	0	0	0	0
	CD217	0.25	0	0	0	0.5	0	0	0	0.25	0	0	0.25
Brasilia	CV127	0	0.25	0	0.5	0	0	0	0	0	0	0	0
	Isoline	0.25	0	0	0.25	0.25	0	0	0.25	0.5	0	0	0
	M8001	0.25	0.25	0	0	1	0	0	0	0.5	0	0	0.25
	CD217	0.5	0	0	0.25	0	0	0	0	0	0	0	0.25

Table VIII-12. The Mean Number of Coleopteran (Col), Lepidopteran (Lep), and Hemipteran (Hem) Insects Collected in Each Plot During Field Trials in Brazil in the 2007 Season. No significant differences were determined for any of the mean values for any treatment across all locations and growth stages, except for one instance at the Teresina field site for Coleopterans at the R5 growth stage, where numbers followed by different letters are statistically significantly different at the 5% level.

		Growth Stage											
		V1			**R1**			**R5**			**R7**		
		Insect Order											
Location	Treatment	Col	Lep	Hem	Col	Lep	Hem	Col	Lep	Hem	Col	Lep	Hem
Uberaba	CV127	1.56	0.71	0.71	0.97	0.84	0.71	0.71	0.93	0.84	0.71	0.84	0.71
	Isoline	1.48	0.71	0.71	1.06	0.84	0.71	0.71	1.06	0.84	0.71	0.84	0.71
	M8001	1.56	0.71	0.71	0.84	0.71	0.71	0.71	0.84	0.84	0.71	0.71	0.71
	CD217	1.40	0.71	0.71	0.97	0.97	0.71	0.71	0.97	0.71	0.71	0.71	0.71
Sete Lagoas	CV127	1.06	0.71	0.71	0.84	1.06	0.71	0.71	1.31	0.84	0.71	0.84	0.97
	Isoline	1.18	0.71	0.71	1.06	1.06	0.71	0.71	1.10	0.71	0.71	0.84	0.71
	M8001	1.22	0.71	0.71	0.97	1.18	0.71	0.71	0.84	0.97	0.71	0.97	0.71
	CD217	1.10	0.71	0.71	0.97	0.97	0.71	0.71	1.06	0.97	0.71	1.06	0.71
Sto. Ant. de Goiás	CV127	1.06	0.84	0.71	0.84	0.71	0.71	0.71	0.71	0.84	0.71	0.71	0.71
	Isoline	1.06	0.71	0.71	0.84	0.84	0.71	0.71	0.71	0.84	0.71	0.71	0.84
	M8001	0.93	0.84	0.71	0.84	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
	CD217	0.93	0.71	0.71	0.71	0.84	0.71	0.71	0.71	0.84	0.71	0.71	0.71
Brasilia	CV127	0.71	0.84	0.71	0.71	0.84	0.71	0.71	0.71	0.71	0.71	0.71	0.84
	Isoline	0.84	0.71	0.71	0.84	0.71	0.71	0.71	0.84	0.97	0.71	0.71	0.84
	M8001	0.84	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
	CD217	0.97	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Vilhena	CV127	0.97	0.71	0.71	1.49	0.71	0.71	1.61	0.84	1.27	0.71	0.71	1.22
	Isoline	1.10	0.71	0.71	1.65	0.71	0.71	1.31	1.10	1.40	0.71	0.71	1.10
	M8001	1.06	0.71	0.71	1.49	0.71	0.71	1.79	1.13	1.44	0.71	0.71	1.18
	CD217	0.97	0.71	0.71	1.40	0.71	0.71	1.59	0.84	1.49	0.71	0.71	1.06
Teresina	CV127	0.71	2.04	0.71	0.71	0.84	0.71	0.71 b	0.71	0.84	0.71	0.71	0.71
	Isoline	0.71	1.90	0.71	0.71	0.97	0.71	0.71 b	0.71	0.71	0.71	0.71	0.71
	M8001	0.71	1.83	0.71	0.71	0.71	0.71	1.79 a	1.13	0.71	0.71	0.71	0.84
	CD217	0.71	1.63	0.71	0.71	0.71	0.71	0.71 b	0.71	0.71	0.71	0.71	0.71

D.3. Nitrogen Fixation Characteristics

An important agronomic characteristic of soybeans is the ability to support a nitrogen fixing symbiosis with the soil bacterium *Bradyrhizobium japonicum*. *B. japonicum* cells in the soil stimulate the development of root nodules on soybean plants. The *B. japonicum* cells populate the interior of the nodule and the plant creates an environment within the nodule that is conducive for nitrogen fixation by the endosymbiont. Through this symbiosis, *B. japonicum* is able to convert atmospheric nitrogen to fixed forms of nitrogen and reduce the soybean host's dependency on nitrogen fertilizer. It is a common practice in Brazil and Argentina for farmers to inoculate soybean seed with *B. japonicum* prior to planting to ensure the development of effective root nodulation and strong nitrogen fixing activity. The purpose of the nitrogen fixation study was to confirm that CV127 soybean is equivalent to the isoline control and to conventional soybean varieties with respect to nitrogen fixation and nitrogen accumulation in the plant. Characteristics of nitrogen fixation in CV127 soybean were evaluated and compared with the characteristics of the isoline control and conventional soybean varieties, including measurements of soil populations of *B. japonicum*, plant growth and nodulation and total nitrogen and ureide nitrogen accumulation in the plants. These determinations were made on plants grown in field studies during the 2006/2007 growing season at seven geographically-distinct field locations in Brazil which are representative of areas of commercial soybean production and to which the soybean varieties included in the study are adapted. Identical evaluations of the *B. japonicum* nitrogen fixing symbiosis were also made at six trial locations in the 2007 short growing season in Brazil. The field trial locations are the same as described in section VIII.C. ("Agronomic and Phenotypic Evaluations in Replicated Field Trials"). Plants were harvested at the R2 stage of plant development for analysis of nitrogen fixation parameters. Statistical analyses were conducted by ANOVA, and means comparisons across treatments at each location were determined using Least Significant Difference (LSD) statistical procedures, and across locations using the Tukey's test (Steel and Torrie, 1980)

The results of these studies showed that CV127 soybean is equivalent to the isoline control with respect to biological nitrogen fixation, including nodule number, nodule dry weight, shoot dry weight, total N content and the contribution of nitrogen fixation to the total nitrogen (N) accumulated in tissues as evaluated by the N-ureide content of the shoot (Tables VIII-13 and VIII-14). Comparison of CV127 soybean and the isoline control as a group with the conventional soybean varieties showed some differences in nitrogen fixation parameters, which were attributed to basic genetic differences between these soybean varieties in biological nitrogen fixation capabilities. A more detailed description of the methodology used in the study and the results obtained are presented in Appendix F.

Table VIII-13. Effect of Different Treatments on Nitrogen Fixation Parameters in the 2006/2007 Growing Season. Measurements included nodule number (NN, number plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), concentration of N in shoots (CNS, g N kg^{-1} plant^{-1}), total N accumulated in shoots (TNS, mg N plant^{-1}), % N as N-ureide (%NU)[2] and total N as N-ureide (TNU, mg N-ureide plant^{-1})[3] in soybean harvested at R2 in the seven trials performed in the summer of 2006/2007[1].

Treatment	Parameter						
	NN	NDW	SDW	CNS	TNS	%NU	TNU
CV127	52.6 a	168.4	20.78 a	48.13 ab	1031.0 a	75.04 a	772.6 a
Isoline	46.2 ab	163.6	21.84 a	50.66 ab	1153.4 a	76.70 a	894.9 a
M8001	36.7 b	158.9	16.95 b	52.82 a	928.8 b	67.49 b	626.7 b
CD217	33.8 b	124.2	17.16 b	48.78 ab	886.8 b	66.26 b	598.6 b

[1]The data represent the mean of 20 replicates, with ten plants per replicate. Means followed by the same letter do not differ statistically significantly by the Tukey's test at 5% probability.
[2] N-ureides were measured in stem and petiole tissues only and expressed as a percent (%NU) of total N (N-ureides plus nitrate) in these tissues. N-ureides are an estimate of the contribution of nitrogen fixation to total N accumulated by the plant.
[3] The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot (TNU) for treatment comparison purposes.

Table VIII-14. Effect of Different Treatments on Nitrogen Fixation Parameters in the 2007 Growing Season. Measurements included nodule number (NN, number plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), concentration of N in shoots (CNS, g N kg^{-1} plant^{-1}), total N accumulated in the shoots (TNS, mg N plant^{-1}), % N as N-ureide (%NU)[2] and total N as ureides (TNU, mg ureide-N plant^{-1})[3] in soybean at the R2 stage in field trials conducted in Brazil at six locations in the 2007 short growing season[1].

Treatment	Parameters						
	NN	NDW	SDW	CNS	TNS	%NU	TNU
CV127	29.4	150.0 a[1]	9.06 ab	42.5	396	69.1 ab	293 ab
Isoline	34.6	165.4 a	10.01 a	42.0	421	74.3 a	324 a
M8001	21.4	120.2 ab	9.12 ab	40.9	388	71.0 a	297 ab
CD217	21.8	112.9 b	7.96 b	39.4	313	67.0 b	212 b

[1] Data are means of 24 replicates and analyses in duplicate. Means followed by the same letter do not differ statistically significantly by the Tukey's test at 5% probability.
[2] N-ureides were measured in stem and petiole tissues only and expressed as a percent (%NU) of total N (N-ureides plus nitrate) in these tissues. N-ureides are an estimate of the contribution of nitrogen fixation to total N accumulated by the plant.
[3] The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot (TNU) for treatment comparison purposes.

E. Conclusions from Agronomic, Phenotypic and Ecological Evaluations of CV127 Soybeans

The purpose of the studies summarized in this section was to:
1) Evaluate the agronomic, phenotypic, and phenologic characteristics of CV127 soybean relative to an isoline control and to other conventional soybean varieties;
2) Determine the ecological impact (interaction with diseases, insects and factors relevant to nitrogen fixation) of cultivating CV127 soybean relative to cultivating the isoline control and the conventional soybean varieties in the plant and soil environments.

CV127 soybean was compared to the isoline control and two other conventional soybean varieties with respect to key vegetative and reproductive developmental characteristics associated with competitiveness and survival of plant species. In field trials, characteristics including seed germination rate, seedling vigor, days to reach key developmental stages, plant height, and grain yield, as well as susceptibility to and interactions with diseases and insects were evaluated. In addition, the impact of cultivating CV127 soybeans on factors relevant to the nitrogen fixing symbiosis with *Bradyrhizobium japonicum* were assessed and compared between CV127, the isoline control and conventional soybean varieties. Furthermore, laboratory and greenhouse-based studies on seed germination, pollen number and pollen germination were also conducted and comparisons of these characteristics made between CV127 soybeans, the isoline control and the two other conventional soybean varieties.

Data from these studies demonstrate that CV127 soybean has no different agronomic or phenotypic characteristics, disease or insect interactions, seed germination, pollen number and pollen germination characteristics, or ability to support a nitrogen fixing symbiosis with *Bradyrhizobium japonicum* than do the isoline control or the comparator conventional soybean varieties. The only trait for which there was a trend toward a difference between CV127 and the isoline control was seed size. For all other traits measured there were no consistent statistically significant differences between CV127 and the isoline control. Further, where seed size differences were observed between CV127 and the isoline control, this did not confer a selective advantage with respect to germination, seedling vigor, plant stand or any other agronomic, phenotypic or ecological interaction trait.

In addition to agronomic, phenotypic and ecological interaction evaluations, extensive analyses were conducted on the key nutrients and antinutrients of the grain and forage derived from CV127 soybean and the isoline control and conventional soybean varieties (section VII). Results of these studies demonstrated that grain and forage from CV127 soybean is substantially equivalent in its nutrient and antinutrient content to the grain and forage from conventional soybean varieties that have a long history of safe use for the production of food and feed products. Collectively, from results of all the above studies it is concluded that the cultivation of imidazolinone-tolerant CV127 soybean poses no greater environmental or plant pest risk than does the cultivation of conventional soybeans.

IX. Environmental Assessment of CV127 Soybeans

The potential for CV127 soybean to exhibit plant pest or weediness characteristics as well as an evaluation of interactions of CV127 soybean with both pest and non-pest organisms in the environment are discussed in this section. The potential for gene flow from CV127 and the impact of the cultivation of CV127 soybeans in the environment are also addressed.

CV127 soybean was developed for cultivation primarily in Brazil and Argentina. The major weeds in soybean cultivation in these countries are sensitive to the imidazolinone herbicides containing imazapyr and imazapic, making this product concept an attractive proposition for soybean cultivation in South America. Therefore, regulatory approvals for this product are sought in Brazil and Argentina for production as well as for food and feed uses, and in the U.S. and other countries for importation of grain from CV127 soybean for food, feed, and processing uses. However, the purpose of this discussion is to establish that, in the unlikely event that CV127 soybeans were to be introduced in to the U.S. environment, CV127 soybeans would present no different environmental impact compared to conventional soybean varieties with regard to cultivation practices, interactions with pest and non-pest organisms, and weed control measures, other than the ability to use imidazolinone herbicides for weed control in the CV127 crop.

A. Safety of the Imidazolinone-tolerance Trait

The imidazolinone herbicides inhibit plant growth due to their inhibition of a plant enzyme, acetohydroxyacid synthase (AHAS), which has an important function in the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine. CV127 soybean is tolerant to the imidazolinone class of herbicides as a result of the introduction of the *csr1-2* gene from *Arabidopsis thaliana* encoding an AHAS enzyme that is tolerant to imidazolinone herbicides. Several *AHAS* genes that encode AHAS enzymes that are tolerant to imidazolinone herbicides have been discovered in plants as naturally-occurring mutations or through the process of chemically-induced mutagenesis. The mutation in the *csr1-2* gene which results in the substitution of a serine residue with an asparagine at position 653 [S653N] (relative to the amino acid sequence of the AHAS enzyme from *Arabidopsis thaliana*) is one of the five most common single-point mutations in AHAS genes that have been found to result in tolerance to imidazolinones in plants (Tan *et al*., 2005). This mutation confers tolerance to imidazolinone herbicides with no cross-tolerance to other AHAS inhibitors such as the sulfonylurea herbicides (Lee *et al*., 1999). To date, imidazolinone-tolerant maize (*Zea mays* L.), rice (*Oryza sativa* L.), bread wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* and *B. juncea* L. Czern.), lentil (*Lens culinaris*) and sunflower (*Helianthus annuus* L.) have been developed through mutagenesis or introgression from natural sources, selection, and conventional breeding technologies and have been commercialized under the **Clearfield®** brand name since 1992, 2003, 2002, 1996, 2006, and 2003, respectively. Of these, **Clearfield®** varieties of maize, rice, bread wheat, and oilseed rape have been commercialized that contain the same S653(At)N AHAS amino acid substitution that is present in CV127 soybean. These crops have been cultivated for many years without adverse environmental impact and without the development of weediness or other plant pest characteristics (Tan *et al*., 2005). Furthermore, the grain and other food or feed components

produced from the **Clearfield®** crops described above have been demonstrated to be as nutritious and as safe as the same food or feed components produced from their respective imidazolinone-sensitive counterparts. Therefore, there is a history of safe production as well as safe food and feed use of products containing the same S653(At)N AHAS amino acid substitution that is present in CV127 soybean.

In two separate growing seasons in field trials in Brazil, CV127 soybean was compared to the isoline control and two other conventional soybean varieties with respect to key vegetative and reproductive development characteristics associated with the competitiveness and survival of plant species (section VIII of this petition). Also, CV127 was assessed for disease susceptibility and interactions with different orders of insects, as well as the impact of cultivating CV127 soybeans on factors relevant to the nitrogen fixing symbiosis with *Bradyrhizobium japonicum.* Data from these studies demonstrated that CV127 soybean has no different agronomic or phenotypic characteristics, disease or insect interactions or ability to support a nitrogen fixing symbiosis with *Bradyrhizobium japonicum* than do conventional soybean varieties. In combination, all of these studies support a conclusion that CV127 soybean poses no different plant pest or weediness risk or environmental impact compared to that from the cultivation of conventional soybean varieties. Also, extensive analyses of the nutrient and antinutrient content of the grain and forage produced from CV127 soybeans described in section VII of this petition confirmed that these products are as nutritious and safe as grain and forage produced by conventional soybean varieties.

All plants possess a biochemically-active AHAS enzyme that is necessary for the production of the branched-chain amino acids required for protein synthesis. The imidazolinone-tolerant AtAHAS enzyme encoded by the *csr1-2* gene that was introduced into CV127 soybeans encodes an AtAHAS enzyme that has the same biochemical characteristics as other plant AHAS enzymes except for its tolerance to imidazolinone herbicides. The AtAHAS protein expressed in CV127 soybean is structurally and biologically closely related to the AHAS proteins found in all plants. For example, there is 79% amino acid identity between the AtAHAS and endogenous soybean AHASL2 (JGI locus Glyma13g31470.1) proteins. Also, the AtAHAS protein expressed in CV127 soybean does not share any biologically relevant amino acid sequence homology or biological characteristics with known toxins or allergens. Finally, the source organism of the *csr1-2* gene that encodes the AtAHAS protein in CV127 soybean, *Arabidopsis thaliana*, is not a known human or animal pathogen and is not known to cause allergic reactions in humans. Similarly, *A. thaliana* is not known to produce toxic substances. Therefore, results of these studies show that neither the AtAHAS protein nor CV127 soybeans are likely to pose any increased safety risk to other plants and organisms in the environment compared to other plant-produced AHAS proteins or other conventional soybean plants.

B. Ecological Characteristics of CV127 Soybean

B.1. Weediness Potential of CV127 Soybean

The commercial varieties of soybean that are cultivated in the U.S. do not possess any characteristics that are commonly associated with weeds, such as seed dormancy or persistence of seed in the soil, or the ability to widely disperse, invade diverse habitats, compete with native plant species, and become a dominant species in different habitats (CFIA, 1996). Cultivated soybean seed rarely display any dormancy characteristics and only under certain environmental conditions will they grow as a volunteer in the year following cultivation (Raper and Kramer, 1987; CFIA, 1996; OECD, 2000). In managed ecosystems, soybean does not compete effectively with other cultivated plants or primary colonizers. Further, soybeans are not frost-tolerant and do not typically survive freezing temperatures during winter.

Volunteer soybean management is normally not a significant management concern in North America because of environmental conditions, crop rotation, the numerous herbicide control options available, and the fact that soybeans do not compete well with the succeeding crop (CFIA, 1996; OECD, 2000). Since soybean seed does not survive the freezing temperatures of winter, soybean seed do not overwinter and are typically not viable in the following season (CFIA, 1996). When a rotational crop such as winter wheat is planted after soybean harvest, it is possible for soybean seed to be incorporated into the soil and to germinate that fall. These volunteer plants will be controlled by freezing temperatures in the fall and do not become a competitive consideration. Therefore, normally volunteer soybean is not a target for weed control measures in winter wheat.

When corn is the rotational crop with soybean, the first option available to control volunteer soybeans the following spring is pre-plant tillage or the use of a pre-plant burn-off herbicide such as glyphosate. The most common herbicide active ingredients applied to corn in the United States in 2010 are listed in Appendix G, Table G-1; there were 58 active ingredients reported as used on corn. Herbicides from group 4 (auxinic herbicides), group 5 (triazines), group 10 (phosphorylated amino acids) and group 27 or group 28 (4-hydroxyphenyl pyruvate dioxygenase or HPPD inhibitors) all will control volunteer soybeans in corn. Seventeen of the 58 active ingredients applied to corn (29% of the total) are members of one of these herbicide groups. Therefore there are numerous tools available to control volunteer soybeans in corn. Thus, in the event that CV127 soybean volunteers were to become established, they would not compete well with succeeding crops and they could be controlled by either mechanical means or by herbicides other than the group two herbicides that target the AHAS enzyme.

In Roundup Ready® (RR) corn, volunteer conventional or imidazolinone-tolerant soybeans will be controlled by any of the glyphosate products (group 9) applied. In some cases the glyphosate is tank-mixed or used in sequence with another broadleaf herbicide. Any of the broadleaf herbicide products from groups 4, 5 or 27 will also control volunteer soybeans. Therefore, virtually all the herbicide options available in Roundup Ready® corn will control volunteer soybeans.

The agronomic evaluations that were conducted with CV127 soybean in two growing seasons suggest no different characteristics compared to conventional soybeans that would impart the ability to be invasive or weedy. CV127 soybean was equivalent to the isoline control and to two conventional soybean varieties with respect to seed germination, quality and dormancy, as well as pollen number and pollen germination and key phenotypic characteristics including seedling vigor, days to flowering and to maturity and seed production. Also, no differences in the frequency of volunteers between CV127 soybean, the isoline control, and the two conventional soybean varieties were observed in the post-harvest monitoring of the field trials described in this petition, providing further support for the conclusion that CV127 soybean has no different survivability or persistence characteristics than conventional soybean varieties. Also, since wild species of *Glycine* are not indigenous to, or known to exist in, the U.S., it is biologically improbable that CV127 soybean would out-cross to weedy plants and transfer its imidazolinone herbicide tolerance trait to compatible weedy species. Based upon the results of these studies it was concluded that CV127 soybean has no increased weediness potential compared to conventional soybean.

B.2. Interactions of CV127 Soybean with Other Organisms in the Environment

Soybean like every other plant is known to interact with other organisms in the environment including microorganisms, viruses, insects, birds, and mammals. A list with examples of potential interactions of soybean with pathogens, consumers, symbionts, and others is given in the consensus document for *Glycine max* (L.) Merr. (OECD, 2000). Also, soybeans develop symbiosis with *Bradyrhizobium japonicum*, a plant-associated nitrogen-fixing bacterium. When soybeans are grown in new production areas, seeds are normally inoculated with *B. japonicum* prior to planting.

CV127 soybean was evaluated for susceptibility to a number of important soybean diseases in Brazilian agriculture, including Asian soybean rust (*Phakopsora pachyrhizi*), Downy mildew (*Peronospora manshurica*), Powdery mildew (*Erysiphe diffusa*), and end-of-cycle diseases (DC) caused mainly by *Septoria glycines* (brown spot or Septoriose) and *Cercospora kikuchii* (soybean leaf spot). CV127 soybean was also evaluated for interactions with several different insect orders common in commercial soybean production fields in Brazil that included the Coleopterans (*Diabrotica* and *Aracanthus* spp.), Lepidopterans (*Anticarsia*, *Agrotis*, *Pseudoplusia*, and *Spodoptera* spp.), and Hemipterans (*Euschistus*, *Piezodorus*, and *Nezara* spp.). Finally, the impact of cultivating CV127 soybeans on factors relevant to the nitrogen fixing symbiosis with *Bradyrhizobium japonicum* were assessed. In all of these studies, CV127 soybean was compared to the isoline control and two conventional soybean varieties.

Results of these studies demonstrated that CV127 soybean has no different interaction with various diseases, insects, or *Bradyrhizobium japonicum* nor any difference in nitrogen fixation capacity compared to the isoline control. Therefore, these results further confirmed that cultivation of CV127 soybeans poses no different environmental impact than the cultivation of conventional soybean varieties.

B.3. Potential for Gene Flow in Soybean

Soybean is considered to be a self-pollinating species that is propagated commercially by seed. Commercial cultivars are developed through breeding by artificial hybridization. The longevity of soybean pollen is generally two to four hours at most (Andersson and de Vicente, 2010). The soybean flower stigma is receptive to pollen for a period that begins 24 hours prior to anthesis and ends 48 hours thereafter (Carlson and Lersten, 1987; Carlson and Lersten, 2004). Typically the anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybeans exhibit a high percentage of self-fertilization and cross pollination is usually less than 0.5% (Caviness, 1966; Ray *et al*., 2003; Yoshimura *et al*., 2006). In addition, the seed pod and individual seed do not possess morphological characteristics that encourage the spread of the seed by animals.

For a trait to become incorporated into a species' genome, recurrent backcrosses of plants of the species with hybrid intermediates and survival and fertility of the resulting offspring are necessary. The wild relatives of *G. max* include *G. soja* (Sieb. and Zucc.) (2n=40) and *G. gracilis* Skvortz. (2n=40) that are native to Asia. *G. soja* is a wild viny annual with small, narrow trifoliate leaves, purple flowers, and small round black-brown seeds. It grows wild in Korea, Taiwan, Japan, and the Yangtze river valley in northeast China and areas near the border with Russia. *G. gracilis* has been observed growing in northeast China (Skvortzow, 1927). Interspecific fertile hybrids between *G. max* and *G. soja* (Sieb. and Zucc.) (Hadley and Hymowitz, 1973; Ahmad *et al*., 1977) and between *G. max* and *G. gracilis* (Karasawa, 1952) are readily obtained.

In addition to the subgenus *Soja*, the genus *Glycine* also contains the subgenus *Glycine*. The subgenus *Glycine* contains 22 wild perennial species, including *G. clandestine* Wendl., *G. falcata* Benth., *G. latifolia* Benth., *G. latrobeana* Meissn. Benth., *G. canescens* F.J. Herm., *G. tabacina* Labill. Benth., and *G. tomentella* Hayata. These members of the subgenus *Glycine* are indigenous to Australia, the South Pacific Islands, China, Papua New Guinea, the Philippines, and Taiwan (Hermann, 1962; Newell and Hymowitz, 1978; Hymowitz and Newell, 1981; Grant, 1984; Tindale, 1984 and 1986). Hybridization between the diploid perennial *Glycine* species produces normal meiosis and fertile offspring.

Attempts at hybridization between members of the annual subgenus *Soja* and the perennial subgenus *Glycine* have proven unsuccessful. Although hybridization results in the initiation of pod development, the pods eventually abort and abscise (Ladizinksy *et al*., 1979; Hood and Allen, 1980). Later, intersubgeneric hybrids between *G. max* and *G. clandestine* Wendl. and between *G. max* and *G. tomentella* Hayata were obtained *in vitro* through embryo rescue (Singh and Hymowitz, 1985; Singh *et al*., 1987). Successful hybridization between *G. max* and *G. canescens* was also achieved using transplanted endosperm as a nurse layer (Broué *et al*., 1982). In all cases, the progeny of these intersubgeneric hybrids were obtained with difficulty and only with human intervention and all resulting progeny were sterile. Soybean is not able to cross with other plants outside of the *Glycine* subgenus *Soja* and so intergeneric hybridization does not occur.

The potential for the genetic transfer of the imidazolinone-tolerance trait from CV127 soybean is limited to members of the subgenus *Soja* due to biological barriers. The wild relatives of the subgenus *Soja* do not exist in North or South America. Consequently, the possibility of natural gene transfer between CV127 soybeans and compatible relatives or wild species of the subgenus *Soja* does not exist in North or South America. In the unlikely event transfer of the trait to weedy species should occur, the acquisition of the imidazolinone-tolerance trait would not be expected to enhance the weediness of the recipient plants. Such hypothetical imidazolinone-tolerant weedy plants could be controlled by many other herbicides other than those of the imidazolinone class or by cultivation.

There is no evidence that genetic information can transfer from soybean to other organisms that cannot sexually interbreed with soybean (horizontal gene transfer). Therefore, genetic information in CV127 soybean will not be transferred by horizontal gene flow to other organisms in the environment.

C. Cultivation of Soybean

Soybean is the leading oilseed crop produced and consumed in the world today. The current world production of soybean greatly exceeds that of all other edible oilseed crops (Wilcox, 2004; Soy Stats, 2011). In 2011, 258.4 MMT of soybeans were produced worldwide representing 58% of the world's total oilseed production (Soy Stats, 2011). Soybean has been the dominant oilseed produced in the world since the 1960s (Smith and Huyser, 1987) and in the past twenty years (1989 through 2009) soybean production has increased by 116 MMT (FAOSTAT, 2011). For the past 50 years, the United States has been the world's leading producer of soybeans and in 2010 it produced 90.6 MMT, or about 35% of the world's total production (Soy Stats, 2011). The second and third largest producers of soybean are Brazil and Argentina who produced 70.0 and 49.5 MMT of soybeans, respectively, in 2010 (Soy Stats, 2011). The two largest soybean producers in Asia are the People's Republic of China (15.2 MMT in 2011) and India (9.6 MMT).

Due to the ability of soybean to support a nitrogen fixing symbiosis with *Bradyrhizobium japonicum* and to convert atmospheric nitrogen to fixed forms of nitrogen, soybeans are often planted in rotation with corn, wheat, cotton, rice and other crops. Studies have demonstrated that the rotation of soybean with other crops generally leads to increased yields for the soybean and subsequent crops (Johnson, 1987; Wesley, 1999). Other benefits for the farmer from rotation with soybeans include reduced nitrogen input in the following crop, increased residue cover, and reduction of pest and weed cycles.

For many years, genetically modified (GM) soybean varieties have constituted the largest percentage of GM crops planted worldwide. In 2010 the area planted to GM soybeans was 73.3 million hectares or 50% of the global area planted to soybeans (James, 2010). The vast majority of this acreage was planted in herbicide-tolerant soybean varieties. In 2010, herbicide-tolerant soybeans comprised about 93% of the soybean acreage planted in the United States, almost 100% in Argentina, and 75% in Brazil (James, 2010). The rapid and widespread adoption of herbicide-tolerant soybeans by growers worldwide has been due to increased yields, the reduced cost of effective weed control, and a simplified, more flexible weed control program (Reddy, 2001; Gianessi, 2005) that is offered by the cultivation of herbicide-tolerant crops. Perhaps more

importantly to the grower, the benefits from the cultivation of herbicide-tolerant soybeans have increased the income of those growers that have planted them instead of conventional varieties. It has been estimated that the cultivation of herbicide-tolerant soybeans increased the income of farmers by $2.84 billion in 2005 (Brookes and Barfoot, 2006). Given that the U.S. soybean crop value in 2005 was $17,337 million (Soy Stats, 2011), this represents an increase equal to approximately 16% of the total value of the soybean crop.

The primary herbicide-tolerant soybeans grown today are tolerant to the herbicide glyphosate. Glyphosate-tolerant soybeans were first grown commercially in 1996 and the cultivation of this crop has steadily increased every year since introduction (Gianessi and Reigner, 2006). In recent years, several weed species have evolved resistance to glyphosate (Heap, 2011; Nandula *et al*., 2005). It should be noted that most of the cases of glyphosate-resistant weeds occurred in non-transgenic crops. Exposure to glyphosate started well before the introduction of glyphosate-tolerant crops and thus, attributing the development of glyphosate-resistant weed species to the use of glyphosate-tolerant crops is not warranted (Dill, 2005). The introduction of imidazolinone-tolerant soybeans will provide the grower with the opportunity to cultivate soybeans that are tolerant to a herbicide with a different mode of action compared to glyphosate as a means to control weeds that may be tolerant to glyphosate and to help prevent the continued evolution of more weed species that are glyphosate-tolerant. The rotation of herbicide-tolerant crops that are resistant to different herbicide classes in combination with the application of the corresponding herbicides is an effective strategy for managing the development of herbicide tolerance in weed populations.

C.1. Cultivation of Soybean in Brazil. Brazil is the largest producer of soybeans in South America and the second largest producer in the world behind the U.S. The first soybean varieties produced in Brazil were varieties that were bred for production and commercialization in the U.S. However, after the early introduction of U.S. varieties, research and breeding programs in Brazil, including the public agricultural research organization 'Empresa Brasileira de Pesquisa Agropecuaria' (Embrapa), began to develop varieties with increased yield potential and resistance to pathogens specifically adapted to all major agricultural regions of Brazil. Today, many of the most productive Brazilian varieties developed in these programs contain an ancestor that was developed in the US. As a result of the Brazilian breeding effort, soybean production in Brazil has steadily increased from about 0.5 MMT in 1965 to 31 MMT in 1999 and 70 MMT in 2010 (Wilcox, 2004; Soy Stats, 2011) and yield on a per hectare basis has doubled from 1.2 to 2.4 tons/ha (Wilcox, 2004).

Soybeans may be planted in any region of Brazil as a full-season crop in late October or early November with harvest occurring in March. In some tropical and sub-tropical regions, soybean may be planted as a short-season crop in late February or early March with harvest in July. The cultivation of soybean plants in Brazil is similar to the cultivation of soybean plants in the U.S. as the plant's requirements from the environment are not different. However, the input requirements to produce a crop in Brazil may be different from those required to produce a crop in the U.S. due to environmental differences that may exist between the two countries. Soybeans are commonly grown in rotations with grain crops such as maize or winter wheat. The use of reduced tillage that has been facilitated by the introduction of herbicide-tolerant varieties of

soybean and the correction of soil acidity and improved seed quality have all contributed to increased soybean production in Brazil.

C.2. Cultivation of Soybean in Argentina. Argentina ranks second to Brazil in the production of soybeans in South America and third worldwide (Soy Stats, 2011). There are many similarities in soybean production in Argentina and Brazil, including the fact that soybeans were also first introduced into Argentina from the U.S. However, since Argentina is located at latitudes that are more similar to those in the regions of soybean cultivation in the U.S., the American cultivars were better adapted for cultivation in Argentina. Soybean varieties in Maturity Groups III to IX are cultivated from latitude 23 to 39°S. As in Brazil, production of soybeans in the past few decades has increased tremendously in Argentina, from 1.4 MMT in 1977, to 19.9 MMT in 1999, and 49.5 MMT in 2010 (Wilcox, 2004; Soy Stats, 2011). The use of herbicide-tolerant soybean varieties has been common practice for many years and this has led to the widespread adoption of minimum- or no-tillage cultivation practices. About 65% of soybeans that are cultivated in Argentina are grown as a full-season crop with the remaining 35% being double cropped with wheat. Soybean is also commonly rotated with maize, sorghum, pasture, peanut, and flax (Wilcox, 2004).

C.3. Cultivation of Soybean in the U.S. The U.S. is the world's largest producer of soybeans. As in the other major soybean producing countries of Brazil and Argentina, production has increased dramatically in the U.S. in recent years. While production of soybean has increased 70% in the U.S. since 1985, from 51 to 87 MMT in 2006, during this period the area planted to soybean increased only about 14%, from 25.6 to 29.3 million hectares (Wilcox, 2004). From 1985 to 2010, soybean yields on a per-acre basis increased from 34.1 bushels per acre to 43.5 bushels per acre in the U.S. (Soy Stats, 2011). Soybean is grown primarily in the eastern half of the U.S. from the Gulf of Mexico to the Canadian border where rainfall is sufficient to sustain the crop. This area includes 31 states in which at least one million acres of soybean were planted in 2011, including the following states: IA, IL, MN, IN, MO, NE, OH, SD, AR, ND, KS, MI, MS, WI, NC, KY, TN, and LA (USDA-NASS, 2011). The most common rotational crops for soybeans in the United States are corn, other row crops, and small grains. In 2002 approximately 80% of the soybeans were grown in rotation with corn (USDA-ERS, 2006b). Soybean is normally grown as a full-season crop that is seeded in the spring, but a small percentage of the crop is planted mid-June to early July as a second crop following winter wheat, rice or winter canola in the more southerly regions of the U.S.

Soybeans with the non-recombinant DNA-derived STS™ trait are tolerant to sulfonylurea herbicides. The STS™ trait does not provide cross-tolerance to imidazolinone herbicides and it is currently marketed by NK®, among others, in the United States as a stacked trait together with glyphosate tolerance. Use of STS™ soybeans allows effective weed control by sulfonylurea herbicides in soybean production areas where glyphosate-tolerant weeds are present. [

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All of the GM soybeans commercially planted to date in the U.S. contain herbicide tolerance traits (Table IX-1, Table IX-2). In 2011 approximately 94% of the U.S. soybean crop was genetically modified for herbicide tolerance (USDA-ERS, 2011a). Since 2007 over 90% of the

soybean cultivated in the U.S. in recent years contains one or more biotechnology-derived traits and if CV127 soybean were to be cultivated in the U.S., it would not be expected to significantly alter the area of biotechnology-derived soybeans planted in the U.S.

As in other regions of the world where herbicide-tolerant soybeans have been widely adopted, including Brazil and Argentina, the practice of minimum or no-till cultivation practices have increased in the U.S. Minimum- and no-till cultivation practices have lead to reduced: i) soil erosion, ii) contamination of surface waters with agricultural fertilizers and chemicals, iii) fuel consumption, and iv) release of greenhouse gases into the atmosphere.

Table IX-1. Commercialization Status of Genetically-Modified Soybeans in the United States in 2011

OECD Unique Identifier	Soybean Trait	Applicant	USDA Approval Date	U.S. Commercial Cultivation in 2011[1]?
MON-04032-6	Glyphosate tolerant	Monsanto	May 1994	**yes**
ACS-GMØØ5-3	Glufosinate tolerant	Bayer CropScience	July 1996	**yes**
ACS-GMØØ4-2	Glufosinate tolerant	Bayer CropScience	July 1996	no
ACS-GMØØ2-9	Glufosinate tolerant	Bayer CropScience	July 1996	no
ACS-GMØØ1-8	Glufosinate tolerant	Bayer CropScience	July 1996	no
DD-Ø26ØØ5-3	Increased oleic acid content	Du Pont	May 1997	no
ACS-GMØØ6-4	Glufosinate tolerant	Bayer CropScience	June 1998	no
ACS-GMØØ3-1	Glufosinate tolerant	Bayer CropScience	November 1998	no
MON-89788-1	Glyphosate tolerant	Monsanto	July 2007	**yes**
DP-356Ø43-5	Glyphosate and acetolactate synthase tolerant	Pioneer	July 2008	no
DP-3Ø5423-1	High oleic acid content	Pioneer	June 2010	no[2]
BPS-CV127-9	Imidazolinone tolerant	BASF Plant Science L.P.	n.a.	no
MON-87701-2	Lepidopteran resistant	Monsanto	n.a.	no
MON-87769-7	Stearidonic acid produced	Monsanto	n.a.	no
MON-877Ø5-6	Improved fatty acid profile	Monsanto	n.a.	no
MST-FGØ72-3	Glyphosate and isoxaflutole tolerant	Bayer CropScience and MS Tech	n.a.	no
DAS-68416-4	2,4-D and glufosinate tolerant	Dow AgroSciences	n.a.	no
MON-877Ø8-9	Dicamba tolerant	Monsanto	n.a.	no
MON-87712-4	Increased yield	Monsanto	n.a.	no

[1] Source: www.biotradestatus.com, accessed August 31, 2011.

[2] Plenish™ high oleic soybeans will be grown under contract in 2011 for ongoing field and oil testing. Commercialization of Plenish™ high oleic soybeans is anticipated in 2012, upon full regulatory approval**. Source:** http://www.plenish.com/about_plenish.aspx, accessed September 3, 2011.

Table IX-2. Planted Acres of Soybeans and of Biotechnology-Derived Soybeans in the United States in 2001 through 2011 (USDA-ERS, 2011a; USDA-ERS, 2011b; USDA-NASS, 2011).

Year	Acres Planted	Biotech Percentage of Planted Acres*	Acres of Biotech Soybeans Planted*
2011	75208000	0.94	70695520
2010	77404000	0.93	71985720
2009	77451000	0.91	70480410
2008	75718000	0.92	69660560
2007	64741000	0.91	58914310
2006	75522000	0.89	67214580
2005	72032000	0.87	62667840
2004	75208000	0.85	63926800
2003	73404000	0.81	59457240
2002	73963000	0.75	55472250
2001	74075000	0.68	50371000

* Since there are not currently any non-herbicide tolerant biotechnology-derived products commercialized, the acreage of biotechnology-derived soybeans presented here is synonymous with the acreage of biotechnology-derived herbicide-tolerant soybeans.

Since a large percentage of the soybeans that are cultivated currently in the U.S. are tolerant to glyphosate, this herbicide is the leading herbicide used on soybeans in the U.S. (Table IX-3). In addition, soybean is naturally resistant to some imidazolinone herbicides, including imazamox, imazethapyr, and imazaquin due to lower uptake and higher metabolism of these herbicides by soybean. These three imidazolinone herbicides are registered for use on soybeans in the United States under brand names such as Raptor®, Pursuit®, and Scepter®, among others. The 2010 usage of all herbicides on soybeans in the U.S. is presented in Table IX-3.

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C.4. Comparison of the Cultivation of Soybean in Brazil and the U.S.

The following section reviews the similarities between soybean genotype, environment, and agronomic practices used in soybean production in Brazil and the U.S. This comparison demonstrates that the data generated in field trials conducted in Brazil to support the environmental and food and feed safety of CV127 soybean are equally applicable to the environmental and food and feed safety assessments of CV127 in the U.S. environment.

Soybean genotype comparison

Based on their photosensitivity, soybean varieties are divided into maturity groups (MG) 000 to X. Varieties in the earliest maturity group (000 to II) are adapted to the northernmost soybean production regions of North America and the southernmost production regions of South America. Varieties in the latest MG (VII-X) are adapted to the southernmost soybean production regions of North America and the northernmost production regions of South America. Thus, specific varieties are grown at certain latitudes because they flower and mature within an optimal time period required for commercial production at that latitude. A recent study conducted in Brazil using soybean varieties developed in Brazil and other varieties developed in the U.S. concluded that the maturity classification system used in North America was an efficient method for describing relative maturity in Brazil on a broad environmental basis (Alliprandini *et al.*, 2009).

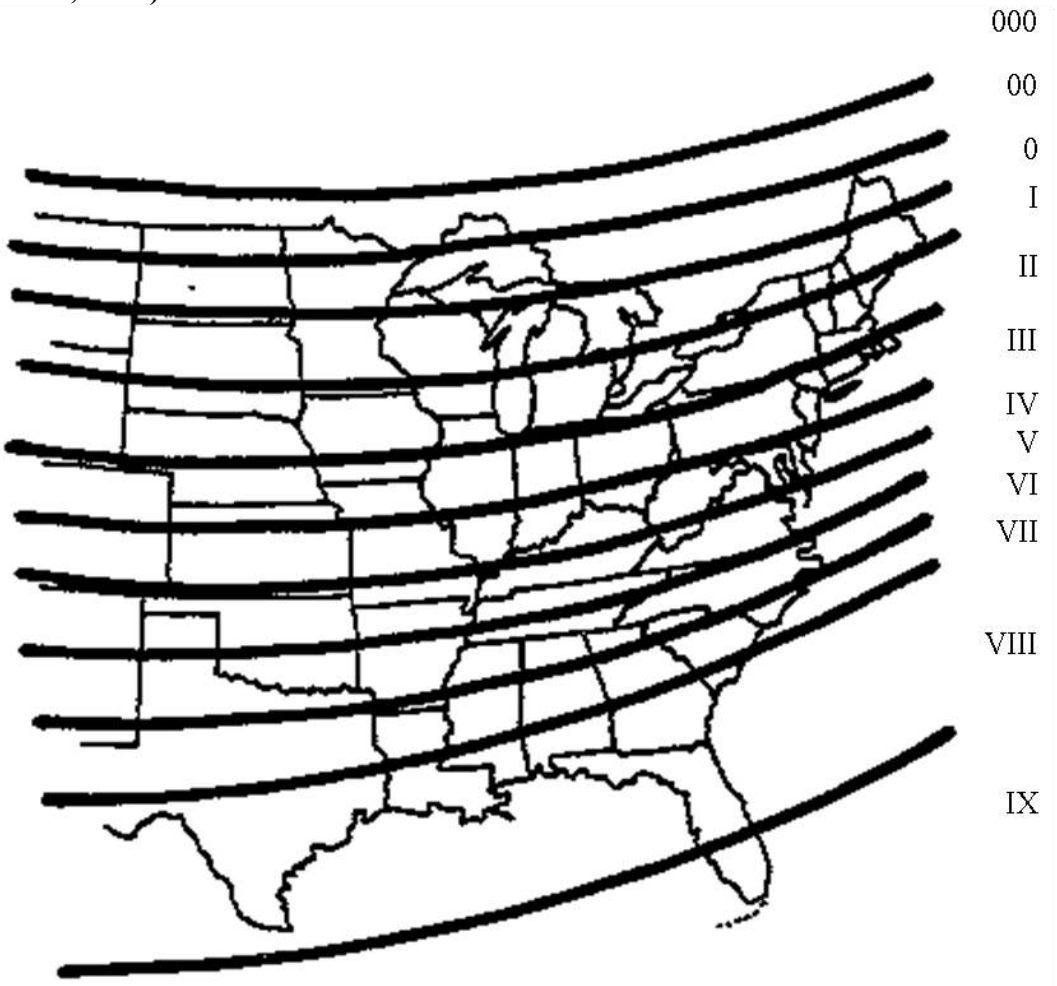
Differences between early maturing (MG 000 to IV) and late maturing (MG V to X) soybean varieties with respect to time to flowering and maturity are controlled by at least five genes at the 'E' locus: *E1* and *E2* (Bernard, 1971), *E3* (Buzzell, 1971), *E4* (Buzzell and Voldeng, 1980), and *E5* (McBlain and Bernard, 1987). Three of these genes (*E1*, *E3*, *E4*) are also involved in photoperiod sensitivity (Cober *et al.*, 1996). Soybean breeders, using classical methodologies, routinely recombine genes at the E locus to modify time to maturity and develop varieties adapted to specific environments. Another main genetic difference normally observed between early maturing and late maturing varieties occurs at the locus (dt) for stem determinancy. Early maturing varieties generally have an indeterminate growth habit with genotype dt1/dt1 and late maturing varieties generally have a determinate growth habit with genotype Dt1/Dt1 (Bernard, 1972). This common relationship between maturity and determinancy is a result of selection for the best combination of genes required to maximize production in a specific region. For example, in a study which included later-maturing determinate and indeterminate varieties adapted to the southern U.S., it was concluded that the indeterminate growth habit may confer a yield advantage over the determinate growth habit in 10 to 15% of the environments with limited yield and growth potential, but that the determinate growth habit provided a yield advantage over a larger percentage of the environments. Thus, while indeterminate late-maturing varieties are genetically possible and available, the yield advantage an indeterminate may have over a determinate growth habit in a late-maturing variety is small (Kilgore-Norquest and Sneller, 2000).

In response to the variation in day length and climatic conditions in soybean production regions of the U.S., varieties grown in the U.S. range from MG I to MG IX (Figure IX-1). In Brazil, the variation in environmental conditions across the soybean production regions supports the production of varieties in MG V to X (Alliprandini *et al.*, 2009). In general, the soybean

genotypes produced in Brazil are similar to those produced in the midsouthern and southeastern U.S. where variety maturities range from IV to VIII. In fact, the original soybean varieties cultivated in Brazil during the early 20th century were varieties developed for cultivation in the southern U.S (refer to Figure II-1 as an example of the breeding lineage of the commercial Brazilian variety Conquista). Later, these U.S.-developed varieties became the parental genotypes used in Brazilian-based breeding programs to develop modern Brazilian soybean varieties. Therefore, soybean varieties grown in the U.S. and Brazil share common genetic backgrounds.

The lines of CV127 soybean that were evaluated in the field trials described in this petition are MG VIII. These lines are adapted to the northern soybean production regions of Brazil and the southern soybean production regions of the U.S. However, using conventional breeding methods, it is possible to use lines of CV127 soybean to develop early (MG I to IV) or late (MG V to VIII) maturing soybean varieties with determinate or indeterminate growth habits. The specific production environment ultimately dictates which combination of traits is required in that environment to enable the plant to be most productive.

Figure IX-1. Map Showing Regions in the Continental United States and Southern Canada where Various Soybean Maturity Groups (MG) Are Adapted for Full-Season Growth. (Willis, 1989).



Environmental comparison

Climate: The varieties of CV127 soybean that have been developed for cultivation in Brazil are in maturity group VIII. In the U.S., soybeans in maturity group VIII are recommended for cultivation from the eastern coastal plains regions of the Southeastern states to Texas. The average monthly temperatures in this region during the growing season are shown in Table IX-4. Also shown for comparison are the average monthly temperatures at locations near the individual locations where CV127 field trials were conducted in Brazil. The average monthly temperatures during the soybean growing season in Brazil are less variable across the season compared to those in the soybean growing regions of the southeastern U.S. (Table IX-4). In the U.S. locations, the average monthly temperatures are comparable to those in Brazil.

Table IX-4. Comparison of the Average Monthly Temperatures (Degrees Fahrenheit) during the Soybean Growing Season. Different locations in the southeastern United States where soybeans in maturity group VIII are cultivated and cities closest to the individual field trial locations in Brazil where CV127 soybean was evaluated are shown.

US Locations[1]	Lat.	Apr.	May	June	July	Aug.	Sep.
Charleston, SC	33° N	65	74	78	82	81	76
Macon, GA	33° N	64	72	78	81	80	75
Houston, TX	30° N	70	76	81	84	84	80
Mobile, AL	31° N	68	75	80	82	82	78
Jackson, MS	32° N	65	72	78	82	81	76
Baton Rouge, LA	30° N	68	75	81	82	82	78
Gainesville, FL	30° N	68	74	78	81	81	78
Brazil Locations[2]	**Lat.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**
Ponta Grossa, PR	25° S	64	66	69	71	71	69
Sto. Ant. de Posse (Campinas), SP	23° S	71	73	75	75	77	75
Uberaba, MG	19° S	75	75	75	75	77	75
Sete Lagoas, MG	20° S	71	71	71	73	73	73
Sto. Ant. de Goiás (Goiânia), GO	16° S	73	73	73	71	73	73
Brasília, DF	16° S	69	69	69	69	69	69

[1]Source: http://countrystudies.us/united-states/weather/, accessed September 21, 2011.
[2]Source: http://www.weatherreports.com/ /Brazil, accessed September 22, 2011. Average monthly temperature over five to 36 years, depending on location, is reported.

There are differences in the total amount of annual rainfall and in the distribution of rainfall throughout the year between the regions in Brazil where soybeans are cultivated and the southeastern U.S. where maturity group VIII soybeans are cultivated (Table IX-5). Soybeans in Brazil are cultivated primarily in the high plateau or savannah region in central Brazil and in the southern, more temperate region. The average rainfall in this area ranges from 26 to 60 inches

per year. In the savannah, rainfall is highest during the growing season (November through March) and is much lower during the winter months (May through September). As rainfall is plentiful during the growing season in Brazil, irrigation is not typically required for soybean cultivation. In the southeastern U.S. where rainfall can occasionally be inadequate during the soybean growing season, irrigation, if available, is used during dry periods to insure that the crop receives adequate water for growth. A similar comparison of the monthly average rainfall during the growing season in the southeastern U.S. with locations near the individual Brazilian CV127 field trial locations is shown in Table IX-5. In general, rainfall during the soybean growing season in the primary soybean cultivation regions in Brazil is higher compared to the rainfall in the soybean-producing southern U.S. However, with the use of supplemental irrigation for soybean production in the southern U.S., the total amount of water available to the crop grown in the two countries is comparable. In areas of soybean cultivation in the southeastern U.S. where irrigation is not available, soybean yield can be reduced during periods of inadequate rainfall.

Table IX-5. Average Monthly and Total Rainfall (Inches) during the Soybean Growing Season. Different locations in the southeastern United States where soybeans in maturity group VIII are cultivated and cities closest to the individual field trial locations in Brazil where CV127 soybean was evaluated are shown.

U.S. Locations[1]	Apr.	May	June	July	Aug.	Sep.	Total
Charleston, SC	2.7	4.0	6.4	6.8	7.3	4.7	31.9
Macon, GA	3.5	3.6	3.6	4.3	3.6	2.8	21.4
Houston, TX	3.1	5.3	6.4	4.8	4.5	5.5	29.6
Mobile, AL	4.5	5.7	5.0	6.9	7.0	5.9	35.0
Jackson, MS	5.6	5.1	3.3	4.5	3.8	3.6	25.9
Baton Rouge, LA	5.4	4.9	4.5	6.7	6.0	4.9	32.4
Gainesville, FL	2.6	3.8	6.8	6.8	8.0	5.3	33.3
Brazil Locations[2]	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Total**
Ponta Grossa, PR	5.25	3.32	4.08	6.07	4.64	3.25	26.61
Sto. Ant. de Posse (Campinas), SP	4.33	6.42	9.35	12.23	7.98	6.01	46.32
Uberaba, MG	4.13	4.88	6.98	8.81	7.11	5.47	37.38
Sete Lagoas, MG	2.58	5.79	9.34	7.63	4.81	4.85	35.00
Sto. Ant. de Goiás (Goiânia), GO	4.25	9.79	13.78	13.50	10.57	8.22	60.11
Brasília, DF	2.94	6.40	6.71	5.04	5.10	5.75	31.94

[1]Source: http://countrystudies.us/united-states/weather/, accessed September 21, 2011.
[2]Source: http://weather.uk.msn.com/region.aspx?wealocations=Brazil, accessed September 22, 2011.

In summary, the climatic conditions for soybean production in the major growing regions of Brazil are comparable to those in the growing regions of the U.S., and where differences between environments in Brazil and the US were observed, these were generally not different from the range of environments encountered across the soybean production regions within the southern

US. In any environment, the soybean plant's requirements for resources from the environment are not significantly different. However, in those environments in which certain resources are limiting, the input requirements to produce the crop may be different.

Soil: Most of the soils in the new agricultural areas in Brazil are classified as tropical soils, or oxisols. The oxisols contain only hydrated oxides of iron and aluminum, are highly weathered with low pH and low in fertility and organic matter. The low pH of the soils often limits the availability of phosphorus to the soybean plant. Because soybeans require relatively large amounts of phosphorus and a soil pH in the range of 5.0 to 5.5 (Guidelines for GAP, 2002) for optimal growth and development compared to other crops such as corn or wheat, Brazilian soybean growers typically add lime and phosphorus to the soils, and with this treatment oxisol soils can be very productive for soybean cultivation (Leibold *et al*. 2001). Also, addition of lime and phosphorus helps minimize potential aluminum toxicity to growth and development of the soybean plant. Similarly, in the U.S., soybean growers often apply phosphorus as well as potash to increase crop production, but this practice is highly variable from state to state. In both Brazil and the U.S., the full nitrogen needs of soybean can be supplied through the nitrogen fixing, root nodule symbiosis with *Bradyrhizobium japonicum* and nitrogen application is not necessary nor does it usually result in increased yield (Guidelines for GAP, 2002; Whitaker *et al*., 2011).

Agronomic comparison

Cultivation Practices. The planting dates for soybean vary depending on the latitude and region. In Brazil, soybeans are typically planted from mid-October to mid-December and in the more northern areas, in late December. The optimum planting rate and row-spacing for soybean cultivation in Brazil varies depending on the climate of the various soybean cultivating regions. A planting rate of 220,000 to 320,000 seed/ha with row spacing of 0.4 to 0.5 m is typical for soybean cultivation in Brazil (Guidelines for GAP, 2002). Planting depth for soybeans in Brazil is typically 3 to 5 cm deep. Planting deeper decreases germination while planting more shallow results in losses due to high surface-soil temperatures and water stress. Harvest of soybeans in Brazil is at the R8 growth stage (full maturity) when the soybeans have a moisture content of 13 to 15%. In the southeastern U.S. where soybeans in MG VII to VIII are planted (e.g., Georgia), the optimal planting date for soybeans is May 10 to June 10 (Whitaker *et al*., 2011). Seed should be planted at a depth of 2.5 to 3 cm (1 to 1.25 inches) in moist soil. The recommended planting rate and row spacing for soybean cultivation in the U.S. is 10-20 seed/m of row (3-6/foot of row) with a row spacing of 0.5 – 0.9 m (20 – 36 inches) to achieve a final stand of 200,000 to 250,000 plants/ha (85,000 to 100,000 plants/acre). In order to help minimize disease and insect pests, it is recommended in both Brazil and the U.S. that soybeans are rotated with grain crops such as wheat and corn.

Weeds and Weed Control Practices: The major pest problems in the cultivation of soybeans in the U.S. are weeds followed by insects and diseases (Aref and Pike, 1998). Since weeds compete with soybeans for light, nutrients and moisture, weed control is an important factor in optimizing yield. The main factors affecting yield loss due to weeds are the predominant weed species, the weed density, and the length of the competition by weeds during the growing season. If weeds are allowed to grow with a soybean crop for the entire growing season, yield losses can be as high as 75% of the crop (Dalley *et al*., 2001). In addition, the impact of different weed

species on the yield of soybeans has shown that yield losses vary with the predominant weed species and level of infestation (Coble *et al*., 1981; Conley *et al*., 2003). Soybeans grow quickly to close the crop canopy, after which they are very competitive with weeds. However, soybeans are very sensitive to weed competition in the first weeks after emergence from the soil. It is recommended by weed specialists that weeds be controlled in soybeans within three to five weeks after emergence in order to prevent irreversible yield losses due to early competition from weeds. The practice of narrow row planting has been used to shorten the time for soybean to close the canopy and thereby gain an advantage over weeds. However, weeds that are taller than soybeans can still break through the canopy and cause yield losses. Common weeds in soybean cultivation in the U.S. include the annual grasses and broad-leaf weeds such as foxtail, ragweed and lambsquarter, winter annuals such as marestail and biennial wormwood, and perennials such as quackgrass and Johnsongrass. With the adoption of no-till or reduced-tillage cultivation systems, the weed spectrum has shifted to favor the winter annual, biennial, and perennial weeds.

Brazilian soybean farmers tend to have fewer weed control problems than farmers in the U.S. This is partly due to the fact that the agricultural lands in Brazil do not have as long a history of farming as those in the U.S. However, the practice of no-till farming in Brazil is common, just as it is in the U.S., and weed pressure is expected to increase over time in Brazil. For example, soybean farmers in the southern regions of Brazil are faced with greater weed pressure than farmers in other soybean growing regions of Brazil because this area has a longer history of crop cultivation. Another challenge to soybean production in Brazil is that temperatures stay warm after the soybean leaf canopy has fallen, which encourages weeds to start growing before the crop is harvested. While this late season weed growth has little impact on soybean yield, it can be a challenge to harvest crops with young green weeds growing. However, it is not common for either Brazilian or U.S. farmers to apply herbicides at this late stage of crop development. The fact that it rarely freezes in Brazil during the winter months means that perennial weeds pose a greater weed problem in Brazil compared to the weed spectrum in soybeans in the U.S.

Prior to the use of herbicides, the primary method of weed control in all crops was by mechanical cultivation. The first herbicides used in soybean were soil-incorporated and pre-emergence herbicides were introduced in the 1960's (Carpenter and Gianessi, 1999). These were replaced in the 1980's by post-emergent herbicides that were not active on soybeans and their use increased dramatically until recently. The use of post-emergence herbicides has greatly facilitated the adoption of reduced- or no-tillage practices since farmers could effectively control weeds with one or two early treatments. This has both economic and environmental advantages, including more effective weed control leading to enhanced yield and reductions in tractor passes, fuel consumption, soil erosion and the release of greenhouse gases. The more recent advent of genetically-modified (GM) soybeans that are tolerant to post-emergent treatment with broad-spectrum herbicide has further facilitated the adoption of reduced- or no-tillage practices (Gianessi, 2005).

The cultivation of glyphosate-tolerant soybeans treated with glyphosate has been widely adopted in the U.S., Argentina, and Brazil. In 2010, 60% of the United States treated acres were treated with glyphosate (Table IX-3). Similarly, nearly 100% of the soybean crop in Argentina is glyphosate-tolerant and the percentage of cultivation of glyphosate-tolerant soybeans cultivated in Brazil is rapidly increasing since regulatory approval in 2005 (approximately 75% in Brazil in

2010, [James, 2010]). Therefore, the uses of glyphosate herbicide to control weeds in soybeans is the most common practice in both the U.S. and Brazil. These trends suggest that there is a clear need for tolerance in soybeans to herbicides with different modes of action as a means to better manage the development of herbicide resistance. Chemical carryover is less of a problem in Brazil compared to the U.S. due to prolonged warmer soil temperatures and higher rainfall in Brazil. However, there is still a need to rotate crops and chemical modes of action to minimize the probability of developing herbicide-resistant weeds in Brazil. In addition to glyphosate, other herbicides used for weed control in soybeans are similar between the U.S. and Brazil (Leibold *et al*., 2001).

The broad use of any class of herbicide with a specific mode of action is known to eventually lead to the development of resistance to the herbicide in weed populations. Some weeds commonly found in soybeans have developed resistance to glyphosate, namely horseweed (*Conyza canadensis)*, common waterhemp (*Amaranthus rudis*), common ragweed (*Ambrosia artemisiifolia*), and giant ragweed (*Ambrosia trifida*) (Heap 2011). Also, the growing use of glyphosate with glyphosate-tolerant soybeans in Brazil has led to a shift in the species of prevalent weeds with those that are more tolerant to glyphosate predominating. The most common weeds in this category include Benghal dayflower (*Commelina benghalensis* L.), morning glory (*Ipomoea* spp.), Brazil pusley (*Richardia brasiliensis*), and winged false buttonweed (*Spermacoce alata*). These weeds are sensitive to imidazolinone herbicides. An effective method of managing and reducing the development of resistance is the rotation of herbicides with different modes of action (Loux *et al*. 2004; Loux and Stachler 2006). Therefore, the introduction of imidazolinone-tolerant CV127 soybeans will provide soybean growers an additional tool for the management of herbicide resistance in weeds.

Insect Control: In the U.S., the control of insects in soybean with insecticides is second only to weed control in total pesticide use. In 2006, insecticides were applied to 16% of the U.S. soybean acreage (USDA-NASS, 2007). The three most commonly-used insecticides included lambda-cyhalothrin, chlorpyrifos, and esfenvalerate that were applied to 6, 5, and 3% of the planted soybean acres, respectively (USDA-NASS, 2007). In the U.S., the number of soybean insect pest species and their densities follows a north-south gradient with a greater insect pressure in the southern states bordering the Gulf of Mexico and the Atlantic Ocean (Way, 1994). This is the same region in which soybeans in maturity group VIII such as CV127 are adapted for cultivation. The major insect pests of soybean in the southern U.S. include the lepidopterous defoliators, velvetbean caterpillar (*Anticarsia gemmatalis*) and soybean looper (*Pseudoplusia includens*); the coleopterous defoliator, bean leaf beetle (*Cerotoma trifurcata*); the pod-feeding stink bugs, complex-southern green stink bug (*Nezara viridula*) and green stink bug (*Acrosternum hilare*); and the corn earworm (*Helicoverpa zea*) that attacks foliage and pods. The midwestern U.S. suffers only sporadically from severe outbreaks of insect pests in soybeans (Boethel, 2004). Some of the insect pests that occasionally are problematic in soybeans in the Midwest include the green cloverworm (*Plathypena scabra*), Mexican bean beetle (*Epilachna varivestis*) and bean leaf beetle. More recently, the soybean aphid (*Aphis glycines*) that is a native of Asia has become a significant soybean insect pest since its discovery in the U.S. in 2000.

In Brazil the warmer winter temperatures result in a larger number of insects, typical of the southern U.S. The main insect pests of soybean in Brazil, as in the southern U.S., include the velvetbean caterpillar and the green stink bug (Turnipseed and Kogan, 1976). The lepidopterous cutworm *Agrotis ipsilon* is known to cause sporadic problems in soybean production in Brazil (Turnipseed and Kogan, 1976). The lesser cornstalk borer, *Elasmopalpus fignosellus*, is widely distributed from the southern U.S. through Mexico, to southern Brazil and Argentina where stand reductions frequently necessitate replanting in late-planted soybean in sandy soils, particularly under drier conditions (Turnipseed and Kogan, 1976). While the similarities in the insect pest species between the U.S. and Brazil are greater for the southern U.S., the insect pests of soybean are fewer in the midwestern U.S. due to colder winters in this region.

The imidazolinone-tolerant CV127 soybean has been assessed in multi-location, replicated field trials conducted in two growing seasons in Brazil and has been shown to be no different in its agronomic characteristics or environmental interactions compared to conventional soybeans. The susceptibility of CV127 to insects was found to be no different in CV127 soybean compared to conventional soybean varieties. Protease inhibitors produced by plants have been shown to play a role in the defense against insect predation and disease infestation (Hilder *et al*., 1987; Boulter, 1993; Williamson and Hussey, 1996; Joshi *et al*. 1998). Levels of soybean trypsin inhibitor were measured in the grain of CV127 soybean and the isoline control in field trials conducted during two growing seasons in Brazil and it was determined that there were no differences in the level of trypsin inhibitor in the grain from these soybeans (Section VII, Table VII-9).

A key component of the protection of crop plants from insects is the development of genetic resistance to insects that has been introduced into modern soybean varieties through breeding. The strong genetic similarity between soybeans cultivated in the U.S. and Brazil suggests that the same insect resistance traits are incorporated into both. The commercial cultivation of CV127 soybean in the U.S. would require the imidazolinone tolerance trait to be transferred by breeding into soybean cultivars that are adapted to the various climatic and environmental conditions of the soybean cultivating regions of the U.S. Based on the above considerations and on the extensive experience with many different imidazolinone-tolerant **Clearfield®** crops that have not demonstrated different sensitivity to insects compared to their corresponding imidazolinone-sensitive conventional counterparts and on the extensive knowledge of the biological mode of action of the AHAS enzyme, it is not expected that CV127 soybean would demonstrate a different response to insects were it to be cultivated in the U.S. compared to soybean varieties that are currently cultivated in the U.S.

Disease Control: Diseases caused by viruses and fungi can occasionally cause serious damage leading to significant loss of soybean yield in both the U.S. and Brazil. A list of the major viral diseases of soybean that occur in the U.S. and Brazil is presented in Table IX-6 . Of the 14 major viral diseases listed, seven are commonly found in both the U.S. and Argentina, Brazil, or South America (Tolin and Lacy, 2004). Of the others, five are present in the U.S. but not in South America and only two are present in South America but not in the U.S. (Table IX-6).

Bacterial pathogens are widespread on soybean but do not generally cause serious problems that result in significant yield reductions (Tolin and Lacy, 2004).

Over 40 fungal pathogens of soybean are reported to cause significant disease problems worldwide. The environment and crop management practices have a large impact on the prevalence and severity of these diseases (Yang and Feng, 2001). A key component in the management of soybean diseases is the incorporation of genetic resistance to important pathogens through breeding. However, resistance genes are not known for some pathogens and for these proper management practices are important. Due to economic considerations, the widespread application of foliar fungicides on soybeans in the U.S. is not common, but fungicides are commonly applied as seed treatments. In Brazil where Asian soybean rust (*Phakopsora pachyrhizi*) is especially problematic, foliar fungicides are commonly used to treat this pathogen. The major soybean pathogens in the top ten soybean-producing countries are listed in Table IX-7. Also presented in Table IX-7 are the soybean yield losses in the U.S. and Brazil in 1998 that were caused by these diseases (from Wrather *et al*., 2001). It should be noted that soybean rust (*Phakopsora pachyrhizi*) was not a major disease of soybeans in South or North America in 1998 but it has since developed into a major disease problem in Brazil in recent years. To date, effective genetic resistance to Asian rust has not been identified and so control relies on the use of foliar fungicides where infestations are severe. Asian rust has not developed into a significant disease of soybean in the U.S. or Argentina. All of the major soybean diseases that caused significant soybean yield losses in Brazil in 1998 also caused significant losses of soybean yield in the U.S. with the exception for powdery mildew (*Microsphaera diffusa*) and target spot (*Corynespora cassiicola*). Both of these diseases are found in the U.S. (Grau *et al*., 2004), but were not the source of significant losses in the U.S. in 1998.

Table IX-6. Important Viral Diseases of Soybean in the U.S. and South America (from Tolin and Lacy, 2004). Viruses that occur in both the U.S. and South America are denoted with a grey background.

Family, Genus or Virus	Distribution	Major Plant Symptoms	Genetic Resistance in Soybean
Tospovirus			
Tomato spotted wilt virus	U.S. (WI), Brazil, Iran	Systemic chlorosis and necrosis	NR †
Comoviridae			
Bean pod mottle	U.S., South America	Chlorotic mottle, green stems after pod maturity	None found
Bean rugose mosaic	Brazil, Argentina, Central America	Puffy areas along veins, chlorotic blotches	NR
Cowpea severe mosaic	Brazil, Puerto Rico, U.S. (AR, LA, IL)	Severe necrosis and bud blight	NR
Tomato ringspot virus	U.S., China	Bud blight, curved terminal bud and lateral bud proliferation	Observed
Potyvirus			
Bean yellow mosaic	Worldwide	Yellow mottling, rusty necrotic spotting	Observed
Peanut mottle	U.S., China, Thailand, Argentina	Mosaic with green islands; line pattern	*Rpv1; Rpv1, rpv1*
Soybean mosaic virus	Worldwide	Green to yellow mosaic, leaf curl	*Rsv1, Rsv3, Rsv4*
Bromoviridae			
Alfalfa mosaic	Worldwide	Bright yellow mosaic and mottle	NR
Cowpea chlorotic mottle	U.S. (AR, GA, SC)	Stunting, chlorosis, yellow stipple	Single dominant gene
Peanut stunt	U.S. (IL, KY, VA), Japan	Mild mottle and leaf crinkle	Observed
Tobacco streak	U.S. (IA, OH, OK, WI, VA)	Bud blight; axillary branches, stunting	NR
Others			
Cowpea mild mottle	Brazil, S.E. Asia, Africa	Vein clearing, leaf roll; vein necrosis	NR
Tobacco mosaic-soybean	U.S., Eastern Europe	Very mild vein clearing, chlorotic mosaic	NR

†No report of a search for resistance

Table IX-7. Causal Organisms of Commonly-Occurring Soybean Diseases in the Top Ten Soybean-Producing Countries and Soybean Yield Losses Caused by Them in the U.S. and Brazil in 1998 (Wrather *et al*., 2001).

Common Name	Causal Organism	Yield Loss ('000 metric tons)	
		US	Brazil
Anthracnose	*Colletotrichum truncatum*	188.9	2.0
Bacterial diseases	*Pseudomonas savastanoi* pv. *glycinea* and *Xanthomonas axonopodis* pv. *glycines*	27.0	—
Brown spot	*Septoria glycines*	167.0	2194.9
Brown stem rot	*Phialophora gregata*	398.8	—
Charcoal rot	*Macrophomina phaseolina*	1036.8	750.0
Diaporthe–phomopsis complex	*Diaporthe* and *Phomopsis* spp.	24.1	—
Downy mildew	*Peronospora manshurica*	106.3	—
Frog-eye leaf spot	*Cercospora sojina*	86.6	—
Fusarium root rot	*Fusarium* spp.	243.8	2.5
Phytophthora root and stem rot	*Phytophthora sojae*	1149.0	—
Pod and stem blight	*Diaporthe phaseolorum* var. *sojae*	143.6	627.1
Powdery mildew	*Microsphaera diffusa*	—	156.8
Purple stain of seed and cercospora leaf blight	*Cercospora kikuchii*	112.4	940.7
Rhizoctonia foliar blight	*Rhizoctonia solani*	111.5	14.4
Rhizoctonia–pythium root rot	*Rhizoctonia solani* and *Pythium* spp.	128.0	313.6
Sclerotinia stem rot	*Sclerotinia sclerotiorum*	509.0	1.5
Seed diseases	*Alternaria* spp., *Cercospora* spp., *Corynespora, Cladosporium*, *Phomopsis*, and *Fusarium* spp.	45.8	2.0
Seedling diseases	*Rhizoctonia*, *Pythium*, and *Fusarium* spp.	776.9	1.5
Southern blight	*Sclerotium rolfsii*	9.2	—
Stem canker	*Diaporthe phaseolorum* var. *caulivora* and var. *meridionalis*	30.0	10.0
Sudden death syndrome	*Fusarium solani* f. sp. *glycines*	900.6	200.0
Target Spot	*Corynespora cassiicola*	—	130.0

Note: Soybean rust (*Phakopsora pachyrhizi*) was not a major soybean pathogen in Brazil in 1998, but has developed into a major pathogen in recent years.

Differences in environmental conditions, whether between the U.S. and Brazil or between different regions within Brazil or the U.S., may result in differences in how the soybean crop is managed. For example, in regions of the U.S. or Brazil where rainfall is insufficient, irrigation is used to provide sufficient water for the crop and to prevent yield losses. Similarly, if soils are acidic, limestone may be applied. If weeds are a problem in soybean production, herbicides are

applied. In cases of heavy insect infestations, insecticides may be applied. Whether soybean is cultivated in the U.S. or in Brazil, it has the same general needs for growth and development. In some environments these needs are met naturally and in others they must be met through human intervention. There are many similarities in environmental conditions and agronomic practices used in soybean production between the southern U.S. and Brazil, including temperatures and rainfall, the maturity groups of soybean cultivated, as well as weed, insect and disease control practices. Furthermore, some environmental conditions during the growing season such as average temperatures are comparable between U.S. and Brazilian soybean production. In the event that CV127 soybean was cultivated in the U.S. environment, due to the close genetic relationship between the soybean varieties cultivated in the U.S. and Brazil as well as similarities in environmental conditions and agronomic practices that exist between the U.S.and Brazil, CV127 soybean would not be expected to present any new environmental risks relative to the cultivation of soybean varieties that are currently grown in the U.S. The long history of cultivation and safe use of **Clearfield®** crops also supports the conclusion that the introduction of the imidazolinone-tolerance trait into CV127 soybean will have no impact on its interaction with disease pathogens or the environment. Based on these considerations, the data generated in field studies conducted in Brazil to support the environmental as well as food and feed safety of CV127 soybeans are equally applicable to the environmental, food and feed safety assessment of CV127 in the U.S. environment.

D. Imidazolinone Herbicide Use in the U.S.

Imidazolinone-tolerant crops have been cultivated in the U.S. since the introduction of imidazolinone-tolerant corn in 1992 and are currently commercialized under the **Clearfield®** brand name. **Clearfield®** crops are developed by seed mutagenesis or other non-recombinant DNA techniques and contain mutations in the *AHAS* gene(s) that result in the production of an imidazolinone-tolerant AHAS. [

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] As previously noted, soybean is naturally tolerant to some imidazolinone herbicides (imazaquin, imazethapyr, and imazamox), (Tecle *et al.*, 1993; Nelson *et al.*, 1998) and these herbicides are applied to soybeans in the U.S. (Table IX-3). In addition, some other crops - field peas, snow peas, dry beans, snap beans, adzuki beans, fenugreek, seedling and established alfalfa, bird's foot trefoil and chickling vetch- possess a natural tolerance to individual imidazolinone herbicides and imidazolinones are used on these crops for weed control (Health Canada Pest Management Regulatory Agency, http://pr-rp.hc-sc.gc.ca/ls-re/index-eng.php). Soybeans are sensitive to imazapyr and imazapic and CV127 soybean was developed to be tolerant to these compounds. Imazapyr and imazapic are registered in the U.S. for weed control in pastures, rangeland, and other noncrop areas. Imazapic is registered in the United States for use in the control of weeds on pastures and peanuts, while imazapyr is registered for use on pastures and corn. A list of the crops in the U.S. for which the use of different imidazolinone herbicides is registered and the total area treated is presented in Table IX-8.

BASF developed CV127 soybean for commercial cultivation in Brazil and Argentina to address weed control challenges facing soybean farmers in these countries. However, in the event that CV127 soybean may be introduced into the U.S., it is not anticipated that CV127 soybean varieties will cause an increase in the total number of soybean acres planted in the U.S., and it is not expected that the herbicide tolerance trait will confer an incentive to grow the crop outside of its normal geographic range or habitat (e.g. cultivated agricultural lands). Furthermore, based on the use of imidazolinone herbicides for weed control on the different crops listed in Table IX-8, introduction of CV127 soybean in the U.S. is unlikely to have any significant effect on the total use of imidazolinone herbicides for weed control in U.S. agriculture.

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E. Potential Impact of Introduction of CV127 Soybean on Agronomic Practices

The introduction of soybean CV127 in Argentina and Brazil is expected to provide enhanced soybean yield potential and offer growers excellent weed control options in addition to the environmental benefits that result from the cultivation of imidazolinone herbicide-tolerant

soybeans. The imidazolinone herbicides possess several environmentally-beneficial characteristics compared to other herbicide classes (Tan *et al*., 2005). Imidazolinone herbicides control a wide spectrum of grass and broadleaf weeds. The growing use of glyphosate with glyphosate-tolerant soybeans in Argentina and Brazil has led to a shift in the species of prevalent weeds with those that are more tolerant to glyphosate predominating. The most common weeds in this category include Benghal dayflower (*Commelina benghalensis* L.), morning glory (*Ipomoea* spp.), Brazil pusley (*Richardia brasiliensis*), and winged false buttonweed (*Spermacoce alata*). These weeds are sensitive to imidazolinone herbicides, so introduction of CV127 soybean will provide growers an effective means to control these weeds at relatively low herbicide application rates.

Furthermore, with increased grower adoption of glyphosate-tolerant crops and use of glyphosate for weed control purposes, it has been reported that several weed species have evolved resistance to glyphosate (Heap, 2011; Nandula *et al*., 2005). Therefore introduction of CV127 soybeans will provide growers with the opportunity to cultivate soybeans that are tolerant to a herbicide with a different mode of action compared to glyphosate as a means to control weeds that may be tolerant to glyphosate and to help prevent the continued evolution of more weed species that are herbicide tolerant. The introduction of CV127 soybean varieties is not only expected to offer soybean growers an additional tool for controlling weeds, but also provide growers an important option for weed resistance management.

The environmental safety of CV127 was shown to be comparable to conventional soybean varieties which do not exhibit plant pest or weediness characteristics. CV127 soybean was compared to conventional soybean varieties with respect to important agronomic characteristics including seedling vigor, days to reach key developmental stages, plant height, disease and insect susceptibility, and grain yield. In addition, the impact of cultivating CV127 soybeans on factors relevant to the nitrogen fixing symbiosis with *Bradyrhizobium* was assessed. Furthermore, laboratory- and greenhouse-based studies on seed germination as well as pollen number and pollen germination were conducted and comparisons of these characteristics made between CV127 soybeans and the isoline control and, for the seed germination studies, two other conventional soybean varieties. The results of these comparative studies demonstrated that other than tolerance to imidazolinone herbicides, there are no biologically meaningful differences between CV127 and the conventional soybean varieties with respect to the parameters measured as described above.

Although it is not anticipated that CV127 soybean will be cultivated in the U.S., cultivation of CV127 soybean in the U.S. would not be expected to present any different environmental risks compared to the cultivation of herbicide-sensitive, conventional or glyphosate-tolerant soybean varieties. As noted above and elsewhere in this petition, extensive assessments of agronomic characteristics of CV127 soybean and its interaction with the environment have demonstrated that CV127 soybean is not significantly different from conventional soybean varieties in these characteristics. In addition, the Brazilian soybean variety, Conquista, that was transformed with the *csr1-2* gene cassette to produce CV127 soybean is closely related to soybean varieties that are currently cultivated in the U.S. (refer to Figure II-1). Therefore, it is expected that if CV127 soybeans were cultivated in the U.S., they would interact with the environment in a similar manner to commercial soybeans that are currently cultivated in the U.S.

Under the Canadian regulatory system, crops that are developed to be tolerant to herbicides for which the parental variety is susceptible, are considered novel, regardless of the methods by which they are developed. These Plants with Novel Traits require review and approval by the Canadian Food Inspection Agency (CFIA) and/or Health Canada for environmental release and food and feed use. All **Clearfield®** crops have undergone the required regulatory review in Canada and have been assessed to be safe for food and feed uses. In addition, in each case, after review of pertinent environmental data, the CFIA has concluded that **Clearfield®** crops do not present an altered risk to the environment compared to the herbicide-sensitive crop from which they were developed. A list of the **Clearfield®** crops that have been commercialized to date is presented in Table IX-9. In the more than 19 years in which **Clearfield®** crops have been cultivated in North America, other than their tolerance to imidazolinone herbicides, none of these crops have demonstrated any different environmental characteristics compared to their imidazolinone-sensitive counterparts. Similarly, there is no reason to believe that the imidazolinone-tolerant CV127 soybean will present an altered risk to the environment compared to conventional soybeans.

The agronomic and environmental assessments and the results and data from other studies of CV127 soybean that are described in this petition were generated using CV127 soybean lines 127 and 603 which are from maturity group VIII. Currently there are breeding programs in Brazil and Argentina that are breeding the imidazolinone-tolerance trait of CV127 soybean into genetic backgrounds that are earlier maturing and better-adapted for cultivation in Argentina and the southern regions of Brazil which have shorter growing seasons. Because of their earlier maturity, these varieties will also be adapted to soybean-cultivating areas of the midwestern U.S. (Figure IX-1). Given the common ancestry that exists among Brazilian and U.S. soybean varieties (Pardey *et al*., 2004), it is unlikely that the earlier-maturing varieties developed in Brazil or Argentina would interact differently in the U.S. environment or present any different environmental risk compared to soybean varieties that are currently cultivated in the midwestern U.S. In addition, the experience with the safe cultivation of the many different **Clearfield®** crops in the U.S. and other countries supports the conclusion that CV127 soybeans that are adapted for cultivation in the midwestern U.S. would not present any different environmental risks compared to soybean varieties currently cultivated in this region.

Table IX-9. Identity, Year of Market Launch and the CFIA Decision Document Number for Imidazolinone-tolerant Clearfield® Crops.

Crop	First Market Introduction for Crop	Line Name	Decision Document[1]
Corn	1992	XA-17	DD96-10
		XI-12	DD98-29
Canola	1996	NS1471	DD95-03
		S006	DD2008-73
Wheat	2002	SWP 965001	DD1999-31
		AP602CL	DD2003-44
		AP205CL	DD2004-47
		Teal 11A	DD2004-48
		BW255-2 BW238-3	DD2006-60
		DW1	DD2006-63
		DW2 DW6 DW12	DD2007-64
		BW7	DD2007-66
Sunflower	2003	X81359	DD2005-50
		CLHA-PLUS	DD2010-80
Lentil	2006	RH44	DD2004-46
Rice	2003	CL121 CL141 CFX51	DD2002-40
		PWC16	DD2002-42
		IMINTA 1 IMINTA 4	DD2006-62

[1]Decision documents are available on the Canadian Food Inspection Agency (CFIA) website at the following address: http://www.inspection.gc.ca/english/plaveg/bio/dde.shtml.

Based on the above characterizations of CV127 soybean, introduction of CV127 soybean is not expected to have any negative impact on current agronomic practices in soybean production in Brazil and Argentina, or in the U.S. for the following reasons:

a) CV127 soybean will offer growers an additional tool for weed control as well as herbicide resistance management in weeds as part of an integrated weed management program, and is not expected to change weed control practices currently followed by soybean growers.
b) CV127 soybean exhibited no different disease susceptibility or insect interactions compared to the conventional soybean varieties, so no change in disease and insect control practices in each different country is expected.
c) Conquista, the parental soybean variety that was used to develop CV127 soybean, is derived from and closely related to, soybean varieties cultivated in the southern U.S. and therefore possesses agronomic characteristics similar to U.S. soybean varieties.
d) A large variety of imidazolinone-tolerant crops currently commercialized under the **Clearfield®** brand name have been cultivated in North America since 1992 (Table IX-9). Each of these has been evaluated and reviewed by the Canadian Food Inspection Agency (CFIA) and has been determined to present no different environmental risk compared to their conventional, imidazolinone-sensitive counterparts. The collective experience of cultivating **Clearfield®** crops in North America and other regions of the world has confirmed that these crops do not present any additional environmental risks compared to corresponding conventional crops. Likewise, there is no reason to believe that imidazolinone-tolerant CV127 soybean will present any different environmental risk compared to imidazolinone-sensitive soybean varieties.
e) Volunteer soybean management is normally not a significant management concern in North America because of environmental conditions, crop rotation, the numerous herbicide control options available, and the fact that soybeans do not compete well with the succeeding crop. However, the current practices used for management of volunteer soybeans are equally applicable to CV127 soybean. In the event that CV127 soybean volunteers were to become established, they could be controlled by either mechanical means or by herbicides other than those that target the AHAS enzyme.
f) As outlined in section IX.F. of this petition and detailed in Appendix G, BASF is committed to the stewardship or Best Management Practice (BMP) program for management of weed resistance to imidazolinone herbicides in CV127 soybean crops. As detailed in Appendix G, grower compliance with this program is essential to delay the onset of imidazolinone herbicide tolerance in weeds in CV127 soybean crops. The guiding principles of the BMP for CV127 soybeans require that growers do not exceed a maximum of two exclusive Group 2 herbicide applications on any one field in any four-year period. Therefore, the introduction of CV127 soybeans is expected to expand the options to growers for integrated weed management programs and not have any negative impact on integrated weed management programs that growers currently follow that include the use of a wide range of herbicides, cultural practices and crop rotations to manage weed populations.

F. Herbicide Resistance Management for Imidazolinone-tolerant CV127 Soybean

The objectives of herbicide resistance management are to achieve weed control while preserving the value of each herbicide and each herbicide group for the longer term. An integrated

approach to weed control is the Best Management Practice to delay the onset of weed resistance to herbicides. Integrated weed management involves the use of a range of methods available to the grower in order to provide effective weed control in the crop. The use of herbicides is one of a number of useful tools available to growers.

BASF is committed to maintaining the efficacy of all of its herbicides in order to provide growers with effective, high-performance, environmentally-sound products for many years. The key to the performance of the Imidazolinone-tolerant Soybean Production System is effective weed resistance management. BASF is committed to delivering sustainable cropping systems that incorporate best practice principles. The Imidazolinone-tolerant Soybean Production System provides alternative options for growers within a well-managed rotation. As part of its weed resistance management program, BASF has developed a detailed weed resistance management plan for implementation with imidazolinone-tolerant CV127 soybean. An outline of this product stewardship plan is presented below and the complete details of the plan are presented in Appendix G.

Key Points of the Imidazolinone-tolerant CV127 Soybean Resistance Management Recommendations:

- DO NOT exceed a maximum of two exclusive AHAS-inhibiting herbicides on any one field, in any four-year period.

- ALWAYS follow an Integrated Weed Management (IWM) program that includes a wide range of herbicides, cultural practices and crop rotations in order to manage weed populations and minimize weed seed development.

- ALWAYS control volunteers in the season following an imidazolinone-tolerant soybean crop.

- USE practices which minimize the likelihood of out-crossing to similar crops or related weeds.

- Scout fields for weeds or volunteer crops that are uncontrolled by herbicides.

- FOLLOW the Best Management Practices outlined in the Imidazolinone-tolerant Soybean Production System Stewardship Guide (presented in Appendix G).

G. Summary of the Agronomic and Environmental Assessments for CV127 Soybean

The agronomic characteristics and environmental interactions of CV127 soybean were thoroughly evaluated in Brazil during two different growing seasons. In addition, the molecular insert in CV127 soybean was completely characterized, the level of AHAS protein in various tissues of CV127 soybean was analyzed, and the nutrient and antinutrient composition of grain and forage was evaluated. Review of the data from these studies supports the conclusion that CV127 soybean presents no different plant pest potential or environmental risk compared to that of conventional soybean varieties. This conclusion is further supported by the long history of the

cultivation of other imidazolinone-tolerant crops containing the same S653N AHAS amino acid substitution as found in CV127 soybeans. None of these crops have demonstrated enhanced plant pest tendencies or environmental risks compared to their corresponding imidazolinone-sensitive counterparts.

The AHAS enzyme is ubiquitous in plants and its biological mode of action is well studied and is not known to be toxic or allergenic. The food safety of the imidazolinone-tolerant AtAHAS introduced into CV127 soybean is further supported by the long history of safe consumption of food and feed products derived from the many different **Clearfield®** crops that contain the S653N AHAS mutation and by the grain composition studies of CV127 soybean that demonstrate that the grain from CV127 soybean is as nutritious as grain from conventional soybeans.

The environmental safety of CV127 was shown to be comparable to conventional soybean varieties which do not exhibit plant pest or weediness characteristics. CV127 soybean was compared to conventional soybean varieties with respect to important agronomic characteristics including seedling vigor, days to reach key developmental stages, plant height, disease and insect susceptibility, and grain yield. In addition, the impact of cultivating CV127 soybeans on factors relevant to the nitrogen fixing symbiosis with *Bradyrhizobium* were assessed. Furthermore, laboratory- and greenhouse-based studies on seed germination, pollen number and pollen germination were conducted and comparisons of these characteristics made between CV127 soybeans and the isoline control and, for the seed germination studies, two other conventional soybean varieties The results of these comparative studies demonstrated that other than tolerance to imidazolinone herbicides, there are no biologically meaningful differences between CV127 and the conventional soybean varieties with respect to the parameters measured as described above. Therefore, these results reinforce the conclusion that the cultivation of CV127 soybean poses no different plant pest or weediness potential and will have no different environmental impact than the cultivation of conventional soybean varieties.

Other than the additional option to apply imidazolinone herbicides for weed control in CV127 soybeans, the commercial introduction of CV127 soybeans is not expected to change the typical agronomic practices used for soybean production, will have no different environmental interactions compared to other commercially-produced soybean varieties, or any impact on the nutritional quality and safety of soybean food and feed products compared to those from conventional soybean varieties. The introduction of imidazolinone-tolerant CV127 soybeans is expected to expand the tools available to growers for the management of herbicide resistance in weed populations in soybean cultivation.

X. Adverse Consequences of Introduction

BASF Plant Science L.P. is not aware of any information, study results or observations that would indicate that CV127 soybean poses any different weediness or plant pest potential and no different environmental impact than conventional soybean varieties. There are no adverse environmental consequences anticipated with the introduction of CV127 soybean.

The environmental as well as food and feed safety of CV127 soybeans was confirmed based on the results of a series of interrelated safety assessment studies. The detailed molecular characterization of CV127 soybean confirmed that CV127 contains a single functional *csr1-2* gene cassette integrated in the soybean genome. Biochemical characterization of the imidazolinone-tolerant AtAHAS protein expressed in CV127 soybean showed that the AtAHAS protein is typical of other AHAS proteins in this ubiquitous protein family as well as most dietary proteins with a history of safe use in food and feed products and lacks any of the characteristics associated with known allergenic or toxic proteins. Extensive phenotypic and agronomic evaluations as well as ecological interactions of CV127 show that cultivation of CV127 soybean poses no different plant pest or weediness risk and no greater potential environmental impact than cultivation of conventional soybean varieties. Finally, the composition and nutritional equivalence of CV127 soybean compared to conventional soybean varieties was demonstrated by analysis of key nutrients and antinutrients in both grain and forage, and nutritional equivalence to conventional soybeans was further confirmed in a poultry feeding study. The results of these studies show that CV127 soybeans are as safe as conventional soybeans for environmental release as well as for food and feed uses.

CV127 soybean was developed for cultivation primarily in Brazil and Argentina. Introduction of CV127 soybean will allow growers to treat the soybean crop with imidazolinone herbicides for weed control without causing injury to the soybean plant at normal field application rates. Introduction of CV127 soybean varieties is expected to offer soybean growers an additional tool for controlling weeds, as well as an important option for weed resistance management. Furthermore, it is expected that growers planting CV127 soybeans will be able to reduce the number of herbicides used to control weeds in their soybean fields and benefit from reduced weed control costs. The reduction in herbicide use is also expected to benefit the environment.

XI. References

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XII. Appendices

Appendix A: **Materials and Methods for the Molecular Characterization of CV127 Soybean**

Appendix B: **Characterization of AtAHAS Protein Produced in Imidazolinone-tolerant CV127 Soybean and Comparison with AtAHAS Protein Expressed in Recombinant *Escherichia coli***

Appendix C: **Methods for Determining Levels of the AHAS and SEC61γ Subunit Proteins in Soybean Tissues**

Appendix D: **Methods Used and Results of the Grain and Forage Composition Analyses**

Appendix E: **Methods and Results of Seed Germination, Dormancy and Seed Quality Studies of CV127 Soybean**

Appendix F: **Materials and Methods and Results from the Agronomic, Phenotypic, and Ecological Interaction Evaluations of CV127 Soybean**

Appendix G: **Best Management Practice (BMP) Program for the Imidazolinone-tolerant CV127 Soybean Production System**

Appendix H: **Digestive Fate of the AtAHAS Produced in CV127 Soybean and in the Recombinant *E. coli* Expression System**

Appendix A

Materials and Methods for the Molecular Characterization of CV127 Soybean

Source of plant materials for DNA analyses. Young leaf tissue of CV127 soybean was provided to DNA Landmarks, Inc. (Montreal, Canada) by BASF SA for DNA isolation and characterization. Control DNA was isolated from leaf tissue of the non-transgenic soybean variety Conquista that is closely related to CV127 soybean. All plants were grown under greenhouse conditions in Londrina, Brazil, and young, fully-expanded trifoliate leaves were harvested for molecular analyses.

DNA isolation and quantification methods. DNA was isolated from soybean leaf tissue via a modified cetyl trimethyl ammonium bromide (CTAB) method (Carlson *et al*., 1991). Silica gel-desiccated leaf tissue was frozen with liquid nitrogen and ground with an Autogrinder (Autogen; Holliston, MA). The ground tissue was incubated with preheated extraction buffer consisting of 2% (w/v) CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 1% (w/v) polyvinylpyrrolidone (PVP), 20 mM ethylenediamine tetraacetic acid (EDTA), pH 9.5 (5 ml/60 mg dried leaf tissue) and β-mercaptoethanol (10 µl/ml buffer) at 74°C for 20 min. After centrifugation at 2440 x *g* for 10 min, the supernatant was extracted twice with an equal volume of chloroform/isoamyl alcohol (24:1). DNA was precipitated with 0.7 volume of isopropanol and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) with 0.5 mg/ml RNase A (Invitrogen; Carlsbad, CA) added to a final concentration of about 500 ng/µl. The isolated DNA was quantified with Hoechst 33258 dye (Invitrogen) with calf thymus DNA (Invitrogen) used as the DNA standard on a Packard FluoroCount™ BF10000 Microplate Fluorometer (Packard Instrument Company; Meriden, CT) according to the fluorometer user manual.

Probe isolation and labeling methods. The DNA fragments used as transgene-specific probes are indicated in Figure IV-1 of the application. The vector backbone probes are indicated in Figure V-3 of the application. Together these 5 overlapping probes span the entire plasmid. Specifically, probe 1 spans the At*AHAS* promoter region, probe 2 the *csr1-2* coding sequence (CDS), probe 3 the At*AHAS* terminator region, and probes 4 and 5 together cover the complete vector backbone (VB). The probe DNA fragments were generated by polymerase chain reaction (PCR) amplification using plasmid pAC321 as a template (Table A-1). The probes (25 – 50 ng each) were radiolabeled with 50 µCi of (α-^{32}P)-dCTP (3000 Ci/mmol) (MP Biomedicals; Irvine, CA) using the Rediprime™ II DNA Labeling System (Amersham; Piscataway, NJ) according to the manufacturer's instructions. The labeled probes were purified with a Spin-X® Centrifuge Tube Filter (Corning Costar Corporation; Acton, MA).

Table A-1. Primers Used to Generate Probes for Southern Blot Analysis

Purpose	Direction	Primer Sequence	Position in pAC321
Probe 1 5' UTR	Forward Reverse	TGCGTTATCCCCTGATTCTG TGTTGGGGTTTAGGGAG	8261-8280 2597-2613
Probe 2 At*AHAS* CDS	Forward Reverse	CGAAGGCTCAATCACAAATAC AGCAGGCAGATCAACAAC	2269-2289 4604-4621
Probe 3 3' UTR	Forward Reverse	GAACATGTGTTGCCGATGAT CGCAACTGTTGGGAAGGG	4416-4435 5949-5966
Probe 4 VP1	Forward Reverse	GTTTTACAACGTCGTGACTG CGGTTAGCTCCTTCGGTC	5839-5858 6997-7014
Probe 5 VP2	Forward Reverse	CACTGCGGCCAACTTACT CTTGGCGTAATCATGGTC	6962-6979 8592-8609

Restriction digestions and Southern blot analyses. Southern blot analyses were used to determine the number of copies and the integrity of the *csr1-2* expression cassette as well as to confirm the absence of plasmid backbone in CV127. Restriction enzymes NcoI, SpeI and XbaI were used to digest the genomic DNA of CV127 and non-transgenic control Conquista. The pAC321 PvuII transformation fragment is aligned with the CV127 insert in Figure V-1 of the application. A single NcoI restriction site in the *csr1-2* cassette is located at the 5' end of the *csr1-2* coding sequence and digestion of genomic DNA of CV127 with NcoI was expected to generate two fragments that contain DNA from the *csr1-2* cassette. Both fragments are defined by the NcoI site in the *csr1-2* cassette and by the nearest NcoI sites in the flanking soybean genomic sequence. There is one SpeI restriction site in the 5' flanking soybean genomic sequence and two SpeI restriction sites downstream of the At*AHAS* 3' UTR in CV127. The XbaI restriction sites flank the complete *csr1-2* expression cassette. The number and sizes of the DNA fragments expected to be detected by Southern hybridization are listed in Table V-1 of the petition.

Genomic DNA (7 µg) from the F_8 generation of CV127 line 603 (Figure III-1 of the petition) and from the non-transgenic control Conquista was digested overnight in a volume of 40 µl with the restriction enzymes listed above (8 units/µg DNA) under the conditions specified by the enzyme manufacturers (New England Biolabs; Ipswich, MA; or Amersham). Two additional non-transgenic Conquista genomic DNA samples were spiked with one- and two-genome copy equivalents[2] of pAC321 plasmid DNA (27 and 54 pg, respectively) and used as

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[2] Calculation of copy number equivalents

Assumptions:

- The haploid content of the soybean genome is 1.115 X 10^9 bp (Arumuganathan and Earle, 1991).
- Plasmid pAC321 is 8669 bp.

Since the insert in Cultivance® Event 127 is homozygous and 7 µg of soybean DNA is used per digest in the Southern blot analysis, the mass of one copy equivalent of pAC321 is:

$$\frac{\text{mass of pAC321 DNA}}{\text{7 µg genomic DNA}} = \frac{\text{8669 bp transgene DNA}}{(1.115 \text{ X } 10^9 \text{ bp genomic DNA}) \text{ x } 2}$$

mass of pAC321 DNA = 27 pg

positive controls. Restriction digests were separated by electrophoresis in 10 cm long 0.8% agarose gels. The DNA was further fragmented by soaking the gels in 0.25 N HCl for about 20 min and was denatured with 0.4 N NaOH for about 30 min. The gels were rinsed with 2X NaCl/sodium citrate solution (SSC) and the denatured DNA was transferred onto Hybond N+ nylon membrane (Amersham) using 0.4 N NaOH as a transfer buffer. Southern hybridization was carried out according to Sambrook *et al*. (1989). The membranes were prehybridized at 65°C for 2-4 h and hybridized at 65°C overnight in 20 – 30 ml (about 0.2 ml/cm^2) of hybridization buffer (2x SSC, 0.6% SDS, 50 mM Na_2HPO_4, 1x Denhardt's solution, 2.5 mM EDTA, 5% dextran sulfate, pH 7.2) in a Hybaid MAXI 14 Hybridization Oven (Thermo Electron Corporation). After hybridization, the membranes were washed with 2x SSC, 0.5% SDS (1 ml/cm^2) at room temperature for 15 min, 2x SSC, 0.1% SDS (4 ml/cm^2) at 65°C for 30 min, and finally with 0.1x SSC, 0.1% SDS (4 ml/cm^2) at 65°C for 15 min. After washing, the membranes were wrapped in plastic wrap and exposed to Hyperfilm™ MP film (Amersham) for 2-5 days, depending on the radioactive signal intensity, in cassettes with intensifying screens at –80ºC.

Stability of the DNA insert across breeding generations. Southern blot analyses were also conducted as described above to monitor the stability of the insert across multiple generations. Plant material was obtained from the T_4, F_4, F_8 and F_9 generations of CV127 line 603 (Figure III-1). Genomic DNA from these samples was digested with NcoI and SpeI (as described above) and Southern blot analysis was carried out as described above.

Complete sequence of the DNA insert. Six PCR-generated amplicons were designed to span the entire insert as well as the junctions with the adjacent soybean genomic sequences (Figure V-5). The primer pairs used to generate these six amplicons are described in Table A-2. The complete sequence of the inserted DNA was obtained by PCR amplification of these six overlapping fragments followed by DNA sequence analysis. PCR amplicons containing sequence discrepancies relative to the sequence of the transformation fragment were re-amplified with rTth DNA polymerase XL. PCR products were purified with the Zymo DNA Clean & Concentrator™-5 and were sequenced on both strands to a quality level of Phred 40 by direct sequencing and primer walking. DNA sequencing was performed as described above.

Table A-2. Primers Used for CV127 DNA Insert Amplification

Purpose	Direction	Primer Sequence	Position in pAC321
PCR1	Forward	GCTTGATATGCCTTTTGGTTC	5265-5285
	Reverse	TTGTCTTCCCTCATTGGAC	6150-6168
PCR2	Forward	GACGAGATATTCCCGAAC	4544-4561
	Reverse	GTCTGATTAGTGCTTCTGG	5525-5543
PCR3	Forward	CCCTGTTGCGAGTACGTTGA	3739-3758
	Reverse	CTTCCGTTATGACATCGTTG	4732-4751
PCR4	Forward	AACCACTCCCTCTCCAAC	2980-2997
	Reverse	CTGATGATAGCCACTGCC	4266-4283
PCR5	Forward	TTCGTTCGCTCTGGTGTC	2062-2079
	Reverse	ACGGTTTCTACGCCTTG	3089-3105
PCR6	Forward	GAAAATAGGAAGTTTAGGCTTG	1000-1021
	Reverse	GGGCTGATAATGTCGTTTG	2229-2247

Reverse transcription-polymerase chain reaction (RT-PCR) analysis of the* At*SEC61γ* *subunit transcript. Young leaf tissue of silica gel-desiccated young leaves derived from F_7 generation plants of CV127 soybean and from leaves of the non-transgenic parental soybean variety Conquista was provided to DNA Landmarks, Inc. (Montreal, Canada), by BASF SA for RNA isolation and characterization. All plants were grown under greenhouse conditions in Londrina, Brazil, and young, fully-expanded trifoliate leaves were harvested for molecular analyses.

Total RNA was extracted from silica gel-desiccated young leaves with the Qiagen RNeasy Mini Kit (Qiagen; Valencia, CA). About 25 mg of silica gel-desiccated leaf tissue was frozen with liquid nitrogen and ground with an Autogrinder. The total RNA isolation procedure was carried out according to the manufacturer's directions. On-column DNase digestion was performed with RNase-Free DNase (Qiagen) to eliminate any soybean genomic DNA from the total RNA preparation according to the recommendation in the RNeasy Mini Kit user manual. The isolated RNA was quantitated by measuring the absorbance at 260 nm using a BioMate™ 3 spectrophotometer (Thermo Electron Corporation; Waltham, MA).

RT-PCR analysis was conducted to determine if the At*SEC61γ* subunit gene present in CV127 was transcribed. The primers used for this RT-PCR analysis are described in Table A-3. Total RNA was used as template for RT-PCR using the Qiagen OneStep RT-PCR Kit (Qiagen). For RT-PCR analysis of the At*SEC61γ* subunit coding sequence, *Arabidopsis* total RNA samples from leaves and roots were also used as positive controls. The *Arabidopsis* total RNA samples were provided by BASF Plant Science (Research Triangle Park, NC). The *Arabidopsis* total RNA samples were prepared with TRIzol reagent (Invitrogen) without DNase treatment. The RT-PCR reactions contained 1x Qiagen OneStep RT-PCR Buffer, 400 µM of each dNTP, 0.6 µM of each primer, 2 µl of Qiagen OneStep RT-PCR Enzyme mix, and 500 ng or 125 ng of total RNA in a total volume of 50 µl. The RT-PCR was conducted using the GeneAmp PCR System 9700. Following a 30 min reverse transcription step at 50°C, PCR amplification was carried out under the following conditions: one 15 min denaturation step at 95ºC; 30 cycles at 94ºC for 30 sec, 64ºC for 30 sec, and 72ºC for one min; and one ten-min extension at 72ºC.

The endogenous soybean *SEC61γ* subunit (Gm*SEC61*) and *Iota* genes were used as positive controls. The soybean *Iota* subunit gene is expressed constitutively and ubiquitously in soybean (Yamamoto and Knap, 2001).

Table A-3. Primers Used for RT-PCR Analysis of the At*SEC61γ* subunit transcript

Purpose	Direction	Primer Sequence	Position in pAC321
RT-PCR At*SEC61*	Forward	ACGAACCTGCTGAAACCCTAAT	1338-1359
	Reverse	TAAGAATGGAGAATTTGGCTACA	2280-2302
RT-PCR Iota	Forward	TGAAGCAGCAGCTGAGTTTCGC	N/A*
	Reverse	GGCAGTCTGAACCGTCTCCTC	N/A
RT-PCR Gm*SEC61*	Forward	GCTTGGGAGACAGAGAAAGAGA	N/A*
	Reverse	CCTTTTGCTTGACAACCTGAAT	N/A
RT-PCR ORF501	Forward	TTGGAATGCATGGGACTGT	3807-3825, 5733-5751
	Reverse	TGTCTTCCCTCATTGGACTG	6148-6167

*N/A – not applicable. This is a positive control; primer set is expected to amplify cDNA derived from an endogenous soybean transcript unrelated to the CV127 insert.

RNA ligase-mediated rapid amplification of 5' complementary DNA ends (RLM-5'-RACE) of the* At*SEC61γ subunit transcript. The test material for this study was RNA isolated from greenhouse-grown CV127 soybean plant material. The control material for this study was RNA isolated from greenhouse-grown conventional soybean variety Conquista plant material. The reference material for this study was RNA isolated from *Arabidopsis thaliana* ecotype Columbia (Col-0). Leaf tissue from the CV127 eighth filial (F_8) generation and its comparator non-transgenic line, Conquista, was obtained from young (25-day-old) greenhouse-grown plants. Plant tissue from greenhouse-grown Arabidopsis was also used.

Messenger RNA (mRNA) was isolated from Arabidopsis and soybean leaf tissue using the FastTrack MAG Maxi mRNA Isolation Kit (Invitrogen GmbH; Karlsruhe, Germany). Approximately 200 mg of leaf tissue was ground in liquid nitrogen using a mortar and pestle. Pre-mixed Lysis Buffer L4/Protein Degrader (510 µl) was added to each sample and samples were suspended by vortexing. Samples were centrifuged for five minutes at approximately 16,100 x g at room temperature. Supernatants were transferred to new tubes and incubated for 15 minutes at 45˚C. FastTrack MAG Beads (200 µl per sample) were pre-washed and bound to each sample in accordance with the manufacturer's instructions. The recommended wash procedure was followed with the inclusion of an optional DNase I digestion. Two additional washes (beyond the standard protocol) with Wash Buffer W7 were performed immediately before elution of the mRNA. Messenger RNA was eluted under the recommended conditions in 20 µl RNase-free water followed by a second elution in 10 µl RNase-free water. The two mRNA eluates were combined and stored at –80˚C.

Messenger RNA was diluted in 1 M Tris-HCl, pH 7.5 and quantitated by measuring the absorbance at 260 nm using a BioPhotometer (Eppendorf AG; Hamburg, Germany).

RNA ligase-mediated rapid amplification of 5' cDNA ends (RLM-5'-RACE) was conducted using the GeneRacer Kit (Invitrogen) in accordance with the manufacturer's instructions unless otherwise noted. Messenger RNA (250 ng) was dephosphorylated with calf intestinal phosphatase (CIP) to remove the 5' phosphate from any truncated mRNA or non-mRNA present in the mRNA preparation. Because CIP has no effect on capped full-length mRNA, this dephosphorylation step was expected to eliminate, or at least reduce, the contribution from non-full-length messages in later steps of the RLM-5'-RACE procedure.

This dephosphorylated RNA was then treated with tobacco acid pyrophosphatase (TAP) to remove the 5' cap structure from full-length mRNA. In order to determine the contribution from non-full-length messages in this RLM-5'-RACE experiment, a second set of samples was processed identically to the experimental samples with the exception that TAP was excluded from these control reactions. If the RLM-5'-RACE experiment is only amplifying capped full-length message, then no PCR products would be evident in these "minus TAP" controls.

TAP treatment generates a free 5' phosphate which is required for the subsequent step: ligation of the GeneRacer RNA Oligo to the 5' end of the de-capped full-length mRNA with T4 RNA ligase. The ligated mRNA was reverse transcribed into first-strand cDNA using SuperScript III RT (Invitrogen) and the GeneRacer Oligo dT Primer in a 75 minute reaction. The region of the first-strand cDNA corresponding to the GeneRacer RNA Oligo contains binding sites for the GeneRacer 5' Primer and the GeneRacer 5' Nested Primer which will be used in the primary and secondary PCR amplifications, respectively.

Primary PCR amplification was performed using 2 µl of each reverse transcription reaction as a template for amplification by Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen) under the reaction conditions specified in the GeneRacer Kit instructions. In this primary PCR, the GeneRacer 5' Primer was paired with either gene-specific primer RS41 (Integrated DNA Technologies; Coralville, Iowa) or gene-specific primer RS46 (BASF AG Oligonucleotide Synthesis Facility; Ludwigshafen, Germany). Gene-specific primer RS41, when used in combination with the GeneRacer 5' Primer, was expected to amplify the 5' end of cDNAs derived from endogenous soybean *SEC61γ* subunit transcripts. Due to the lack of a published full-length cDNA sequence, soybean *SEC61γ* subunit-specific primers were designed based on sequences from The Institute for Genomic Research's *Glycine max* gene index of likely genes and their variants. Gene-specific primer RS46, when used in combination with the GeneRacer 5' Primer, was expected to amplify the 5' end of cDNAs derived from Arabidopsis *SEC61γ* subunit (locus At3g48570) transcripts. Although there are two additional putative *SEC61γ* subunit genes in Arabidopsis, primer RS46 was designed to bind specifically within the 3' untranslated region (UTR) of only the locus At3g48570 cDNA to reduce background amplification in control RLM-5'-RACE experiments using Arabidopsis mRNA.

Table A-4. Gene-Specific Primers Used in RLM-5'-RACE Experiment: Primary PCR

Name	Primer Sequence and Description
RS41	CATCCTAACCAGATCCGACGATGATGTTG *SEC61γ* subunit, soybean. Reverse primer
RS46	CAAAGGGCTGATAATGTCGTTTGGTTCGTTCTTC *SEC61γ* subunit, Arabidopsis. Reverse primer

Primary PCR amplification was carried out on a PrimusHT thermal cycler (MWG-Biotech AG; Ebersberg, Germany) using the following thermal cycling conditions:

Table A-5. Primary PCR Thermal Cycling Parameters

Temperature	Time	Number of Cycles
94˚C	2 minutes	1
94˚C 72˚C	30 seconds 2 minutes	5
94˚C 70˚C	30 seconds 2 minutes	5
94˚C 60˚C 68˚C	30 seconds 30 seconds 2 minutes	25
68˚C	10 minutes	1
8˚C	∞	

In order to increase both yield and specificity, a secondary PCR amplification was performed using nested primers. The secondary PCR utilized 1 µl of each primary amplification reaction as a template for amplification in 50 µl reactions containing final concentrations (or quantities) of 2.5 units of Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen), 1X High Fidelity PCR Buffer, 2 mM magnesium sulfate, 0.2 µM of each primer, and 0.2 mM of

each dNTP (Invitrogen). In this secondary PCR, the GeneRacer 5' Nested Primer was paired with either nested gene-specific primer RS43 (Integrated DNA Technologies) or nested gene-specific primer RS42 (Integrated DNA Technologies). Nested gene-specific primer RS43, when used in combination with the GeneRacer 5' Nested Primer, was expected to amplify the 5' end of cDNAs derived from endogenous soybean *SEC61γ* subunit transcripts. Nested gene-specific primer RS42, when used in combination with the GeneRacer 5' Nested Primer, was expected to amplify the 5' end of cDNAs derived from Arabidopsis *SEC61γ* subunit (locus At3g48570) transcripts. Although there are two additional putative *SEC61γ* subunit genes in Arabidopsis, primer RS42 was designed to bind specifically within the 3' untranslated region (UTR) of only the locus At3g48570 cDNA to reduce background amplification in control RLM-5'-RACE experiments using Arabidopsis mRNA.

Table A-6. Gene-Specific Primers Used in RLM-5'-RACE Experiment: Secondary PCR

Name	Primer Sequence and Description
RS42	CCTACTCCTCGGAGCATTGCCTCGTA *SEC61γ* subunit, Arabidopsis. Reverse primer, nested. Spans intron.
RS43	CAGTACGGACGGCAACCTTGGAGAAT *SEC61γ* subunit, soybean. Reverse primer, nested.

Secondary PCR amplification was carried out on a PrimusHT thermal cycler (MWG-Biotech AG) using the following thermal cycling conditions:

Table A-7. Secondary PCR Thermal Cycling Parameters

Temperature	Time	Number of Cycles
94˚C	2 minutes	1
94˚C 65˚C 68˚C	30 seconds 30 seconds 2 minutes	20
68˚C	10 minutes	1
8˚C	∞	

Secondary PCR products (20 µl) were separated by electrophoresis at 10 V/cm in a 1% (w/v) agarose gel containing 0.2 µg/ml ethidium bromide in TAE (40 mM Tris-acetate, 1 mM EDTA).

The CV127 RLM-5'-RACE secondary PCR products generated with Arabidopsis *SEC61γ* subunit-specific primers were cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen). TOPO vector pCR4-TOPO (1 µl) was incubated with an aliquot of the secondary PCR (4 µl) and 1 µl of a 1.2 M sodium chloride, 0.06 M magnesium chloride solution for 30 minutes at 23˚C. An aliquot of the cloning reaction (2 µl) was transformed into One Shot TOP10 Chemically Competent *Escherichia coli* (Invitrogen) according to manufacturer's instructions and plated on Luria-Bertani agar plates containing 50 µg/ml kanamycin.

Twelve transformants were screened for positive clones by colony PCR. Each colony was suspended in 4 µl of sterile water. One microliter of this suspension was added to a 25 µl reaction containing final concentrations of 1X Eppendorf MasterMix (Eppendorf AG;

Hamburg, Germany) and 0.2 µM of each primer. Due to the bidirectional nature of the cloning, a gene-specific primer (RS42) was paired with either vector-specific primer RS7 (Integrated DNA Technologies) or vector-specific primer RS8 (Integrated DNA Technologies) to determine the insert orientation.

Table A-8. Primers Used in PCR Screen for Positive Clones

Name	Primer Sequence	Description
RS7	CGCCAGGGTTTTCCCAGTCACGAC	M13 forward (-41) primer
RS8	AGCGGATAACAATTTCACACAGG	M13 reverse (-48) primer
RS42	CCTACTCCTCGGAGCATTGCCTCGTA	*SEC61γ* subunit, Arabidopsis. Reverse primer, nested. Spans intron.

PCR amplification was carried out on a PrimusHT thermal cycler (MWG-Biotech AG) using the following thermal cycling conditions:

Table A-9. PCR Screen Thermal Cycling Parameters

Temperature	Time	Number of Cycles
95˚C	5 minutes	1
95˚C 55˚C 72˚C	15 seconds 15 seconds 30 seconds	30
72˚C	7 minutes	1
8˚C	∞	

Aliquots of each reaction (5 µl) were separated by electrophoresis at 10 V/cm in a 1% (w/v) agarose gel containing 0.2 µg/ml ethidium bromide in TAE (40 mM Tris-acetate, 1 mM EDTA).

All transformants identified by the PCR screen as containing positive clones were cultured in 2.5 ml of Luria-Bertani broth containing 50 µg/ml kanamycin. Plasmids were purified from these cultures using the QIAprep Spin Miniprep Kit (Qiagen GmbH; Hilden, Germany) in accordance with manufacturer's instructions.

Double-stranded sequencing of selected clones was performed at the BASF AG Sequencing Facility (Ludwigshafen, Germany). Sequencing data was assembled using Sequencher, version 4.5 (Gene Codes Corporation; Ann Arbor, Michigan).

RT-PCR analysis of the 501 bp open reading frame (ORF). RT-PCR was conducted to determine if the 376 bp duplication of a portion of the *csr1-2* coding sequence present in CV127 was transcribed. Tissues (roots, leaves, and leaf buds) were collected from greenhouse-grown CV127 and Conquista plants and, together with seed, were used as the source of RNA and genomic DNA samples for this study.

Genomic DNA for PCR analysis was isolated from leaf, root, leaf buds and seed tissue using the NucleoSpin® Plant II kit (Macherey-Nagel; Bethlehem, PA) in accordance with the manufacturer's protocol with the following modifications. Powdered tissue sample (100 mg) was mixed with 400 µl Buffer PL1. Following vortexing, 10 µl RNase A was added and after

mixing, the sample was incubated at 65ºC for 30 minutes. The lysate of each sample was loaded onto a single NucleoSpin Plant II Column and centrifuged for 1 minute at 12,000 rpm at room temperature. The flow-through was mixed with 450 µl Buffer PC and loaded onto another NucleoSpin Plant II Column and centrifuged for 1 minute at 12,000 rpm. The flow-through from this spin was discarded and the column was washed consecutively with 400 µl PW1, 700 µl PW2 and 200 µl PW2. Finally, the DNA was eluted with 50 µl Elution Buffer PE. All genomic DNA samples were stored at 4ºC.

Genomic DNA was quantitated using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen; Carlsbad, CA) in accordance with the manufacturer's instructions. Lambda DNA provided with the kit was used to generate a standard curve from 1 ng/ml to 1000 ng/ml. Fluorescence was measured with a Turner Quantech Digital Filter Fluorometer (Barnstead International; Dubuque, IA), λ_{ex}= 490 nm, λ_{em}= 515 nm. All genomic DNA samples were quantitated in duplicate.

RNA was isolated from 100 mg of powdered tissue using the Qiagen RNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) and treated with DNase as described in the Mini Kit instructions. Powdered tissue (100 mg) was mixed with Buffer RLT and the lysate was transferred to a QIAshredder spin column. The flowthrough was mixed with 0.5 ml ethanol, loaded onto the RNeasy mini spin column, centrifuged at 11,000 rpm for 1 min and washed with 350 µl Buffer RW1. DNase I was added directly onto the column and incubated at room temperature for 15 min. The column was washed with 350 µl Buffer RW1 followed by two washes with 500 µl Buffer RPE. RNA was eluted in 35 µl RNase free water and quantitated using 1.5 µl of the RNA sample on the NanoDrop ND-1000 spectrophotometer.

To monitor the presence of the ORF 501 transcript in the CV127 soybean plants, RT-PCR was conducted using primers specific for this open reading frame (anticipated size 435 bp) and control primers to amplify the transcript of an endogenous, constitutively-expressed soybean gene, cyclophilin (Jian *et al*., 2008, anticipated amplicon size 315 bp). As an additional control, reactions in which the reverse transcriptase was omitted were included to confirm that amplification was not occurring from contaminating genomic DNA.

Table A-10. Primers Used for RT-PCR Analysis of the 501 bp ORF

Purpose	Direction	Primer Sequence (5' → 3')	Position in pAC321
ORF 501	Forward	TTGGAATGCATGGGACTGT	3807 – 3825 5733 - 5751
	Reverse	TGTCTTCCCTCATTGGACTG	6148 - 6167
Cyclophilin	Forward	AGCTCTATGCCGACGTGACTCC	N/A*
	Reverse	ACTCCGTCTTCGTCGTGCAGAT	N/A

*N/A – not applicable. Cyclophilin was used as a positive control; the primer set is expected to amplify cDNA derived from an endogenous soybean transcript unrelated to the CV127 insert.

Total RNA (500 ng) was used as a template for RT-PCR using the Superscript First Strand Synthesis System for RT-PCR (Invitrogen; Carlsbad, CA). RNA was converted to cDNA in the first step that included a denaturation of RNA in the presence of random hexamers and dNTPs at 65ºC for 5 minutes and 4ºC for 5 minutes. Each 20 µl RT reaction contained final

concentrations of 1.25 ng/µl random hexamers, 0.5 mM dNTP mix, 1x RT Buffer, 5 mM magnesium chloride, 10 µM DTT, 2 U/µl RNaseOUT, and 10 U/µl Superscript III Reverse Transcriptase. The reaction was incubated at 25ºC for 10 minutes, 50ºC for 50 minutes, 85ºC for 5 minutes and then stored at 4ºC to cool down followed by addition of 1 µl of RNase H and incubation at 37ºC for 20 minutes. The second step is the PCR amplification using 1 µl of the cDNA template generated in the first step. Each 25 µl reaction contained final concentrations of 1x AmpliTaq® Gold 360 Buffer, 2.5 mM magnesium chloride, 0.2 mM of dNTP mix, 0.6 U of AmpliTaq® Gold 360 DNA Polymerase and 0.2 µM of each primer. All primers were synthesized by Integrated DNA Technologies (Coralville, IA). Following a 30 minute reverse transcription step at 50ºC, PCR amplification was carried out under the following cycling conditions: 95ºC for 10 minutes, 30 cycles at 95ºC for 30 seconds, 64ºC for 30 seconds, and 72ºC for one minute, followed by 72ºC for 7 minutes.

PCR of genomic DNA (50 ng) using the same primers as were used in the RT-PCR amplifications was conducted as a positive control. Genomic DNA template was amplified with both the ORF 501 primers and the cyclophilin primers described above.

Appendix B

Characterization of AtAHAS Protein Produced in Imidazolinone-tolerant CV127 Soybean and Comparison with AtAHAS Protein Expressed in Recombinant *Escherichia coli*

The herbicide tolerance in CV127 soybean is the result of a single amino acid substitution of serine to asparagine at position 653 of the AtAHAS protein. In addition, a second amino acid substitution of an arginine replaced by lysine was identified at position 272 of the AtAHAS protein; this amino acid substitution does not impact the enzymatic function of the AtAHAS enzyme.

The full-length AtAHAS protein consists of 670 amino acids and includes the *A. thaliana* native chloroplast transit peptide (CTP) that is predicted to consist of 85 amino acids on the N-terminus (Mazur *et al*., 1987). During transport into the chloroplast, the chloroplast transit peptide is removed to produce the mature AtAHAS enzyme that has a molecular weight of approximately 64,000 and is predicted to consist of 585 amino acids.

As part of the food, feed and environmental safety assessment of CV127 soybean, studies were conducted to confirm that the AtAHAS protein is equivalent to other AHAS proteins that are found ubiquitously among plant species and have a history of safe use in food and feed products. These safety assessments of the AtAHAS protein included a mouse acute gavage study and a digestive fate study conducted with purified AtAHAS protein. The AtAHAS protein is expressed at extremely low levels in tissues of CV127 soybean plants. For example, the AHAS protein is present in soybean grain at levels less than 1.5×10^{-6} % on a dry-weight basis (Schwerz, 2007 and 2008). Therefore, it was not technically feasible to extract sufficient quantities of the AtAHAS protein from soybean tissues for the safety assessment studies of the protein. The same AtAHAS coding sequence introduced in the CV127 soybean genome was introduced into *Escherichia coli* for over-expression of the AtAHAS protein. The AtAHAS protein, as encoded in this expression system, lacks the predicted 85-amino acid N-terminal CTP sequence that targets the protein *in planta* to the chloroplast. The microbially-produced AtAHAS protein contained the same S653N and R272K amino acid substitutions as found in the plant-produced protein.

Microbial fermentation provided sufficient starting material for purification of gram quantities of the AtAHAS protein required for the safety assessment studies. The purpose of the following study was to characterize the AtAHAS protein produced in the transgenic imidazolinone-tolerant CV127 soybean to evaluate key biochemical and functional parameters and to demonstrate that it is substantially similar to the microbially-produced AtAHAS protein which has been used in various safety assessment studies.

The AHAS characterized in this study was obtained from extracts of young leaves of field-grown CV127 plants. In some studies, immuno-purified AHAS was used, and in others, ammonium sulfate precipitated AHAS-enriched extract was utilized. These materials served as the test substances. As a control, the endogenous soybean AHAS from an ammonium sulfate precipitated AHAS-enriched extract of leaves from the isoline control soybean was included in some of the studies. The AtAHAS protein purified from the *Escherichia coli* over-expression system was used as a reference substance. This protein is identified as AtAHAS-0107. Soybean-expressed AHAS protein was evaluated for its molecular weight, immunoreactivity, enzymatic activity, sensitivity to branched-chain amino acid feedback

inhibition and imidazolinone herbicide inhibition. In addition, the soybean-expressed AHAS protein was evaluated for post-translational modifications and amino acid sequence confirmation.

Western blot analyses using antibodies specific for AHAS showed the same molecular weight of approximately 64,000 and immunoreactivity for both the plant-produced and the microbially-produced proteins.

The functionality of the AtAHAS protein produced in CV127 and the microbially-produced protein was demonstrated by monitoring the enzymatic activity, its lack of sensitivity to inhibition by imidazolinone herbicide, and feedback inhibition by branched-chain amino acids. While both the plant-produced and microbially-produced proteins demonstrated the expected enzymatic activity with the substrate pyruvate, the specific activity of the AHAS protein in CV127 was found to be higher compared to the microbially-produced protein, most likely a result of enzyme activity losses incurred during extensive purification of the microbially-produced protein. Both CV127-produced AHAS and microbially-produced AtAHAS proteins showed tolerance to inhibition by the imidazolinone herbicide imazethapyr compared to the control plant-produced protein. Sensitivity to feedback inhibition by branched-chain amino acids was retained in the plant extracts but not in the microbially-produced material. Acetohydroxyacid synthase in *Escherichia coli* is composed of two subunits. However, previous studies have demonstrated that when the *A. thaliana* acetohydroxyacid synthase catalytic (large) subunit (AtAHAS) is expressed in *E. coli*, the *E. coli* small (regulatory) subunit is incapable of interacting with the *Arabidopsis thaliana* catalytic or "large" subunit to confer the feedback inhibition observed in *Arabidopsis thaliana* seedlings (Singh *et al*., 1992; Chang and Duggleby, 1997).

No evidence of glycosylation associated with either the plant- or microbially-produced AtAHAS was detected. Mass spectral analysis of the immuno-purified AHAS from young leaves of CV127 plants confirmed the expected amino acid sequence of the protein for 23% of the entire amino sequence of the protein.

This study confirmed that the AHAS protein produced in CV127 plants had the expected size, immunoreactivity, and functionality and was substantially similar to the AtAHAS produced in a microbial over-expression system for use in safety assessment studies. Therefore, this study demonstrates the functional and chemical equivalence of the microbially-produced AtAHAS and the plant-produced AHAS proteins (AtAHAS and the endogenous soybean AHAS proteins) and therefore justifies the use of the microbial-produced protein for safety assessment studies of the AtAHAS protein produced in CV127 soybean.

Preparation of test substance. AHAS expressed in CV127 used in these experiments was either from an ammonium sulfate precipitated AHAS-enriched fraction or it was immuno-purified from young leaves (collected from the F_6 generation of CV127 line 127, grown in regulatory field trials in Santo Antônio de Goiás, GO, Brazil in 2007). Crude extracts were prepared by homogenizing frozen powdered leaves (1:3 w:v) in extraction buffer [50 mM potassium phosphate, pH 7.2, 100 mM sodium pyruvate, 5 mM $MgCl_2$, with HALT™ protease inhibitor cocktail (Pierce Biotechnology, Inc., Rockford, IL)] using a Polytron™ (Brinkmann, Westbury, NY). Extracts were filtered through miracloth and subjected to centrifugation for 15 min at approx. 10,000 x g. The resulting supernatant was considered to be the crude extract. The ammonium sulfate precipitated AHAS-enriched fraction was obtained by adding an equal volume of saturated ammonium sulfate to the crude extract with stirring on ice for 30 min, centrifugation for 15 min at 10,000 x g, and resuspension of the pellet with extraction buffer. Further purification was achieved by using goat anti-AtAHAS antibody (which had been purified using Protein G affinity chromatography followed by chromatography on an AtAHAS affinity column).

Preparation of the control substance. Endogenous AHAS from the isoline control soybean used in these experiments was from an ammonium sulfate precipitated AHAS-enriched fraction prepared from young leaves [collected from the isoline control soybean (F_6 null) corresponding to the F_6 generation of CV127 line 127, and grown in regulatory field trials in Santo Antônio de Goiás, GO, Brazil in 2007], as described above for the test substance.

Table B-1. Reference Substance Description.

Identification	Lot Number	Gene Source	Expression System	Physical Description
Acetohydroxyacid Synthase R272K, S653N [3]	AtAHAS-0107	*Arabidopsis thaliana*	*Escherichia coli* strain BL21(DE3)pLysS	Light yellow proteinaceous powder

AtAHAS was purified by Invitrogen, Inc. and sent as an ammonium sulfate pellet to the Regulatory Science Laboratory at BASF Plant Science, Research Triangle Park, NC, where it was received on February 27, 2007. AtAHAS protein was purified from 2,400 g cell paste after lysis in 20 L buffer (50 mM sodium phosphate, 300 mM NaCl, 10 mM β-mercaptoethanol, 30 mM imidazole) using an Emulsiflex cell disruptor (Avestin, Ontario, Canada). Insoluble material was removed by centrifugation. The His-tagged AtAHAS present in the supernatant was purified by chromatography using Talon® cobalt resin (Clontech, Inc., Mountain View, CA). Once eluted, the fractions containing AtAHAS were pooled and immediately precipitated by ammonium sulfate at 40% saturation. The ammonium sulfate pellet was resuspended in 100 mM Tris-HCl, pH 7.0 and dialyzed vs. 20

[3] The *AtAHASL* gene was cloned into the inducible, over-expression pTrcHis A® vector (Invitrogen, Madison, WI) and expressed in *E. coli* strain BL21(DE3)pLysS. AtAHAS protein as encoded by this vector lacks the 85-amino acid N-terminal leader sequence that targets the protein *in planta* to the chloroplast. This leader has been replaced in this vector with 38 amino acids including a 6x His tag to allow for ease in purification, a gene 10 leader sequence to enhance solubility and an Xpress™ tag for detection. The remainder of the protein is identical in amino acid sequence to that produced by the native gene from *Arabidopsis thaliana* (Mazur *et al*., 1987) except for the mutation at amino acid 653 that results in asparagine replacing serine and a mutation at amino acid 272 that results in lysine replacing arginine. The S653N mutation renders a decreased binding of the herbicide imparting the tolerance. The R272K mutation was found in the AtAHAS protein produced in CV127 soybean but has no apparent effect on activity or sensitivity to imidazolinone herbicide.

mM ammonium bicarbonate buffer, pH 7.9, containing 20 μM FAD. The dialyzed material was then lyophilized and designated AtAHAS-0107. This reference substance was characterized (Privalle, 2007a), and results are summarized in Table B-2.

Table B-2. Characterization of Sample AtAHAS-0107

	AtAHAS Concentration			Specific Activity
Date of Analysis	AtAHAS (g/g sample)	% AtAHAS by weight	AtAHAS as % total protein	Mean units/mg protein ± standard deviation
April 12, 2007	0.524	52.4	90.6	0.790 ± 0.216

Protein quantification. Protein in the AtAHAS reference substance and ammonium sulfate-treated test and control AHAS-enriched fractions from plant tissues was quantified by the BCA™ procedure (bicinchoninic acid procedure; Pierce) in accordance with the manufacturer's instructions, using bovine serum albumin as the standard (SOP BPS 510.04). Samples were prepared such that the expected concentration of protein would be within the standard curve. Samples (25 μl) were loaded onto a multiwell plate in triplicate, reacted with 200 μl of a 1:50 (B:A) mixture of BCA reagent, incubated at 37ºC for 30 min and allowed to cool at room temperature for 10 min. The absorbance at 550 nm was measured using a Multiskan Ascent V1.24 multiwell plate reader (Therma Labsystems, Helsinki, Finland). The results were analyzed using DeltaSoft PC software (Version 1.71.4, Biometallics, Inc.; Princeton, NJ) using the linear regression curve fit.

AHAS quantification. Samples were quantitatively analyzed for AHAS protein by a sandwich enzyme-linked immunosorbent assay (ELISA) [Tijssen, 1985] using immunoaffinity-purified polyclonal rabbit anti-AtAHAS peptide 2 antibody and Protein G-purified goat antibodies specific for AHAS. Nunc 96-well plates (VWR; West Chester, PA) were coated with rabbit anti-peptide 2 and incubated at 4ºC overnight. The plate was washed two times with wash buffer and then blocked with 1% BSA in Tris-buffered saline (25 mM Tris-HCl, 3 mM KCl, 0.14 M NaCl) with 0.05% Tween for 2 hours at 37ºC. After washing twice, samples and standards were applied in triplicate. Plates were incubated at 4ºC for 1.5 hr and 45 min at room temperature, and then washed five times prior to the addition of the goat anti-AtAHAS followed by incubation for 1 hr at 37ºC. Plates were then washed three times and donkey anti-goat-horseradish peroxidase (HRP) was added. After incubation at 37ºC for 1 hr, the plates were washed three times and HRP substrate was added (1 Step Ultra TMB; Pierce). After 20 min at room temperature the absorbance at 620 nm was measured using a Multiskan Ascent V1.24 multiwell plate reader. The results were analyzed using DeltaSoft PC software (Version 1.71.4; Biometallics, Inc.; Princeton, NJ). The four-parameters algorithm was used to generate a curve. The AHAS component of the samples was quantified from the standard concentration curve generated from highly purified AtAHAS protein.

Molecular weight and immunoreactivity determination. To confirm that the AtAHAS produced in CV127 soybean plants had the predicted molecular weight of AtAHAS (*ca.* 64,000), aliquots of the sample preparations were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, SOP BPS 510.02), using an 8 – 16% polyacrylamide gradient Tris-glycine gel (Invitrogen) followed by electroblotting onto PVDF membrane (Invitrogen; SOP BPS 510.03). Samples of the ammonium sulfate AHAS-

enriched preparations and AtAHAS-0107 preparations were loaded onto the gel. After electroblotting the membrane was probed with rabbit anti-AtAHAS peptide 2 polyclonal antibody. Donkey anti-rabbit IgG linked to horseradish peroxidase (Pierce), diluted 1:3,000 in blocking buffer (3% nonfat dry milk in 0.1% Tween-20, 10 mM Tris-HCl, 150 mM NaCl, pH 7.5), was used to bind to the primary antibody and was visualized by development with ECL Plus Western Blotting Detection Reagents (Amersham, GE Healthcare).

Western blot analysis, using rabbit anti-AtAHAS peptide 2 polyclonal antibodies, of extracts prepared from both CV127 and the isoline control soybean contained immuno-reactive bands at a molecular weight of approximately 64,000 (Figure B-1). This was the same apparent molecular weight as the microbially-produced AtAHAS reference substance, AtAHAS-0107, indicating that the AHAS produced in the CV127 and isoline control soybeans was of the same anticipated size and of similar immunoreactivity to the microbially-produced protein. Both plant extracts included a smaller immunoreactive band at approximately 47,000 molecular weight, which is most likely a degradation product of the mature AtAHAS protein. The molecular weights of the microbially-produced AtAHAS and plant-produced AHAS proteins were also shown to be similar using SDS-PAGE and Coomassie blue staining. The approximate molecular weights of the immuno-purified AHAS protein from CV127 soybean plants and the AtAHAS in the AtAHAS-0107 test substance were shown to be approximately 64,000 (Figure B-5).

Based on a sequence comparison of the deduced amino acid sequences of AHAS proteins from several different plant and microbial sources, Mazur *et al*. (1987) predicted that the CTP of the AtAHAS is cleaved at the C-terminal side of residue 85 to produce a mature AtAHAS enzyme that consists of 585 amino acids. In the current study, the microbially-produced AtAHAS protein consisted of the predicted mature AtAHAS (585 amino acids) plus an N-terminal addition of 38 amino acid to facilitate protein purification, folding and solubility. Therefore, it was expected that the molecular weight of the microbially-produced protein would be slightly greater than that of the AHAS proteins produced in either CV127 soybean or the isoline control soybean. Because the apparent molecular weights of the plant-produced AHAS and microbially-produced AtAHAS proteins were equivalent, results of these studies suggest that the mature AtAHAS protein produced in plant tissues contains more than the predicted 585 amino acid residues of the mature AtAHAS protein. These additional amino acids are most likely derived from the defined CTP component at the N-terminal end of the protein due to a different cleavage site in soybeans compared to the predicted cleavage site in Arabidopsis. This conclusion is supported by data presented in the later section of Appendix B entitled “Amino acid sequence analysis”.

Enzymatic activity. The enzymatic activity of AHAS was assayed according to Singh *et al*. (1988) and SOP BPS 510.09. AHAS catalyzes the synthesis of acetolactate (an acetohydroxy acid) by the condensation of two molecules of pyruvate. The acetolactate produced by AHAS in this assay is converted to acetoin in the presence of acid and acetoin is detected colorimetrically ($A^{530\ nm}$) after interaction with creatine and naphthol. One unit of AHAS activity is defined as 1 µmole acetoin produced per minute. The ammonium sulfate AHAS-enriched preparations of CV127 and the isoline control soybean as well as AtAHAS-0107, dissolved in 50 mM Tris-HCl, pH ~7.0 and diluted to the desired concentration, were mixed with an equal volume of 2x assay buffer [100 mM potassium phosphate, pH 7.2, 10 mM $MgCl_2$, 200 mM sodium pyruvate, with 20 µM FAD and 2 mM thiamine pyrophosphate (TPP)] and incubated at 37ºC for 90 minutes prior to the addition of 20 µl 5% H_2SO_4 followed by a 15 min incubation at 60ºC. Background absorbance was determined by

immediately quenching the reactions prior to incubation at 37ºC. Acetoin color was developed by incubating the quenched reactions with creatine (0.17%, final concentration) and 1-naphthol (1.7% in 4 N NaOH, final concentration) for 15 min at 60°C and the absorbance was measured at 550 nm. Absorbance values were compared to an acetoin standard curve and corrected for background absorbance. Several dilutions of a single dissolved sample of AtAHAS were prepared and each dilution was assayed in triplicate; the mean corrected value is reported.

Feedback inhibition of AHAS enzymatic activity by leucine and valine was confirmed by including leucine and valine in the assay mix. Leucine and valine solutions were prepared in 2x assay buffer at twice the desired final assay concentration (1 mM). Fifty µl of 20-fold diluted AtAHAS-0107, the ammonium sulfate AHAS-enriched preparation of CV127 and the ammonium sulfate AHAS-enriched preparation of isoline control soybean were incubated with 50 µl of 2x assay buffer or leucine and valine solution for 120 min at 37°C and AHAS enzymatic assays were performed in triplicate.

AHAS enzymatic activity sensitivity to inhibition by an imidazolinone herbicide was examined using a stock solution (50 mM) of imazethapyr, a commercial imidazolinone herbicide, diluted in 2x AHAS assay buffer to two times the desired final assay concentration, where the final concentration of herbicide ranged from 0 to approx. 500 µM. Fifty µl of the sample was incubated with 50 µl of 2x assay buffer or imazethapyr solution for 90 min at 37°C. The absorbance was measured at 550 nm. Background samples were generated by pre-quenching with acid prior to assay incubation and the absorbances thus generated were subtracted from the test samples. All assays were conducted in triplicate and results are presented as mean values. The amount of enzymatic activity obtained for each sample in the absence of inhibitor was assumed to be 100%.

The microbially-produced AtAHAS and the plant-produced AHAS proteins demonstrated the expected enzymatic activity with the substrate pyruvate, confirming the same functional activity of the AHAS proteins from both microbial and plant sources. However, the specific activity of ammonium sulfate AHAS-enriched plant extracts was considerably higher than the highly purified microbially-produced AtAHAS protein (Table B-3). AHAS is a highly labile protein of low abundance in plants and has been demonstrated to be extremely difficult to purify from plant preparations (Durner and Boger, 1988; Muhitch *et al*., 1987; Singh *et al.*, 1991; Chang and Duggleby, 1997). In these leaf preparations, AHAS was present at very similar levels of 0.516 ng/ml and 0.384 ng/ml in the CV127 and the isoline control soybean extracts, respectively. This corresponds to AHAS representing only 0.06 ng/mg total protein or 0.000006% of the total protein. The AHAS protein is stable in ammonium sulfate fractions but further purification results in greatly reduced enzymatic activity. The microbially-produced AtAHAS was purified extensively for the protein safety studies, whereas the plant AHAS used in these enzymatic studies was derived from an ammonium sulfate purification step. Hence, these differences in the level of purification accounted for the difference in specific activity between the microbially-produced material, AtAHAS-0107, and the ammonium sulfate AHAS-enriched leaf extracts (Table B-3). Activity was very similar between CV127 soybean and the isoline control soybean, 0.33 and 0.30 Units AHAS activity/mg protein, respectively.

Both CV127-produced and microbially-produced AtAHAS proteins showed tolerance to inhibition by the imidazolinone herbicide imazethapyr compared to the isoline control plant-produced protein (Figure B-2). An intermediate level of inhibition of the CV127-produced

AtAHAS protein was observed, and this was as expected due to the mixture of sensitive (endogenous) and tolerant (transgenic) AHAS present in CV127 soybean. In general, the degree of herbicide inhibition observed for the AHAS proteins in the test (i.e. CV127) and isoline control soybeans was lower than anticipated but may reflect the tolerance that soybean naturally has to this particular imidazolinone herbicide, imazethapyr.

Feedback inhibition by the branched-chain amino acids leucine and valine was very similar for both the CV127 soybean AHAS and the isoline control soybean AHAS (Figure B-3). No feedback inhibition was observed for the AtAHAS present in the microbially-produced reference substance as expected. Acetohydroxyacid synthase in *Escherichia coli* is composed of two subunits. However, previous studies have demonstrated that when the *A. thaliana* acetohydroxyacid synthase catalytic (large) subunit (AtAHAS) is expressed in *E. coli*, the *E. coli* small (regulatory) subunit is incapable of interacting with the *Arabidopsis thaliana* catalytic or "large" subunit to confer the feedback inhibition observed in *Arabidopsis thaliana* seedlings (Singh *et al*., 1992; Chang and Duggleby, 1997).

Glycosylation analysis. The DIG Glycan Detection kit (Roche Diagnostics GmbH, Mannheim, Germany) was used in accordance with the manufacturer's instructions (method B, described below) to monitor for glycosylation associated with CV127 soybean-expressed AtAHAS following the SDS-PAGE/electroblotting-to-membrane procedure. This method takes advantage of the adjacent hydroxyl residues in sugars of glyco-conjugates by oxidizing them to aldehyde groups by mild periodate treatment. The spaced-linked steroid hapten digoxigenin (DIG) is then covalently attached to these aldehydes via a hydrazide group. Digoxigenin-labeled glycoconjugates are then subsequently detected by western blot analysis using a digoxigenin-specific antibody conjugated with alkaline phosphatase. Samples were run on SDS-PAGE and transferred to PVDF membrane. After washing with phosphate-buffered saline, PBS (50 mM potassium phosphate, pH 6.5, 150 mM NaCl), the membrane is treated with 10 mM sodium metaperiodate (in sodium acetate buffer, pH 5.5) for 20 minutes at room temperature (oxidation). The membrane is then washed three times with PBS and incubated with DIG-3-O-succinyl-ε-aminocaproic acid hydrazide for 1 hour at room temperature to label the glycoproteins. The membrane was washed three times with TBST (25 mM Tris, 3 mM KCl, 0.14 M NaCl, 0.05% Tween-20, pH 7.4) and blocked for at least 30 minutes, followed by incubation with anti-digoxigenin-AP for 1 hour. The chromagenic reaction with nitroblue tetrazolium was used to monitor the alkaline phosphatase bound to the membrane. Transferrin and creatinase were used as the positive and the negative controls, respectively. All incubations were conducted at room temperature with gentle agitation except for color development (room temperature, no agitation). The limit of detection for this method was determined to be 2 – 4 molecules of glucose equivalents per molecule of AtAHAS under these conditions.

No evidence of glycosylation was detected for either the CV127-produced or microbially-produced AtAHAS protein (Figure B-4). As shown in Figure B-5, there are only four N-glycosylation site motifs [NX(S/T)X, where X can be any amino acid except for proline] in the full-length AtAHAS polypeptide. At least one N-glycosylation site motif is most likely present in the chloroplast transit sequence and would not be present in the mature protein. There are, however, numerous potential O-glycosylation sites (any serine or threonine). It was not anticipated that AHAS would be glycosylated as it does not enter the secretory pathway, a prerequisite of glycosylation, but rather it is destined to be localized in the chloroplast where proteins are not typically glycosylated. Likewise, the AtAHAS produced in the microbial expression system should not be glycosylated as glycosylation does not

occur in prokaryotes. The results shown here demonstrate that no glycosylation was detected above the limit of detection (2 – 4 molecules of glucose equivalents/molecule of AtAHAS).

Amino acid sequence analysis. To further confirm that the AtAHAS protein produced in CV127 soybean was the protein expected, amino acid sequence was determined. The attempts to obtain N-terminal amino acid sequence using Edman degradation methods were unsuccessful. Therefore, a combination of SDS-PAGE and liquid chromatography coupled with tandem mass spectroscopy (LC/MS/MS) was used to obtain amino acid sequence data. Immunoaffinity-purified AtAHAS from CV127 soybean young leaves was subjected to SDS-PAGE and stained with Coomassie blue. The bands were excised and transferred into 0.5 ml Eppendorf cups. The gel pieces were washed consecutively with 100 µl of acetonitrile/ water (1:1, v:v), acetonitrile and acetonitrile/water (1:1). For each of the wash steps, the samples were shaken at 800 rpm for 5 min at 30°C, subjected to 1 min of sonication in an ultrasonic bath, centrifuged at 13,000 x g and the respective supernatants were discarded. Following addition of 10 µl of 100 mM ammonium bicarbonate, the gel pieces were shaken for 5 minutes at 30°C. Then 50 µl of the trypsin solution [0.76 µg trypsin, (Roche Mannheim), 0.12% $CaCl_2$, 8% acetonitrile, 100 mM ammonium bicarbonate] were added and the samples were incubated at 37°C for 16 hours with shaking (300 rpm). After stopping the reaction by addition of 90 µl stop solution (75% acetonitrile, 0.2% trifluoroacetic acid), shaking was continued for 1 hour followed by centrifugation for 1 min at 13,000 rpm. The supernatants were transferred to a fresh tube and 10 µl acetonitrile were added, shaken for 2 min and centrifuged as before. The wash was repeated a second time and the supernatants combined. The combined supernatants containing the peptides were concentrated in a Speed Vac until dry and used for analysis.

The peptide fragments were then subjected to nanoHPLC/ESI-MS/MS in the MS-laboratory of BASF Central Research, Ludwigshafen, Germany. MS-analysis of the trypsin-digested protein samples was carried out on a quadrupole ion-trap mass-spectrometer (LCQ, Fa. Thermofinnigan) with a gold-platinated spray capillary (Fa. New Objektive, Inc.). The spectrometer is directly linked to a µHPLC unit (Fa. SunChrom GmbH), allowing separation of the peptide mix prior to the MS-analysis.

The peptides were separated on the µHPLC unit using a reverse-phase column (Fa. LC-Packings) with 75 µm i.d. and a length of 15 cm. The column was packed with PepMap™ C18[4] material of 100 Å pore diameter and 3 µm particle size. A gradient of 5 – 50% solvent A and B (A: 95% H_2O, 5% ACN, 0.08% HCOOH; B: 95% ACN, 5% H_2O, 0.08% HCOOH) in 60 min was selected for separation of the trypsin-digested peptides. The 10 µl/min flow from an Eldex HPLC pump (Fa. SunChrome) was reduced using a split-system to achieve a column flow-rate of 180 nl/min.

The separated peptide mix was directly eluted into the mass spectrometer. MS-experiments were run in an alternating sequence of "*full MS-scan // MS/MS-scan"*. The full scan experiments provided an overview of the ions present at the respective elution time. In this study, these were mostly intact mono-, di-, and tri-protonated peptide ions. The most abundant ions of the full scan were then selected for MS/MS-analyses. In an MS/MS-experiment a specific ion is selected (by its m/z value) and subjected to collision with helium atoms. This collision brings about a collision-induced dissociation (CID) of the selected peptide ion into fragment ions. In the chosen experimental set-up, dissociation occurs mainly

[4] C18 material, i.e. covalently-bonded octadecylsilane, is used as bonded phase in the column.

at the peptide bonds, with an ideally statistical distribution. Two series of ions are formed: b-ions, where the charge is retained on the N-terminal fragment, and y-ions, carrying the charge on the C-terminus of the fragmented peptide. The m/z-difference between two adjacent signals of one ion series corresponds to one amino acid residue. In theory, the MS/MS-spectra feature the full amino acid sequences of the analyzed proteolytic peptides. But usually not every expected signal is retrieved, so that instead of full-length sequences, sequence tags are obtained. A sequence tag is a data combination, consisting of the m/z-value of the entire peptide, and a partial, accurately localized amino acid sequence of the peptide. Depending on the length/sequence coverage of the tag, a sequence tag of a proteolytic peptide can be highly specific for the originating protein. In summary, the MS-experiments provide the molecular masses of the proteolytic peptides and either full-length sequences or specific sequence tags.

In order to identify the originating protein of the peptide mix, the MS/MS-data were analyzed using the MASCOT MS/MS-ions search. In short, MASCOT compares the experimental MS/MS-data against a comprehensive primary sequence database. For this comparison, the entries of the sequence database are subjected to ion cleavage rules, thus yielding calculated fragment-ion mass-values. The quality of the obtained matches is evaluated by an appropriate scoring algorithm. Depending on the contents of the database, the originating protein or a protein with close homology can be identified. The MASCOT search was carried out without specifying a digesting enzyme. All MASCOT results were checked manually against the experimental data to assure appropriate evaluation. In addition to the automated database search with MASCOT, amino-acid sequences were retrieved by hand from the MS/MS-spectra as far as possible.

Amino acid sequence data was obtained for approximately 23% of the AHAS protein produced in CV127 soybean, and approximately 73% of the microbially-produced AtAHAS. The results (Figure B-5) confirm that the major protein band at approximately 64,000 molecular weight in CV127 soybean is AtAHAS since the amino acid sequences obtained from peptide fragments derived from it were identical to regions of the deduced amino acid sequence from the coding sequence of the *csr1-2* gene in the CV127 soybean genome. Similarly, sequences obtained from peptide fragments derived from the microbially-produced AtAHAS were identical to regions of the deduced amino acid sequence from the coding sequence of the *csr1-2* gene in the *Escherichia coli* over-expression system (data not presented). Also, where AtAHAS peptide fragments overlapped between the plant-produced and microbially-produced sources, the amino acid sequences were the same (data not presented). Since the same DNA coding sequence for the AtAHAS protein was introduced into the genome of CV127 soybean and into the *Escherichia coli* over-expression system, these data confirm that the amino acid sequences of peptides produced from the predicted mature AtAHAS from the *Escherichia coli* over-expression system and from CV127 soybean are the same and correspond to the predicted amino sequence of AtAHAS with the S653N and R272K amino acid substitutions.

Two of the four potential N-glycosylation sites were included in the amino acid sequence obtained, providing confirmatory support for the lack of glycosylation at these sites. If glycosylation had been present, fragments of different masses would have been obtained.

The peptide fragment corresponding to residues 52 to 73 was not expected to be present in the mature AtAHAS protein produced in CV127 soybean. The *csr1-2* gene from *Arabidopsis thaliana* encodes a single polypeptide of 670 amino acids that includes a chloroplast transit

peptide (CTP) on the N-terminus that has been hypothesized, but not confirmed, to consist of 85 amino acids (Mazur *et al*., 1987; Chang and Duggleby, 1997). This hypothesized CTP was deduced by comparing the amino acid sequences of known AHAS proteins and looking for the first conserved residue (i.e. threonine 86). An identical CTP cleavage site was experimentally determined for the AHAS enzyme in maize (B. K. Singh, personal communication). During transport into the chloroplast, the chloroplast transit peptide is removed to produce the mature AtAHAS enzyme. For this reason, the peptide fragment corresponding to residues 52 to 73 was not expected to be present in CV127 soybean. The microbially-produced AtAHAS protein was designed to represent the mature form of the AtAHAS protein and lacks the 85-amino acid N-terminal sequence corresponding to the predicted chloroplast transit peptide sequence. The microbial protein does contain an additional 38 amino acids (see footnote on page 197) to aid in bacterial expression and purification. The molecular weights of the CV127-produced and microbially-produced proteins were shown to be similar and, based on this information, represent 623 amino acids in the *Escherichia coli*-produced AtAHAS and potentially 619 amino acids in CV127-produced AtAHAS. Furthermore, the N-terminal amino acid for one of the endogenous soybean AHAS proteins (AHAS1) has been shown to be the serine 48; thus, the chloroplast transit peptide for that protein is apparently only 47 amino acids in length (Rood *et al*., 2006).

Conclusions. Key biochemical and functional parameters were evaluated to demonstrate that the AtAHAS protein produced in CV127 soybean is substantially similar to the microbially-produced AtAHAS protein which has been used in various safety assessment studies. The molecular weight and immunoreactivity of the AHAS produced in CV127 soybean was shown to be similar to that of the endogenous AHAS from the isoline control soybean and the reference AtAHAS produced in a microbial over-expression system. The specific activity of the AHAS produced in CV127 soybean was very similar to the endogenous soybean AHAS and was higher than the microbially-produced AtAHAS. This was attributed to AHAS being a very unstable protein that loses activity readily upon purification (Chang and Duggleby, 1997). Therefore, it is not unexpected that the AHAS specific activity in an impure state such as in the ammonium sulfate AHAS-enriched fraction is higher than that retained in the much more highly purified microbially-produced material. Both CV127-produced and microbially-produced AtAHAS proteins showed tolerance to inhibition by the imidazolinone herbicide imazethapyr compared to the control plant-produced protein. Sensitivity to feedback inhibition by branched-chain amino acids was retained in the plant extracts but not in the microbially-produced material as expected. Acetohydroxyacid synthase in *Escherichia coli* is composed of two subunits. However, previous studies have demonstrated that when the *A. thaliana* acetohydroxyacid synthase catalytic (large) subunit (AtAHAS) is expressed in *E. coli*, the *E. coli* small (regulatory) subunit is incapable of interacting with the *Arabidopsis thaliana* catalytic or "large" subunit to confer the feedback inhibition observed in *Arabidopsis thaliana* seedlings (Singh *et al*., 1992; Chang and Duggleby, 1997). No evidence of glycosylation was found associated with the AtAHAS in CV127 leaves or the microbially-produced AtAHAS.

To compare the amino acid sequence of the AtAHAS protein produced in CV127 soybean with that produced in the *E. coli* expression system, the amino acid sequence of the AtAHAS protein of CV127 soybean was investigated. Attempts to obtain N-terminal amino acid sequence using Edman degradation methods were unsuccessful. This was most likely due to the low abundance of the AtAHAS protein in plant tissues, coupled with the repetitive amino acid content of the putative CTP. The high content of serine, threonine and proline within the first 85 amino acids of the full-length AtAHAS (the putative CTP segment of the protein)

contributes to the difficulties in obtaining unambiguous results. Serines and threonines negatively impact the yield of amino acid sequence and have high carryover into subsequent chromatograms, leading to ambiguous interpretation of results of the amino acid sequence analysis. Proline is not efficiently cleaved (only 50 – 60%) by the Edman degradation chemistry. This also contributes to the high carryover into subsequent chromatograms. These three amino acids represent 60% (51/85) of the putative CTP. Therefore, the resulting data gave the appearance of N-terminal blockage of the AtAHAS protein.

Because the above approach was not successful in determining the N-terminus of the mature AtAHAS protein, a combination of SDS-PAGE and liquid chromatography coupled with tandem mass spectroscopy (LC/MS/MS) was performed. Peptides were derived from CV127 soybean and compared to the deduced amino acid sequence of the AtAHAS gene in the *E. coli* expression vector. The amino acid sequence obtained covered approximately 23% of the entire AtAHAS amino acid sequence. All amino acid sequence data obtained from the AtAHAS of CV127 soybeans was identical to the corresponding amino acid sequence of the *E. coli*-produced AtAHAS with the exception of a region near the N-terminus that is predicted to contain the chloroplast transit peptide. Based on a sequence comparison of the deduced amino acid sequence of plant and microbial AHAS proteins, Mazur *et al.* (1987) predicted that the chloroplast transit peptide of AtAHAS is cleaved at the C-terminal side of residue 85. Amino acid sequence analysis of the AtAHAS protein produced in CV127 soybean showed a peptide fragment corresponding to amino acid residues 52 to 73. Therefore, the CTP cleavage site of the AtAHAS protein produced in CV127 soybean is most likely not at the predicted site at the C-terminal side of residue 85, but is located at least at the C-terminal side of residue 51. This conclusion was further confirmed because the apparent molecular weights of the CV127-produced AtAHAS protein and the microbially-produced AtAHAS protein were equivalent, yet the microbially-produced protein included an additional 38 amino acids attached to the 585 amino acids of the predicted mature AtAHAS protein.

However, even though the data in this study suggest that the mature AtAHAS protein produced in CV127 soybean consists of the predicted mature protein (585 amino acids) plus approximately 34 additional amino acids from the CTP as predicted by Mazur *et al*. (1987), the microbially-produced AtAHAS protein is considered equivalent to the CV127 protein for the following reasons. The microbially-produced AtAHAS protein was functionally active and had the expected enzymatic activity with the substrate pyruvate. Furthermore, the microbially-produced protein lacked sensitivity to inhibition by the imidazolinone herbicide imazethapyr, similar to the lack of sensitivity of the CV127 protein to the same herbicide. In addition, the microbially-produced AtAHAS protein and CV127-produced AHAS proteins had similar immunoreactivity to antibodies specific to the AHAS protein based on results of both western blot and ELISA analyses. Both the microbially-produced AtAHAS protein and the CV127-produced protein showed no evidence of glycosylation. Finally, both proteins had the same amino acid sequence of the predicted mature protein of 585 amino acids, based on amino acid sequence analysis of overlapping peptide fragments.

Results of these studies justify the use of the microbially-produced AtAHAS protein as an appropriate substitute for the AtAHAS produced in CV127 soybean in safety assessment studies of the AtAHAS protein.

Standard Operating Procedures

BPS 510.02	SDS-Polyacrylamide Gel Electrophoresis
BPS 510.03	Western Blot Analysis
BPS 510.04	Protein Determination Using the BCA Procedure
BPS 510.09	Enzymatic Assay for Acetohydroxyacid Synthase (AHAS)

Table B-3. Specific Activity of AHAS in CV127 Soybean, the Isoline Control Soybean, and the Reference Substance AtAHAS-0107

Source of AHAS	ng AHAS/ml extract	mg protein/ ml extract	AHAS activity nmole/min/ml extract	AHAS specific activity nmole/min/mg protein	AHAS specific activity Units/μg AHAS
CV127	0.516	8.16	2.697	0.33	5.227
Isoline Control	0.384	8.13	2.435	0.30	6.341
AtAHAS-0107	85411.000	0.32	436.003	1379.43	0.005

Figure B-1. Western Blot Analysis of AHAS Protein in CV127 Soybean, the Isoline Control Soybean and Reference Substance AtAHAS-0107.
The immunoreactivity and integrity of AHAS from CV127 soybean and the isoline control, as well as in sample AtAHAS-0107, was evaluated by western blot analysis. Lane 1, 0.4 ng AtAHAS from AtAHAS-0107; lane 2, 76 µg protein from the ammonium sulfate AHAS-enriched fraction from CV127 soybean; and lane 3, 81 µg protein from the ammonium sulfate AHAS-enriched fraction of the isoline control soybean. Blots were probed with rabbit anti-AHAS peptide 2 antibody. The molecular weight of intact AHAS corresponds to *ca.* 64,000 mol. wt. Molecular weight (x 10^{-3}) markers are indicated.

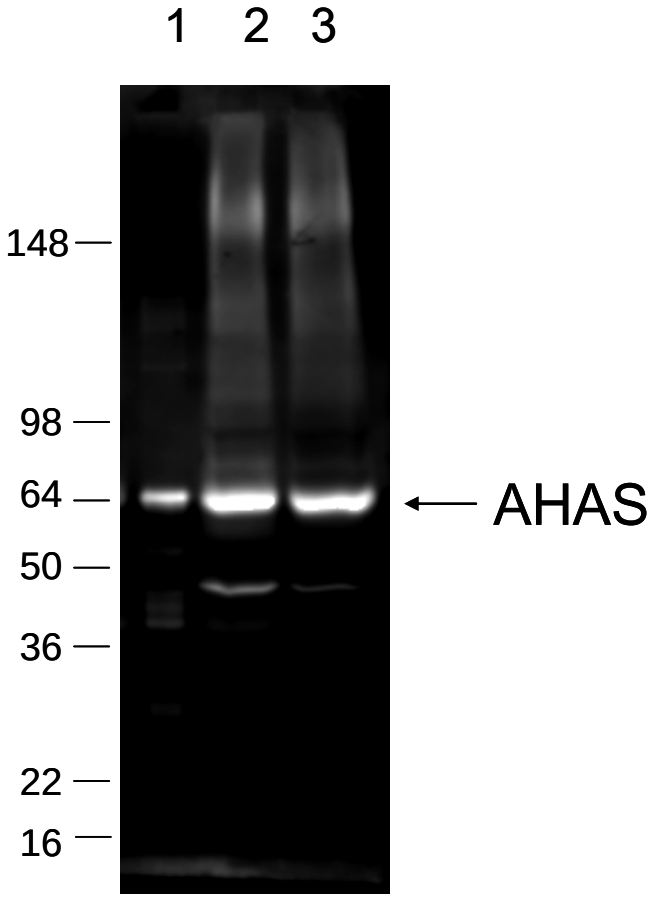


Figure B-2. Sensitivity of the Enzymatic Activity of AHAS from CV127 Soybean, the Isoline Control Soybean and Reference Substance AtAHAS-0107 to Imazethapyr, an Imidazolinone Herbicide.

AHAS enzymatic activity was measured in the presence of increasing concentrations of the imidazolinone herbicide imazethapyr in AHAS extracts enriched by ammonium sulfate precipitation, prepared from CV127 soybean leaves (-▲-) and isoline control soybean leaves (-■-), as well as in the reference AHAS microbially-produced test substance, AtAHAS-0107 (-▼-).

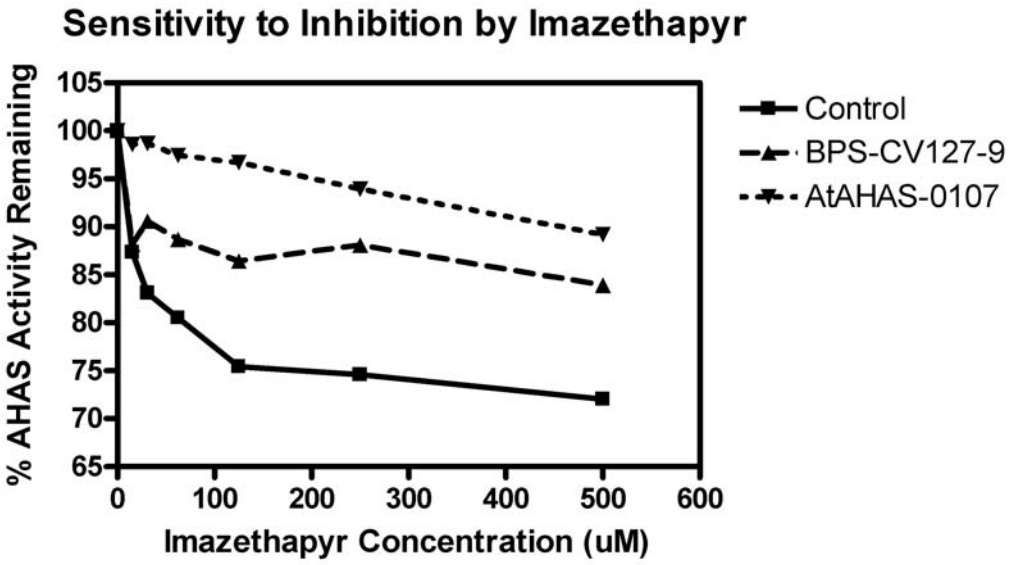


Figure B-3. Sensitivity of the Enzymatic Activity of AHAS from CV127 Soybean, Isoline Control Soybean and Reference Substance AtAHAS-0107 to Feedback Inhibition by Leucine and Valine.
AHAS enzymatic activity was measured in the presence of increasing concentrations of the branched-chain amino acids leucine and valine in AHAS-enriched ammonium sulfate precipitation extracts prepared from CV127 soybean leaves (-▲-) and the isoline control soybean leaves (-■-), as well as in the reference AHAS microbially-produced test substance, AtAHAS-0107 (-▼-).

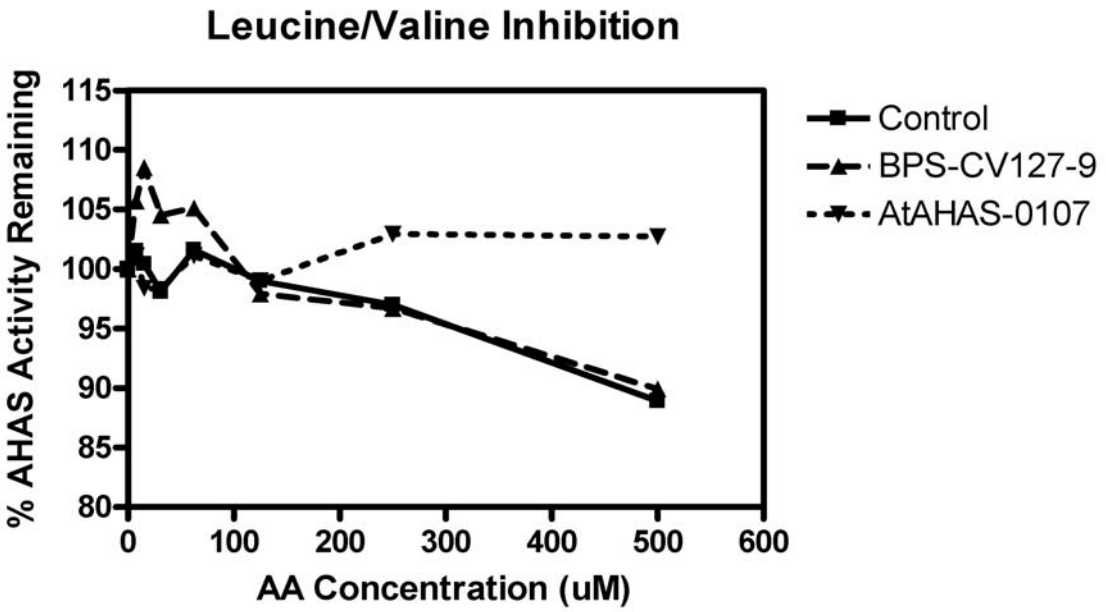


Figure B-4. Examination of Glycosylation of AHAS from CV127 Soybean and Reference Substance AtAHAS-0107.
Samples were subjected to SDS-PAGE on a single 8 – 16% polyacrylamide gel and electroblotted onto a PVDF membrane. The membrane was divided so that lane 1 and part of the marker lane (M) were developed by western blot analysis using goat anti-AtAHAS, Lanes 2 – 4 were developed in the absence of oxidation, and lanes 5 – 10 with oxidation. Lanes 1 and 5, 25 - 50 ng AHAS from the AHAS-enriched CV127 soybean leaf preparation; lane "M", SeeBluePlus™ protein markers; lanes 2 and 10, 100 ng transferrin each and lanes 8 and 9, 5 and 50 ng of transferrin (positive control), respectively; lanes 3 and 6, 2 ng AtAHAS-0107; lane 7, 100 ng creatinase (negative control). The AtAHAS position is indicated by arrows.

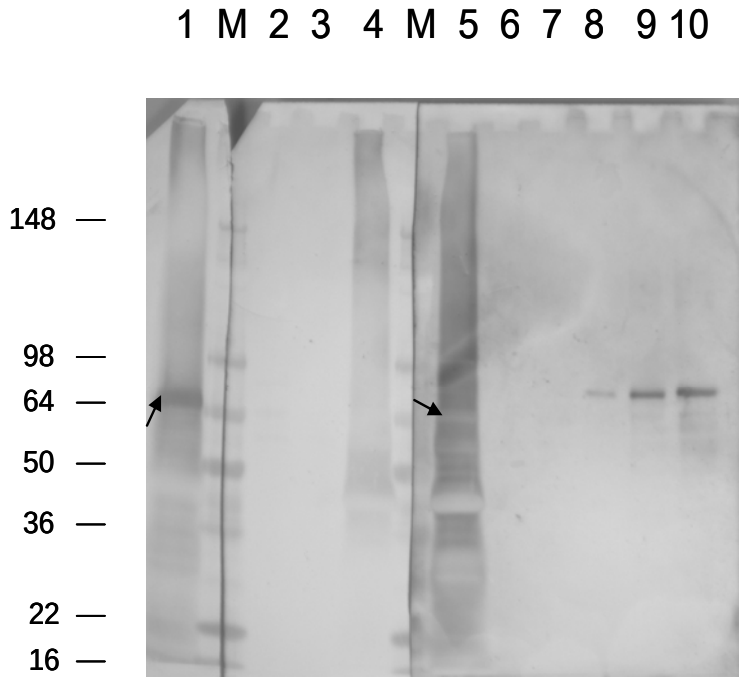
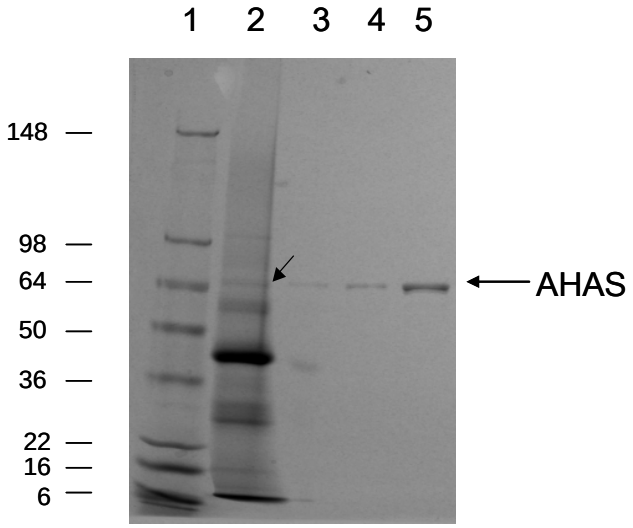


Figure B-5. AtAHAS Amino Acid Sequence.
Panel A. SDS-PAGE with Coomassie blue staining of immuno-purified AtAHAS from CV127 soybean (lane 2, arrow). Lanes 3 through 5, microbially-produced AtAHAS loaded at 25, 50, and 250 ng AHAS, respectively. Molecular weight (x 10^{-3}) markers are indicated. **Panel B**. The predicted amino acid sequence of AtAHAS encoded by the gene transformed into soybean resulting in the event designated CV127. The amino acid sequences underlined in Panel B were obtained by LC/MS/MS analysis of the protein band indicated by the arrow in Panel A, lane 2. The four potential N-glycosylation sites [motif NX(S/T)X] are indicated by shading. The two amino acids that are different from wild-type AtAHAS are indicated in bold (K^{272}, N^{653})

A.



B.

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1   MAAATTTTTT SSSISFSTKP SPSSSKSPLP ISRFSLPFSL NPNKSSSSSR
51  RRGIKSSSPS SISAVLNTTT NVTTTPSPTK PTKPETFISR FAPDQPRKGA
101 DILVEALERQ GVETVFAYPG GASMEIHQAL TRSSSIRNVL PRHEQGGVFA
151 AEGYARSSGK PGICIATSGP GATNLVSGLA DALLDSVPLV AITGQVPRRM
201 IGTDAFQETP IVEVTRSITK HNYLVMDVED IPRIIEEAFF LATSGRPGPV
251 LVDVPKDIQQ QLAIPNWEQA MKLPGYMSRM PKPPEDSHLE QIVRLISESK
301 KPVLYVGGGC LNSSDELGKF VELTGIPVAS TLMGLGSYPC DDELSLHMLG
351 MHGTVYANYA VEHSDLLLAF GVRFDDRVTG KLEAFASRAK IVHIDIDSAE
401 IGKNKTPHVS VCGDVKLALQ GMNKVLENRA EELKLDFGVW RNELNVQKQK
451 FPLSFKTFGE AIPPQYAIKV LDELTDGKAI ISTGVGQHQM WAAQFYNYKK
501 PRQWLSSGGL GAMGFGLPAA IGASVANPDA IVVDIDGDGS FIMNVQELAT
551 IRVENLPVKV LLLNNQHLGM VMQWEDRFYK ANRAHTFLGD PAQEDEIFPN
601 MLLFAAACGI PAARVTKKAD LREAIQTMLD TPGPYLLDVI CPHQEHVLPM
651 IPNGGTFNDV ITEGDGRIKY
```

Appendix C

Methods for Determining Levels of the AHAS and SEC61γ Subunit Proteins in Soybean Tissues

A. Determining the level of AHAS protein in soybean tissues

To assess the level of production (or expression) of the AtAHAS enzyme in CV127 soybean that is encoded by the *csr1-2* gene, the amount of AHAS protein was determined in tissues of CV127 soybean and the isoline control that were grown in Brazil in two different growing seasons. Seven field trials in the 2006/2007 growing season and six in the 2007 short growing season were conducted in geographically distinct locations that were representative of commercial soybean production areas in Brazil. The field sites were located in or near the locations listed as follows:

Field Trial Locations for the AHAS Expression Study		
Location	Trial Season 1 Oct. 2006–Mar. 2007	Trial Season 2 Feb.–July 2007
Sto. Antônio de Posse – SP	√	
Ponta Grossa – PR/South	√	
Londrina – PR/North	√	
Uberaba – MG	√	√
Brasília – DF	√	√
Sto. Antônio de Goiás – GO	√	√
Sete Lagoas-MG	√	√
Teresina - PI		√
Vilhena - RO		√

A detailed description of these field trials is presented in Section VIII.C, and Appendix F.

Tissue sample collection. Plant tissues were collected from two replicate plots of the CV127 soybean plants treated with the imidazolinone herbicide imazapyr (treatment T1) and from two replicate plots of the isoline control that were treated with the conventional soybean herbicide Volt® (bentazon + acifluorfen-sodium at a rate of 1.0 liters/ha; treatment T3) as described in Section VIII.C and Appendix F. Leaf samples were collected at the V2 growth stage at all field trial locations in the 2006/2007 and 2007 growing seasons, as described in Section VI.D. Also, approximately 500 g of grain was harvested from each plot at all of the field test locations in the 2006/2007 and 2007 growing seasons, except for Ponta Grossa in the 2006/2007 growing season. In addition, six whole plants per plot, including roots, were collected at two of the seven field trial locations in the 2006/2007 growing season (Sto. Antônio de Posse and Londrina) and two sites in the 2007 growing season (Brasília and Sto. Antônio de Goiás) at three different developmental stages, including the V2 (plants 15-20 cm tall with three nodes and two unfolded leaflets), R2 (plants in full bloom), and R8 (full maturity) stages. Each whole plant was placed in a separate, labeled plastic bag and was shipped on ice within 24 hr to the test facility:

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Global Environmental and Consumer Safety Laboratory
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Guaratinguetá – São Paulo – Brasil
CEP: 12521-1802

Upon receipt at the test facility, three of the six plants were dissected into plant parts, including leaves, stems, roots, and flowers and pods, if present. The plant parts from each plant were maintained as a single sample except for the flowers at the R2 stage and roots at the V2 stage that were pooled to form a single sample in order to obtain sufficient material for analysis. The other three whole plants from each plot were maintained and processed as a whole plant and formed a single sample. The whole plant and leaf tissue samples were shipped on wet ice within 24 hrs to the test facility where they were stored at -80±6°C until they were processed.

Sample processing and extraction. Leaves, grain, whole plants and plant part samples were ground in a mill with dry ice to produce a homogenous, powdered sample. The samples were lyophilized in a freeze drier and stored at -80±6°C until analysis. A 0.1 g aliquot (±0.001 g) of each sample was weighed and placed in a 10 mL centrifuge tube. Three mLs of extraction solution (14.8 mM KH_2PO_4, 35.2 mM K_2HPO_4, 100 mM Na-Pyruvate, 5 mM $MgCl_2$) were added to each sample followed by incubation on ice for 30 min. The tissues were extracted with a tissue homogenizer at 25,000 rpm with several up and down movements of the homogenizer probe over 10 sec. The extracts were centrifuged at 3000 rpm at 5°C for 10 min. The liquid phase was removed and filtered through miracloth and was centrifuged again at 14,000 rpm at 5°C for 15 min. The liquid phase was recovered and stored at 5°C prior to analysis by ELISA.

Sample analysis by ELISA. The amount of AHAS enzyme in the different plant tissues was analyzed using an Enzyme-Linked Immunosorbent Assay (ELISA) protocol that used a goat anti-AHAS IgG antibody. Due to the high amino acid similarity between the AtAHAS enzyme encoded by the *csr1-2 AHAS* gene and the homologous endogenous soybean AHAS enzymes, the antibody used in the ELISA is not capable of distinguishing between these enzymes but rather measures the total AHAS protein in the samples. Therefore, expression levels of the AHAS protein in CV127 soybean include levels of the endogenous soybean AHAS proteins as well as levels of the AtAHAS enzyme encoded by the *csr1-2* gene. Expression levels of the endogenous AHAS proteins in the isoline control soybean were also determined. The ELISA was carried out in flat-bottom, 96-well microtiter plates and the reaction was quantified using a spectrophotometer with a microplate reader at 450 nm. A dilution series of purified AtAHAS protein was included in every microtiter plate for calibration purposes. The limit of quantification (LOQ) for this assay with soybean tissues is 13 ng AHAS enzyme/g fresh-weight tissue. The limit of detection (LOD) for this assay with soybean tissues is 3 ng AHAS enzyme/g fresh-weight tissue. The laboratory portion of this study was conducted under Good Laboratory Practices (GLP).

B. Determining the level of SEC61γ subunit protein in soybean tissues

Source of plant materials. Leaf tissue was harvested from greenhouse-grown plants of CV127 soybean (grown from F_9 generation seed) and the conventional soybean variety Conquista for microsomal membrane protein isolation. The soybean seeds used to produce the plants from which the leaf material was harvested were used as the grain samples.

Arabidopsis thaliana leaves and seed were produced in greenhouses in Research Triangle Park, North Carolina, United States.

Test material. The test materials for this study were microsomal membrane protein preparations from leaf tissues and grain derived from CV127 soybean. Microsomal membranes include the endoplasmic reticulum and were selected to enrich the SEC61γ subunit in the samples to be analyzed.

Control material. The control materials for this study were microsomal membrane protein preparations from leaf tissues and grain derived from the conventional soybean variety Conquista.

Reference material. The reference materials for this study were microsomal membrane protein preparations from *Arabidopsis thaliana* leaves and seed.

AtSEC61γ protein standard. The At3g48570 gene, encoding the AtSEC61γ subunit protein, was cloned into the expression cassette pGEX-6P (GE Healthcare Bio-Sciences Corp, Piscataway, NJ) and transformed into *Escherichia coli* strain Origami B (DE3)pLysS (EMD Biosciences, San Diego, CA). This expression cassette encodes a glutathione S-transferase (GST)–AtSEC61γ subunit fusion protein (mol. wt. *ca*. 35,000). The GST-AtSEC61γ subunit fusion protein was purified from *E. coli* using an immobilized glutathione column according to the manufacturer's instructions (GE Healthcare). On-column digestion using PreScission Protease™ (GE Healthcare) was performed to release the AtSEC61γ subunit protein (mol. wt. *ca*. 7000). This was the AtSEC61γ subunit protein that was used as a standard on the western blots.

Antibody generation and sensitivity. Rabbit polyclonal antibodies used in western blot analysis were generated against two synthetic peptides which together spanned the entire 69-amino acid AtSEC61γ subunit protein. These peptides were conjugated to a carrier protein and injected into rabbits. IgG was purified from the sera using Protein A affinity chromatography. A 2.6 mg/ml stock was routinely diluted 1:3000 in blocking buffer for use in western blot analysis as described below.

Microsomal membrane protein preparations. Arabidopsis and greenhouse-grown soybean leaf extracts were prepared by homogenizing 6 g frozen powdered leaves in 18 ml extraction buffer [8 mM sodium phosphate, 2 mM potassium phosphate, 0.14 M sodium chloride, 10 mM potassium chloride, pH 7.4 (PBS) with protease inhibitor cocktail (Thermo Scientific, Inc., Rockford, IL) and 2 mM $MgCl_2$]. The homogenates were filtered through miracloth and centrifuged at 12,000 x g for 15 minutes. The supernatant was then centrifuged at 100,000 x g for 1.5 hours. The resulting microsomal membrane pellet was resuspended in 250 µl PBS. For soybean grain and Arabidopsis seed extracts, 1 g powdered grain/seed was extracted with 10 ml extraction buffer. The extracts were filtered through miracloth and microsomal membranes were isolated as described above for leaf extracts. The pellets were resuspended in 250 µl PBS.

Cytochrome c reductase assay. The activity of cytochrome c reductase, a marker enzyme of the endoplasmic reticulum (microsomal membrane fraction), was measured in the crude extract, 100,000 x g supernatant and pellet (microsomal membrane fraction) by monitoring the rate of reduction of cytochrome c (the change in absorbance at 547 nm with time) to provide assurance that the fraction being analyzed for the presence of the SEC61 gamma

subunit protein was indeed the microsomal fraction containing the endoplasmic reticulum. The samples were assayed using the cytochrome c reductase (NADPH) assay kit (Sigma, Saint Louis, MO). The total reaction was 230 µl and contained 300 mM potassium phosphate, pH 7.8, 0.1 mM EDTA, 85 µg NADPH, and 36 µM cytochrome c. The reaction was initiated by the addition of NADPH and the change in absorbance at 547 nm was monitored using a Tecan Sunrise® multiwell plate reader. The results were analyzed using DeltaSoft PC software (Version 1.71.4, BioMetallics, Inc., Princeton, NJ) using the kinetic mode to calculate the rate.

Western blot analysis. Aliquots of the microsomal membrane preparations were subjected to SDS-polyacrylamide gel electrophoresis on an 8 – 16% polyacrylamide gradient gel followed by electroblotting onto a PVDF membrane (Invitrogen; SOPs BPS 510.02 and 510.03). The membrane was probed with rabbit anti-AtSEC61γ polyclonal antibody which can detect approximately 0.4 ng SEC61γ subunit protein. Donkey anti-rabbit IgG linked to horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), diluted 1:20,000 in blocking buffer (3% nonfat dry milk in 0.05% Tween-20, 25 mM Tris-HCl, 140 mM NaCl, 3 mM KCl, pH 7.4), was used to bind to the primary antibody and was visualized by development with the SuperSignal® West Femto Maximum Sensitivity Substrate (Thermo Fisher). The limit of detection of this development method with this antibody was found to be approximately 0.4 ng SEC61γ subunit protein.

Appendix D

Methods Used and Results of the Grain and Forage Composition Analyses

The purpose of these studies was to analyze and compare the composition of soybean grain and forage derived from CV127 soybean, the isoline control, and two conventional soybean varieties. For weed control, CV127 soybean plants were treated with imidazolinone herbicide at 70 g ai/ha (treatment presented as "CV127+imi") and the isoline control and two other conventional soybean varieties commonly grown in Brazil (Monsoy 8001 and Coodetec 217 presented as "Std1" and "Std2", respectively, in the data tables) were treated with bentazon + acifluorfen-sodium (commercial name Volt®) at the rate of 1.0 liter/ha. For grain composition analyses only, an additional treatment of CV127 treated with the conventional herbicide Volt® was included in these studies, and the data are reported in the following data tables in this Appendix for this treatment. However, because this treatment will not be used in the commercial production of CV127 soybean, this treatment was not included in the mean and range analyte data determined across field trial locations and presented in Section VII of the petition.

a) Grain Composition Analyses

For grain production, plants were grown at six locations in Brazil during the 2006/2007 growing season and four locations during the short growing season of 2007. The field trial locations and cultivation practices are described in detail in Appendix F, and are summarized in Table D-1. The plants were grown under standard agronomic practices in a complete randomized block design with four replicate blocks per location. For the CV127 and isoline control treatments, grain was harvested separately from each replicate block at the conclusion of the growing season and approximately 2 kg of each replicate grain sample was separated for compositional analyses. For the two conventional soybean varieties ("Std1" and "Std2"), the same grain harvest and separation procedures were followed, but approximately 500 g of grain was further sub-sampled from each 2 kg replicate grain sample, and the four 500 g replicate samples for each conventional soybean variety from each field location were pooled to make a single sample from each location for compositional analyses. Therefore, statistical analyses of the compositional data were only conducted for the CV127 and isoline control treatments, and data from the two conventional soybean varieties were used for comparative purposes to establish a range of natural variability for each analyte for soybeans grown in Brazil. All compositional analyses were conducted using methods listed in Table D-2 by the Instituto de Tecnologia de Alimentos (ITAL) in Campinas, Brazil or, for cysteine and methionine analyses only, Eurofins (Des Moines, IA). Proximate results are reported on both a fresh- and dry-weight basis; all other analytes are reported on a dry-weight basis unless otherwise stated. All results from grain produced in the 2006/2007 season and the 2007 short season are presented in the following tables of this Appendix (D-3 to D-22). Statistical analysis was conducted using the dry-weight data.

b) Forage Composition Analyses

Imidazolinone-treated CV127 plants together with the isoline control, and two other conventional soybean varieties [Monsoy 8001 ("Std 1") and Coodetec 217 ("Std 2")] were grown at six locations in Brazil during the 2007/2008 growing season. The field trial locations are presented in Table D-1. The plants were grown under standard agronomic practices in a complete randomized block design with three replicate blocks per location.

With the exception of the CV127 plants treated with the imidazolinone herbicide at 70 g ai/ha, all other entries in the study were treated with bentazon + acifluorfen-sodium (commercial name Volt®) at the rate of 1.0 liter/ha. Three plants at the full-flowering stage of development in each replicate plot were harvested by cutting the plants at the base of the stem near ground level. The three plants from each plot or treatment replicate were pooled together in a plastic bag and shipped on wet ice to the Instituto de Tecnologia de Alimentos (ITAL) in Campinas, Brazil, for compositional analysis. At ITAL, the pooled samples consisting of three plants from each replicate plot were chopped and mixed to produce a composite sample. After partial drying and weighing, the samples were reduced to a fine powder using a Waring blender. Aliquots (25 g each) of each sample were removed to separate air-tight containers and were shipped on dry ice to Eurofins (Des Moines, IA) for analysis of cysteine and methionine. Samples were stored in air-tight glass bottles until analyzed. Results were recorded on a fresh-weight (FW) basis and adjusted for moisture content to calculate the corresponding dry-weight (DW) value. Statistical analysis was conducted using the dry-weight data. Details of the methods of compositional analyses are presented below and summarized in Table D-2. Results of forage composition analyses are presented in Tables D-23 and 24. Reference values for forage composition analyses from OECD (2001) are presented in Table D-25.

Table D-1. Field Trial Locations in Brazil for Grain and Forage Compositional Analyses

Location	2006-07 Season - Grain	2007 Season - Grain	2007-08 Season - Forage
Santo Antônio de Posse – SP	√		√
Londrina – PR/North	√		√
Uberaba – MG	√		√
Brasília – DF	√	√	√
Santo Antônio de Goiás – SG	√	√	√
Sete Lagoas-MG	√		√
Teresina - PI		√	
Vilhena - RO		√	

c) Analytical Methods

The specific methods used for composition analyses of the grain and the forage are listed in Table D-2. Below is a general description of the methods used for measurement of each analyte.

Ash. The 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 for this study was 0.1% FW.

Carbohydrates and Calories. The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation: % carbohydrates = 100% - (% protein + % fat + % moisture + % ash). Calories were calculated using the following equation on fresh-weight derived data: (kcal/100 g) + (4 x % protein) + (9 x % fat) + (4 x % carbohydrates). The limit of quantitation for carbohydrates for this study was 0.1% FW.

Fat by Butt Extraction. The sample was weighed into a cellulose thimble. Petroleum ether was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed. The limit of quantitation for this study was 0.1% FW.

Moisture. The sample was dried in a forced-draft oven at 130°C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The limit of quantitation for this study was 0.1% FW.

Protein. Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a $CuSO_4 + K_2SO_4 + Se$ mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a standard acid. The percent nitrogen was calculated and converted to protein using the factor 6.25. The limit of quantitation for this study was 0.100%.

Amino Acid Composition. The methods of analysis estimate the levels of 18 amino acids in the sample: alanine, arginine, aspartic acid (including asparagine), cystine (including cysteine), glutamic acid (including glutamine), glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. This was accomplished for all amino acids except cysteine, methionine, and tryptophan through direct acid hydrolysis with 6N hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantified using an automated amino acid analyzer and detected at 520 nm. The limit of quantitation for this study was 0.1 mg/g. The reference standards were 2.5 µmol/mL per amino acid with the exception of cystine which was 1.25 µmol/mL (Pierce, Rockford IL). Cysteine (including cystine) and methionine levels were determined by first converting these residues in protein to cysteic acid and methionine sulfone, respectively, by oxidation with performic acid. Following acid hydrolysis to release cysteic acid and methionine sulfone, these residues were quantified by ion exchange chromatography with an o-phthalaldehyde post-column reaction. The limit of quantification for this method is 0.01% on an "as is" basis. Tryptophan levels were determined by measuring the absorbance at 590 nm following direct enzymatic hydrolysis with pronase at 40º C for 24 hours. The reference standard

was L-tryptophan, >99% (used as 100%, Sigma Chemical Co., Saint Louis, MO). The limit of quantitation for this method was 0.1 mg/g.

Crude Fiber. Crude fiber was measured as the loss on ignition of dried residue remaining after digestion of the sample with 1.25% solutions of sulfuric acid and sodium hydroxide. The limit of quantitation for this study was 0.1 g/100 g FW sample.

Acid Detergent Fiber (ADF). The sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. After an acetone wash to remove the fats and pigments, the lignocellulose fraction was collected on the frit and quantitated gravimetrically. The limit of quantitation for this study was 0.1g/100g FW.

Neutral Detergent Fiber (NDF). Samples were placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. After an acetone wash to remove the fats and pigments, the hemicellulose, cellulose, and lignin fractions were collected on the frit and quantitated gravimetrically. The limit of quantitation for this study was 0.1g/100g FW.

Total Dietary Fiber. The finely ground sample was gelatinized with Termamyl and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Four volumes of 95% ethyl alcohol were added to precipitate soluble dietary fiber. The total residues were filtered and washed consecutively with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying, the residue was weighed. One duplicate was then analyzed for protein, and the other was incinerated at 525º C for ash determination. Total dietary fiber = weight residue – weight (protein + ash) – blank (containing enzymes only). The limit of quantitation for this study was 0.1g/100g FW.

Fatty Acids. The lipids were extracted and saponified with 0.5 N sodium hydroxide in methanol. The mixture was methylated with a solution of NH_4Cl and H_2SO_4 in methanol based on Hartman and Lago (1973). The resulting methyl esters were extracted with hexane. The methyl esters of the fatty acids were analyzed by gas chromatography using area normalization for quantitation. The 37-Component FAME mix from Supelco (Sigma, Saint Louis, MO) was used for reference standards. The limit of quantitation for this study was 0.01% FW.

Isoflavones. The defatted sample was extracted using an aqueous solution of 70% ethanol with 0.1% acetic acid. The extract was centrifuged and filtered. The sample was analyzed on a high-performance liquid chromatography (HPLC) system with a diode array detector. Quantification was conducted against an external standard curve of known standards. The limit of quantification for each component was 0.3 mg/100g.

Phospholipids. Lipids were extracted from the sample by a mixture of chloroform-methanol (2:1). Lecithins were purified by solid phase extraction on a silica column. Separation and quantification of lecithins were conducted by normal phase liquid chromatography with ultraviolet detection. The limit of quantitation for phospholipids was 0.1 mg/g oil.

Lectin. The sample was suspended in phosphate-buffered saline (PBS), shaken, and filtered. An aliquot of the resulting extract was serially diluted in 10 cuvettes containing PBS. A 2% erythrocyte (from dog blood) suspension was added to an equal volume of

the sample and the mixture incubated at 37º C for 30 minutes followed by another 30-minute incubation at room temperature. The last well to show visible agglutination was considered the point of equivalence. One hemagglutinating unit (HU) was defined as the reciprocal of dilution at the point of equivalence, the specific activity being given as one hemagglutinating unit per µg or mg of protein (HU) in the undiluted sample. The minimum dose was defined as the minimum concentration necessary to show visible agglutination. These assays are only semiquantitative and should be regarded as liable to an error of 25%.

Minerals. The sample was placed in an electric furnace at 450°C and ignited to drive off all volatile organic matter. The minerals remaining were quantitated by inductively coupled plasma-optical emission spectroscopy (ICP-OES). The limit of quantitation for this study was 0.01 mg/kg for all minerals.

Phytic Acid. The sample was extracted using 2.4% HCl. Purification and concentration was conducted on an anion exchange column (Dowex-1 AGX-4, 100-200 mesh). Sample and standards were submitted to a color reaction with Wade reagent, with the absorbance measured at 500 nm. Inositol hexaphosphoric acid was used as a standard.

Sugars (Raffinose and Stachyose). Sugars were extracted from the sample with ethanol + deionized water (1:1). Proteins and lipids which co-extracted were eliminated by precipitation, followed by filtration. Raffinose and stachyose were separated and quantified by HPLC with a refractive index detector. The limit of quantification for this study was 0.2 g/100 g.

Trypsin Inhibitor. Trypsin inhibitor units (TIU) were determined by photometrically measuring the inhibition of the trypsin cleavage of benzoyl-DL-arginine-p-nitroanalide hydrochloride. The sample was ground and/or defatted with petroleum ether, if necessary. A sample of matrix was extracted for 3 hours with 0.1 N sodium hydroxide. Varying aliquots of the sample suspension were exposed to a known amount of trypsin and benzoyl-DL-arginine-p-nitroanalide hydrochloride. The sample was allowed to react for 10 minutes at 37° C. After 10 minutes, the reaction was quenched by the addition of 30% trichloroacetic acid. The solution was filtered or centrifuged and the absorbance at 410 nm was measured. The limit of quantitation for this study was 1.00 TIU/mg.

Urease Activity. This assay is based on an increase in pH as ammonia is released from urea by residual urease enzyme in the soy meal. The urease activity was assayed by measuring the hydrolysis of 3% urea at pH 7.0 at 37º C. A difference between the pH of the test sample and the pH of the blank is an indication and index of urease activity. The optimum pH increase has generally been considered to be 0.05–0.30.

Folates. The VitaFast Folic Acid kit from R-Biopharm was used. The method is based on a microbiological assay which uses microplates and the *Lactobacillus casei* subspecies *rhamnosus* – ATCC 7469. The antioxidants 2-mercaptoethanol and ascorbic acid were used in all preparation stages of the extract. The samples were hydrolyzed in an autoclave with potassium dihydrogen phosphate 0.1 M pH 6.8, followed by consecutive enzymatic hydrolysis steps. Each enzyme was inactivated by boiling the sample before addition of the next enzyme. The enzymes used were: α-amylase for 3 hours, protease for 3 hours, and folate conjugase for 5 hours. After the necessary dilutions, the extracts were applied to the microplates. After the addition of the samples, the plate was kept in a chamber at 37º C for 44-48 hours and the quantification of the turbidity in the microplates was

measured at 630 nm in a microplate reader. Quantitation was by comparison to a standard curve. The limit of quantitation for this study was 0.1 µg/g FW sample.

Vitamin E (Tocopherols). The samples were saponified at 90-95º C with reflux using nitrogen forced through the condenser, with potassium hydroxide and with the antioxidant, ascorbic acid. The extraction of the non-saponifiable material was conducted with diethyl ether. The diethyl ether extract was concentrated in a rotary evaporator at 40º C, dried under nitrogen and the residue was dissolved in n-hexane. The tocopherols were separated by HPLC over a Lichrospher Si60 (125 x 4 mm) column, (Merck, Germany). The mobile phase consisted of n-hexane, ethyl acetate, and n-propyl alcohol in an isocratic system. The detection and quantification was accomplished using a fluorescence detector with excitation at 294 nm and emission at 326 nm. The limit of quantitation for this study was 0.05 mg/g FW sample.

Vitamin Niacin. For the extraction of niacin, the samples were first hydrolyzed by 1 N sulfuric acid hydrolysis in an autoclave for 30 minutes. Then the pH was adjusted to 4.5 with 10 N sodium hydroxide, as recommended by AOAC (2005) method 961.14. The extracts were passed over an ion-exchange resin prior to HPLC on a Lichrospher 100 RP-18 (250 x 4 mm) column (Merck, Germany). The mobile phase consisted of heptanesulfonic acid sodium salt, triethylamine, potassium dihydrogen phosphate, and methanol in a gradient system. Niacin levels were monitored at 265 nm. The limit of quantification for this study was 0.50 mg/100 g FW sample.

Vitamins B1 (Thiamin) and B2 (Riboflavin). For the extraction of the vitamins, the samples were hydrolyzed with 0.01 N hydrochloric acid in an autoclave for 15 minutes; the pH was adjusted to 4.5 with sodium acetate. Enzymatic hydrolysis using diastase and papain was carried out for 12 hours at room temperature. Extracts were subjected to HPLC using a Lichrospher 100 RP-18 (250 x 4 mm) column (Merck, Germany). The mobile phase consisted of potassium chloride, methanol, and water in an isocratic system. A fluorescence detector was used to monitor the natural fluorescence of the riboflavin (excitation 432 nm and emission 545 nm) and thiochrome, after oxidation of thiamine to thiochrome (362 nm for excitation and 464 nm for emission). The limit of quantitation for this study for Vitamin B1 was 0.03 mg/g fresh weight sample. The limit of quantification for this study for vitamin B2 was 0.02 mg/100 g FW sample.

Statistical analysis. Analysis of variance was carried out on the data using SAS Version 9.1 (SAS Institute Inc., Cary, NC) following two procedures, the General Linear Model and the Mixed Model. With the exception of moisture content, all data were expressed on a dry-weight basis for statistical analyses. Differences were assessed across location and by location. The model for across-location analysis was the following:

y = variety + location + variety x location + block(location) + e
Random effects: location, variety x location, block(location).
Where y is the response variable (any analyte measured)

The model for separate analyses by location was the following:

y = variety + block + e
where e is the response error

Contrasts were carried out to compare each of the sprayed and unsprayed CV127 treatments with the isoline control. Differences were considered statistically significant at the 0.05 confidence level.

Table D-2. Methods for Compositional Analyses of Grain Harvested from 2006-07 and 2007 Field Trial Seasons in Brazil and for Forage Harvested from the 2007-08 Field Trial Season. An "X" is used to identify the specific method of analysis for each analyte.

Analyte	Method	Grain Comp. 2006-07	Grain Comp. 2007	Forage 2007-08
Moisture	AOCS (1998) method Bc 2-49	X	X	
	AOAC (2005) method 934.01			X
Ash	AOAC (2000) method 945.38 C	X	X	
	AOAC (2005) method 942.05			X
Fat	AOAC (2000) method 945.38 F	X	X	
	ISO (1973) method 34 B			X
Crude Fiber	Beythien and Diemair (1963)	X	X	X
Protein	AOAC (2000) method 979.09	X	X	
	AOAC (2000) method 945.39			
	AOAC (2005) method 954.01			X
Carbohydrates and calories	USDA (1963)	X	X	X
Total Dietary Fiber	AOAC (2000) method 985.29	X	X	
ADF	AOAC (1995) method 920.85	X		
	AOAC (1975) method 7.057		X	X
NDF	AOAC (1995) method 920.85 and AOAC (2000) method 973.18	X		
	AOAC (1995) method 920.85 and Van Soest *et al*. (1991)		X	X
Fatty Acids	AOCS (1998) methods Ce 1F-96, Ce 1-62, Ce2-66 AOAC (2000) method 996.06 Hartman and Lago (1973)	X	X	
Raffinose and Stachyose	Cicek (2001) and Kennedy *et al.* (1985)	X	X	
Ca, Fe, P, Mg, K	AOAC (2005) methods 985.35, 984.27	X	X	
Vitamin E	Brubacher *et al*. (1985)	X	X	
Vitamin B1	AOAC (2005) methods 942.23 and 970.65	X	X	
Vitamin B2	AOAC (2005) method 970.65 and 942.23	X	X	
Niacin	AOAC (2005) method 961.14	X	X	
Folate	AOAC (2005) method 2004.05	X	X	
Phytate	Latta and Eskin (1980)	X	X	
Isoflavones	Berhow (2002)	X	X	
Amino Acids (except Cys, Met, Trp)	Spackman *et al*. (1958)	X	X	
Cysteine and Methionine	AOAC (2000) method 994.12 mod.	X	X	
Tryptophan	Spies (1967)	X	X	
Trypsin Inhibitor	Rackis *et al*. (1974)	X	X	
Urease	AOCS (1998) method Ba 9-58	X	X	
Phospholipids	Beare-Rogers *et al.* (1992)	X	X	
Lectins	Padgette *et al*. (1996) and Wititsuwannakul *et al.* (1998)	X	X	

Table D-3. Proximate Composition of Grain on a Fresh-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location	Treatment	N	Moisture (g/100 g FW)	Total Dietary Fiber (g/100 g FW)	Protein (g/100 g FW)	Fat (g/100 g FW)	Ash (g/100 g FW)	Carbohydrates (g/100 g FW)	Calories (kcal/100 g FW)
			Mean ± Standard Deviation (range)						
Santo Antônio de Goias	Isoline	4	10.2 ± 0.1 (10.1 – 10.3)	22.00 ± 1.12 (21.30 – 23.66)	35.8 ± 1.2 (35.1 – 37.5)	19.6 ± 0.2 (19.3 – 19.8)	4.4 ± 0.2 (4.2 – 4.5)	8.0 ± 1.3 (6.9 – 9.5)	352 ± 5 (344 – 356)
	CV127	4	9.1 ± 0.3* (8.8 – 9.4)	22.73 ± 0.79 (21.90 – 23.70)	35.9 ± 0.3 (35.6 – 36.3)	21.2 ± 0.2 (21.0 – 21.5)	4.4 ± 0.1 (4.2 – 4.5)	6.7 ± 1.0 (5.4 – 7.9)	362 ± 4 (356 – 366)
	CV127 + imi	4	9.6 ± 0.1* (9.4 – 9.7)	23.99 ± 2.30 (20.23 – 25.41)	36.6 ± 0.3 (36.3 – 36.9)	20.5 ± 0.5 (19.9 – 20.9)	4.2 ± 0.1 (4.2 – 4.3)	5.6 ± 2.3 (3.4 – 8.8)	353 ± 9 (347 – 366)
	Std 1	1	10.5	23.29	35.4	18.1	4.4	8.3	338
	Std 2	1	9.9	22.84	33.5	21.2	4.2	8.4	359
Uberaba	Isoline	4	9.9 ± 0.4 (9.6 – 10.4)	21.45 ± 1.68 (20.05 – 23.74)	37.3 ± 0.9 (35.9 – 38.0)	19.2 ± 0.9 (18.5 – 20.5)	4.3 ± 0.1 (4.2 – 4.3)	8.0 ± 1.7 (6.0 – 9.6)	354 ± 8 (342 – 360)
	CV127	4	8.9 ± 0.2* (8.7 – 9.1)	21.55 ± 1.35 (20.15 – 22.80)	35.9 ± 0.3 (35.4 – 36.1)	20.3 ± 0.4 (19.9 – 20.4)	4.3 ± 0.1 (4.2 – 4.3)	9.2 ± 1.7 (7.3 – 11.0)	363 ± 4 (358 – 367)
	CV127 + imi	4	9.0 ± 0.2* (8.8 – 9.1)	22.03 ± 1.43 (20.19 – 23.52)	36.3 ± 0.1 (36.2 – 36.4)	20.6 ± 0.3 (20.1 – 20.9)	4.2 ± 0.1 (4.1 – 4.3)	7.9 ± 1.7 (6.0 – 10.1)	362 ± 7 (356 – 370)
	Std 1	1	9.5	22.55	34.9	19.1	4.1	9.9	351
	Std 2	1	9.6	24.19	35.0	21.8	4.1	5.3	357
Sete Lagoas	Isoline	4	10.6 ± 0.2 (10.5 – 10.6)	21.47 ± 1.39 (20.13 – 23.42)	37.0 ± 0.2 (36.6 – 37.1)	19.4 ± 0.1 (19.2 – 19.5)	4.4 ± 0.1 (4.3 – 4.4)	7.3 ± 1.6 (5.0 – 8.9)	351 ± 5 (344 – 356)
	CV127	4	9.7 ± 0.3* (9.3 – 10.0)	21.56 ± 0.97 (20.56 – 22.74)	36.0 ± 0.2 (35.8 – 36.2)	20.3 ± 0.5 (19.8 – 20.9)	4.5 ± 0.1 (4.3 – 4.6)	8.0 ± 1.0 (6.8 – 9.0)	359 ± 5 (354 – 366)
	CV127 + imi	4	9.8 ± 0.1* (9.7 – 9.9)	22.17 ± 1.46 (20.73 – 23.55)	35.6 ± 0.3 (35.2 – 35.9)	20.9 ± 0.3 (20.7 – 21.3)	4.4 ± 0.1 (4.4 – 4.5)	7.1 ± 1.4 (5.9 – 8.6)	359 ± 5 (353 – 364)
	Std 1	1	9.8	23.31	34.0	19.4	4.4	9.1	347
	Std 2	1	9.8	22.80	33.7	21.7	4.3	7.7	361

Table D-3. continued.

Location	Treatment	N	Moisture (g/100 g FW)	Total Dietary Fiber (g/100 g FW)	Protein (g/100 g FW)	Fat (g/100 g FW)	Ash (g/100 g FW)	Carbohydrates (g/100 g FW)	Calories (kcal/100 g FW)
			Mean ± Standard Deviation (range)						
Londrina	Isoline	4	9.4 ± 0.2 (9.2 – 9.7)	24.04 ± 1.01 (22.64 – 25.03)	34.7 ± 0.2 (34.4 – 34.9)	21.0 ± 0.2 (20.7 – 21.1)	4.6 ± 0 (4.6)	6.3 ± 1.1 (5.3 – 7.7)	353 ± 3 (350 – 357)
	CV127	4	9.1 ± 0.1 (9.0 – 9.2)	23.06 ± 1.93 (20.27 – 24.48)	34.1 ± 0.4 (33.7 – 34.5)	21.3 ± 0.1 (21.2 – 21.3)	4.6 ± 0.1 (4.6 – 4.7)	7.9 ± 2.0 (6.3 – 10.7)	359 ± 8 (354 – 370)
	CV127 + imi	4	9.4 ± 0.1 (9.3 – 9.5)	22.53 ± 1.30 (21.37 – 24.38)	34.4 ± 0.4 (33.8 – 34.6)	21.4 ± 0.2 (21.2 – 21.5)	4.6 ± 0.1 (4.5 – 4.7)	7.8 ± 0.8 (6.6 – 8.4)	361 ± 6 (352 – 366)
	Std 1	1	9.9	21.30	32.8	20.5	4.7	10.8	359
	Std 2	1	9.4	24.81	34.1	22.5	4.6	4.6	358
Brasília	Isoline	4	10.1 ± 0.1 (9.9 – 10.2)	22.05 ± 1.60 (19.74 – 23.43)	36.7 ± 0.9 (35.7 – 37.9)	18.6 ± 0.5 (18.0 – 19.1)	4.7 ± 0.1 (4.6 – 4.7)	8.0 ± 2.1 (6.3 – 10.9)	346 ± 5 (342 – 352)
	CV127	4	9.1 ± 0.1* (9.0 – 9.2)	21.76 ± 1.47 (19.67 – 23.01)	36.9 ± 0.9 (35.8 – 38.1)	19.2 ± 0.6 (18.4 – 19.7)	4.6 ± 0.1 (4.5 – 4.6)	8.6 ± 1.4 (6.8 – 10.3)	355 ± 8 (345 – 364)
	CV127 + imi	4	9.0 ± 0.1* (8.9 – 9.2)	20.75 ± 0.46 (20.31 – 21.37)	37.1 ± 0.7 (36.4 – 38.0)	18.8 ± 0.7 (18.3 – 19.9)	4.6 ± 0 (4.6)	9.7 ± 1.2 (8.6 – 11.2)	357 ± 3 (354 – 361)
	Std 1	1	9.5	22.63	33.7	19.9	4.5	9.8	353
	Std 2	1	9.5	22.30	32.9	21.5	4.6	9.2	362
Santo Antônio de Posse	Isoline	4	10.3 ± 0.2 (10.0 – 10.4)	21.58 ± 1.21 (19.77 – 22.29)	36.1 ± 0.4 (35.7 – 36.6)	19.3 ± 0.3 (19.1 – 19.7)	4.7 ± 0.1 (4.5 – 4.8)	8.2 ± 1.1 (7.3 – 9.7)	351 ± 6 (347 – 360)
	CV127	4	10.1 ± 0.1 (9.9 – 10.2)	22.90 ± 0.37 (22.39 – 23.29)	34.7 ± 0.3 (34.5 – 35.1)	20.9 ± 0.9 (20.4 – 22.2)	4.7 ± 0.2 (4.6 – 4.9)	6.7 ± 1.1 (5.1 – 7.4)	354 ± 5 (350 – 360)
	CV127 + imi	4	9.6 ± 0.3* (9.3 – 9.9)	23.27 ± 0.73 (22.32 – 23.85)	34.4 ± 0.5 (33.8 – 34.9)	21.3 ± 0.6 (20.7 – 21.9)	4.8 ± 0.1 (4.7 – 4.9)	6.7 ± 0.8 (6.0 – 7.8)	357 ± 6 (351 – 364)
	Std 1	1	10.1	20.04	33.2	19.6	4.5	12.6	360
	Std 2	1	9.7	25.31	33.5	21.6	4.8	5.1	349

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-4. Proximate Composition of Grain on a Fresh-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location	Treatment	N	Moisture (g/100 g FW)	Total Dietary Fiber (g/100 g FW)	Protein (g/100 g FW)	Fat (g/100 g FW)	Ash (g/100 g FW)	Carbohydrates (g/100 g FW)	Calories (kcal/100 g FW)
			Mean ± Standard Deviation (range)						
Santo Antônio de Goiás	Isoline	4	7.7 ± 0.3 (7.6 – 8.1)	19.68 ± 1.96 (17.51 – 21.73)	37.8 ± 0.5 (37.2 – 38.2)	16.7 ± 0.3 (16.4 – 17.2)	4.8 ± 0.2 (4.7 – 5.0)	13.3 ± 1.8 (11.9 – 15.6)	355 ± 7 (346 – 363)
	CV127	4	8.1 ± 0.1* (8.0 – 8.2)	22.20 ± 0.66 (21.36 – 22.96)	37.8 ± 0.5 (37.0 – 38.1)	17.6 ± 0.4 (17.3 – 18.1)	4.9 ± 0.1 (4.8 – 4.9)	9.5 ± 0.6 (8.9 – 10.2)	325 ± 45 (258 – 351)
	CV127 + imi	4	8.1 ± 0.2* (7.9 – 8.2)	23.23 ± 1.54 (22.14 – 25.49)	37.6 ± 0.4 (37.1 – 38.0)	17.9 ± 0.2 (17.7 – 18.2)	4.8 ± 0.1 (4.7 – 4.8)	8.4 ± 1.5 (6.3 – 9.6)	345 ± 7 (335 – 350)
	Std 1	1	7.9	20.16	38.1	15.6	4.8	13.4	346
	Std 2	1	7.7	21.35	37.6	17.8	4.8	10.8	354
Teresina	Isoline	3	7.4 ± 0.2 (7.2 – 7.5)	22.55 ± 1.62 (20.78 – 23.95)	36.5 ± 1.1 (35.5 – 37.7)	21.7 ± 0.5 (21.2 – 22.0)	5.2 ± 0.1 (5.1 – 5.3)	6.7 ± 1.0 (5.8 – 7.8)	368 ± 5 (363 – 373)
	CV127	3	7.6 ± 0.2 (7.4 – 7.8)	22.98 ± 1.84 (20.92 – 24.45)	35.8 ± 1.3 (34.3 – 36.8)	22.3 ± 0.6 (21.7 – 22.9)	5.1 ± 0.2 (4.9 – 5.3)	6.2 ± 1.6 (4.9 – 8.0)	369 ± 8 (363 – 378)
	CV127 + imi	3	7.8 ± 0.2* (7.6 – 7.9)	24.08 ± 3.45 (20.09 – 26.19)	37.5 ± 0.9 (36.6 – 38.3)	20.9 ± 1.2 (19.9 – 22.3)	5.1 ± 0.2 (4.9 – 5.2)	4.7 ± 3.3 (2.6 – 8.5)	357 ± 21 (344 – 382)
	Std 1	1	7.5	22.02	36.9	20.7	5.1	7.8	365
	Std 2	1	7.0	25.36	34.7	23.3	5.1	4.5	367

Table D-4. continued.

Location	Treatment	N	Moisture (g/100 g FW)	Total Dietary Fiber (g/100 g FW)	Protein (g/100 g FW)	Fat (g/100 g FW)	Ash (g/100 g FW)	Carbohydrates (g/100 g FW)	Calories (kcal/100 g FW)
			Mean ± Standard Deviation (range)						
Vilhena	Isoline	4	7.4 ± 0.2 (7.1 – 7.5)	24.29 ± 1.47 (22.44 – 25.94)	36.6 ± 0.4 (36.2 – 36.9)	19.5 ± 0.2 (19.2 – 19.8)	4.7 ± 0.1 (4.7 – 4.8)	7.6 ± 1.4 (6.0 – 9.3)	352 ± 4 (347 – 357)
	CV127	4	7.8 ± 0.1* (7.7 – 7.9)	22.70 ± 0.79 (22.14 – 23.82)	36.1 ± 0.6 (35.5 – 36.8)	19.6 ± 0.4 (19.2 – 20.0)	4.8 ± 0.1 (4.7 – 4.8)	9.1 ± 0.9 (7.7 – 9.7)	357 ± 4 (351 – 361)
	CV127 + imi	4	7.8 ± 0* (7.8)	20.98 ± 1.47 (19.70 – 23.10)	37.0 ± 0.7 (36.2 – 37.6)	19.2 ± 0.4 (18.7 – 19.5)	4.7 ± 0.1 (4.6 – 4.7)	10.4 ± 1.3 (8.7 – 11.6)	363 ± 4 (356 – 365)
	Std 1	1	7.8	22.33	38.7	16.8	4.8	9.6	345
	Std 2	1	7.4	24.60	35.6	20.8	4.6	7.0	358
Brasília	Isoline	4	8.1 ± 0.1 (7.9 – 8.2)	24.12 ± 1.02 (23.3 – 25.61)	33.9 ± 0.4 (33.4 – 34.4)	17.5 ± 0.2 (17.3 – 17.7)	4.7 ± 0.2 (4.4 – 4.9)	11.8 ± 1.0 (10.6 – 12.9)	340 ± 6 (332 – 344)
	CV127	4	7.9 ± 0.1* (7.8 – 8.0)	23.34 ± 0.75 (22.72 – 24.43)	34.9 ± 0.5 (34.5 – 35.7)	17.5 ± 0.2 (17.3 – 17.7)	4.6 ± 0.2 (4.4 – 4.8)	11.8 ± 0.8 (10.7 – 12.6)	344 ± 4 (339 – 347)
	CV127 + imi	4	7.9 ± 0* (7.9)	22.90 ± 1.77 (21.09 – 25.30)	34.5 ± 0.6 (33.8 – 35.3)	17.9 ± 0.4 (17.4 – 18.2)	4.6 ± 0.2 (4.3 – 4.7)	12.3 ± 1.6 (10.0 – 13.7)	349 ± 7 (339 – 357)
	Std 1	1	8.2	23.90	33.8	18.1	4.5	11.5	344
	Std 2	1	8.0	24.70	34.3	18.7	4.7	9.6	344

*Statistically significantly different from the isoline control at $P \leq 0.05$.

Table D-5. Proximate Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location	Treatment	N	Total Dietary Fiber (g/100 g DW)	Protein (g/100 g DW)	Fat (g/100 g DW)	Ash (g/100 g DW)	Carbohydrates (g/100 g DW)	Calories (kcal/100 g DW)
			Mean ± Standard Deviation (range)					
Santo Antônio de Goiás	Isoline	4	24.65 ± 1.22 (23.75 – 26.34)	39.8 ± 1.3 (39.1 – 41.8)	21.8 ± 0.2 (21.5 – 22.0)	4.9 ± 0.2 (4.7 – 5.0)	9.0 ± 1.4 (7.7 – 10.6)	392 ± 6 (383 – 397)
	CV127	4	25.00 ± 0.92 (24.07 – 26.15)	39.5 ± 0.4 (39.2 – 40.1)	23.3 ± 0.2* (23.2 – 23.6)	4.8 ± 0.2 (4.6 – 5.0)	7.4 ± 1.1 (6.0 – 8.7)	398 ± 4 (393 – 401)
	CV127 + imi	4	26.08 ± 2.53* (22.40 – 28.11)	40.5 ± 0.3 (40.1 – 40.8)	22.7 ± 0.5* (22.0 – 23.1)	4.7 ± 0.1* (4.6 – 4.8)	6.1 ± 2.5 (3.4 – 8.8)	391 ± 10 (384 – 405)
	Std 1	1	26.02	39.6	20.2	4.9	9.3	377
	Std 2	1	25.34	37.2	23.5	4.7	9.3	398
Uberaba	Isoline	4	23.79 ± 1.87 (22.18 – 26.28)	41.4 ± 0.9 (40.1 – 42.1)	21.3 ± 1.1 (20.5 – 22.9)	4.8 ± 0.1 (4.6 – 4.8)	8.8 ± 1.8 (6.6 – 10.6)	392 ± 9 (379 – 399)
	CV127	4	23.66 ± 1.53 (22.07 – 25.08)	39.4 ± 0.3 (38.9 – 39.6)	22.3 ± 0.5 (21.8 – 22.9)	4.7 ± 0.1 (4.6 – 4.7)	10.0 ± 1.9 (8.0 – 12.0)	398 ± 4 (394 – 402)
	CV127 + imi	4	24.20 ± 1.59* (22.13 – 25.87)	39.9 ± 0.1* (39.7 – 40.0)	22.6 ± 0.4* (22.0 – 23.0)	4.6 ± 0.1 (4.5 – 4.7)	8.7 ± 1.8 (6.6 – 11.1)	398 ± 7 (392 – 406)
	Std 1	1	24.91	38.6	21.1	4.5	10.9	388
	Std 2	1	26.75	38.7	24.1	4.5	5.9	395
Sete Lagoas	Isoline	4	24.02 ± 1.56 (22.49 – 26.19)	41.4 ± 0.2 (41.1 – 41.5)	21.7 ± 0.2 (21.5 – 21.8)	4.9 ± 0.1 (4.8 – 4.9)	8.1 ± 1.8 (5.6 – 9.9)	393 ± 6 (385 – 398)
	CV127	4	23.87 ± 1.07 (22.84 – 25.18)	39.9 ± 0.3 (39.6 – 40.1)	22.5 ± 0.4* (22.0 – 23.0)	4.9 ± 0.2 (4.7 – 5.1)	8.8 ± 1.1 (7.5 – 10.0)	397 ± 5 (392 – 403)
	CV127 + imi	4	24.57 ± 1.61* (23.01 – 26.07)	39.5 ± 0.3* (39.1 – 39.8)	23.2 ± 0.3* (22.9 – 23.6)	4.9 ± 0.1 (4.9 – 5.0)	7.9 ± 1.5 (6.6 – 9.5)	398 ± 6 (391 – 403)
	Std 1	1	25.84	37.7	21.5	4.9	10.1	384
	Std 2	1	25.28	37.4	24.1	4.8	8.5	400

Table D-5. continued.

Location	Treatment	N	Total Dietary Fiber (g/100 g DW)	Protein (g/100 g DW)	Fat (g/100 g DW)	Ash (g/100 g DW)	Carbohydrates (g/100 g DW)	Calories (kcal/100 g DW)
			Mean ± Standard Deviation (range)					
Londrina	Isoline	4	26.53 ± 1.06 (25.07 – 27.57)	38.3 ± 0.2 (38.1 – 38.5)	23.1 ± 0.2 (22.8 – 23.3)	5.1 ± 0 (5.1)	7.0 ± 1.2 (5.8 – 8.5)	389 ± 4 (385 – 395)
	CV127	4	25.37 ± 2.11 (22.32 – 26.90)	37.5 ± 0.4 (37.0 – 38.0)	23.4 ± 0.1 (23.3 – 23.5)	5.1 ± 0.1 (5.1 – 5.2)	8.7 ± 2.2 (6.9 – 11.8)	395 ± 8 (389 – 407)
	CV127 + imi	4	24.85 ± 1.45 (23.58 – 26.94)	37.9 ± 0.4 (37.3 – 38.2)	23.6 ± 0.2* (23.4 – 23.7)	5.1 ± 0.1 (5.0 – 5.2)	8.6 ± 0.9 (7.3 – 9.3)	398 ± 6 (389 – 404)
	Std 1	1	23.63	36.4	22.8	5.2	12.0	398
	Std 2	1	27.38	37.6	24.8	5.1	5.1	395
Brasília	Isoline	4	24.51 ± 1.79 (21.93 – 26.06)	40.8 ± 1.1 (39.6 – 42.2)	20.7 ± 0.6 (20.0 – 21.2)	5.2 ± 0.1 (5.1 – 5.2)	8.9 ± 2.4 (7.0 – 12.1)	385 ± 5 (381 – 391)
	CV127	4	23.92 ± 1.64 (21.61 – 25.34)	40.6 ± 1.1 (39.3 – 42.0)	21.1 ± 0.6 (20.3 – 21.6)	5.0 ± 0.1 (4.9 – 5.1)	9.5 ± 1.6 (7.5 – 11.4)	390 ± 8 (380 – 400)
	CV127 + imi	4	22.80 ± 0.51 (22.29 – 23.46)	40.8 ± 0.9 (40.0 – 41.9)	20.7 ± 0.8 (20.1 – 21.8)	5.1 ± 0.1 (5.0 – 5.1)	10.7 ± 1.3 (9.4 – 12.3)	393 ± 3 (390 – 396)
	Std 1	1	25.00	37.2	22.0	5.0	10.8	391
	Std 2	1	24.64	36.4	23.8	5.1	10.2	400
Santo Antônio de Posse	Isoline	4	24.04 ± 1.32 (22.06 – 24.78)	40.2 ± 0.4 (39.8 – 40.8)	21.5 ± 0.4 (21.2 – 22.0)	5.2 ± 0.1 (5.0 – 5.3)	9.1 ± 1.2 (8.1 – 10.9)	391 ± 8 (385 – 402)
	CV127	4	25.46 ± 0.42 (24.90 – 25.93)	38.6 ± 0.3 (38.4 – 39.0)	23.2 ± 0.9* (22.7 – 24.6)	5.3 ± 0.2 (5.1 – 5.5)	7.5 ± 1.3 (5.6 – 8.3)	394 ± 5 (390 – 400)
	CV127 + imi	4	25.72 ± 0.86* (24.60 – 26.46)	38.0 ± 0.6* (37.4 – 38.5)	23.6 ± 0.7* (22.8 – 24.2)	5.3 ± 0.1 (5.2 – 5.4)	7.4 ± 0.9 (6.6 – 8.6)	394 ± 6 (388 – 401)
	Std 1	1	22.29	36.9	21.8	5.0	14.0	400
	Std 2	1	28.03	37.1	23.9	5.3	5.6	386

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-6. Proximate Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location	Treatment	N	Total Dietary Fiber (g/100 g DW)	Protein (g/100 g DW)	Fat (g/100 g DW)	Ash (g/100 g DW)	Carbohydrates (g/100 g DW)	Calories (kcal/100 g DW)
			Mean ± Standard Deviation (range)					
Santo Antônio de Goiás	Isoline	4	21.33 ± 2.11 (18.94 – 23.52)	41.0 ± 0.6 (40.3 – 41.6)	18.1 ± 0.4 (17.7 – 18.6)	5.2 ± 0.2 (5.1 – 5.4)	14.5 ± 1.9 (12.9 – 16.9)	385 ± 9 (374 – 393)
	CV127	4	24.14 ± 0.70* (23.26 – 24.95)	41.1 ± 0.6 (40.2 – 41.5)	19.1 ± 0.4* (18.8 – 19.7)	5.3 ± 0.1 (5.2 – 5.3)	10.3 ± 0.8* (9.3 – 11.1)	354 ± 48 (281 – 382)
	CV127 + imi	4	25.27 ± 1.69* (24.12 – 27.76)	40.9 ± 0.4 (40.4 – 41.3)	19.5 ± 0.2* (19.3 – 19.8)	5.2 ± 0.1 (5.1 – 5.2)	9.2 ± 1.6* (6.9 – 10.5)	375 ± 7 (365 – 381)
	Std 1	1	21.88	41.4	16.9	5.2	14.6	377
	Std 2	1	23.13	40.7	19.3	5.2	11.7	384
Teresina	Isoline	3	24.34 ± 1.75 (22.44 – 25.89)	39.4 ± 1.2 (38.4 – 40.7)	23.5 ± 0.5 (22.9 – 23.8)	5.6 ± 0.1 (5.5 – 5.7)	7.2 ± 1.1 (6.3 – 8.4)	398 ± 6 (392 – 403)
	CV127	3	24.86 ± 1.99 (22.62 – 26.40)	38.7 ± 1.5 (37.0 – 39.9)	24.1 ± 0.6 (23.5 – 24.7)	5.5 ± 0.2 (5.3 – 5.7)	6.7 ± 1.7 (5.3 – 8.6)	399 ± 8 (394 – 409)
	CV127 + imi	3	26.12 ± 3.79 (21.74 – 28.43)	40.6 ± 1.0 (39.6 – 41.6)	22.7 ± 1.3 (21.6 – 24.1)	5.5 ± 0.2 (5.3 – 5.6)	5.0 ± 3.6 (2.8 – 9.2)	388 ± 22 (376 – 374)
	Std 1	1	23.80	39.9	22.4	5.5	8.4	394
	Std 2	1	27.26	37.3	25.1	5.5	4.8	395

Table D-6. continued.

Location	Treatment	N	Total Dietary Fiber (g/100 g DW)	Protein (g/100 g DW)	Fat (g/100 g DW)	Ash (g/100 g DW)	Carbohydrates (g/100 g DW)	Calories (kcal/100 g DW)
			Mean ± Standard Deviation (range)					
Vilhena	Isoline	4	26.21 ± 1.56 (24.26 – 28.01)	39.5 ± 0.4 (39.1 – 39.9)	21.1 ± 0.2 (20.8 – 21.4)	5.1 ± 0.1 (5.1 – 5.2)	8.2 ± 1.5 (6.5 – 10.1)	380 ± 5 (378 – 386)
	CV127	4	24.61 ± 0.84 (24.01 – 25.80)	39.2 ± 0.7 (38.5 – 39.9)	21.3 ± 0.4 (20.8 – 21.7)	5.2 ± 0.1 (5.1 – 5.2)	9.9 ± 1.0 (8.3 – 10.5)	387 ± 5 (380 – 391)
	CV127 + imi	4	22.76 ± 1.59* (21.37 – 25.05)	40.1 ± 0.8 (39.3 – 40.8)	20.8 ± 0.4 (20.3 – 21.1)	5.1 ± 0.1 (5.0 – 5.1)	11.3 ± 1.4* (9.4 – 12.6)	393 ± 5* (386 – 396)
	Std 1	1	24.22	42.0	18.2	5.2	10.4	374
	Std 2	1	26.57	38.4	22.5	5.0	7.6	387
Brasília	Isoline	4	26.23 ± 1.14 (25.29 – 27.89)	36.9 ± 0.5 (36.4 – 37.4)	19.0 ± 0.2 (18.8 – 19.2)	5.1 ± 0.2 (4.8 – 5.3)	12.8 ± 1.0 (11.5 – 14.0)	370 ± 6 (362 – 374)
	CV127	4	25.34 ± 0.83 (24.66 – 26.55)	37.9 ± 0.6* (37.4 – 38.8)	19.0 ± 0.2 (18.8 – 19.2)	5.0 ± 0.2 (4.8 – 5.2)	12.7 ± 0.8 (11.6 – 13.4)	374 ± 4 (368 – 376)
	CV127 + imi	4	24.86 ± 1.92 (22.90 – 27.46)	37.4 ± 0.7 (36.7 – 38.3)	19.5 ± 0.4* (18.9 – 19.8)	5.0 ± 0.2 (4.7 – 5.1)	13.4 ± 1.7 (10.9 – 14.9)	379 ± 8 (368 – 388)
	Std 1	1	26.03	36.8	19.7	4.9	12.5	375
	Std 2	1	26.84	37.3	20.3	5.1	10.4	374

*Statistically significantly different from the isoline control at $P \leq 0.05$.

Table D-7. Fiber Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location	Treatment	N	Crude Fiber	Acid Detergent Fiber	Neutral Detergent Fiber
			Mean ± Standard Deviation (range) g/100 g dry weight		
Santo Antônio de Goiás	Isoline	4	9.6 ± 1.2 (8.1 – 11.0)	10.71 ± 1.37 (9.07 – 12.08)	14.82 ± 0.94 (13.95 – 16.10)
	CV127	4	7.3 ± 0.3* (6.9 – 7.6)	10.90 ± 0.94 (9.75 – 11.79)	13.97 ± 0.97 (12.72 – 15.07)
	CV127 + imi	4	7.0 ± 0.2* (6.8 – 7.2)	13.11 ± 1.25* (11.73 – 14.76)	16.91 ± 1.91* (14.06 – 18.12)
	Std 1	1	8.4	11.51	14.82
	Std 2	1	9.0	12.14	16.36
Uberaba	Isoline	4	8.3 ± 0.3 (7.9 – 8.6)	12.06 ± 2.44 (9.27 – 14.95)	15.12 ± 1.14 (13.57 – 16.16)
	CV127	4	8.7 ± 1.3 (7.5 – 10.5)	13.77 ± 1.41 (12.08 – 15.20)	17.50 ± 1.27* (15.61 – 18.31)
	CV127 + imi	4	10.2 ± 1.2* (9.0 – 11.3)	12.34 ± 1.30 (10.96 – 14.06)	16.76 ± 1.60* (15.07 – 18.15)
	Std 1	1	8.5	9.32	11.71
	Std 2	1	9.3	10.64	10.63
Sete Lagoas	Isoline	4	8.7 ± 0.2 (8.5 – 8.9)	10.22 ± 1.05 (8.84 – 11.20)	13.60 ± 1.05 (12.04 – 14.26)
	CV127	4	7.8 ± 0.6 (7.2 – 8.4)	14.40 ± 0.43* (14.15 – 15.04)	18.24 ± 1.09* (16.84 – 19.26)
	CV127 + imi	4	7.2 ± 0.6 (6.4 – 7.9)	13.69 ± 1.49* (12.07 – 15.62)	18.21 ± 1.30* (17.01 – 19.96)
	Std 1	1	7.6	12.74	16.57
	Std 2	1	8.4	10.96	12.72
Londrina	Isoline	4	7.8 ± 0.6 (7.3 – 8.6)	11.70 ± 0.58 (11.18 – 12.51)	14.27 ± 0.52 (13.76 – 14.81)
	CV127	4	9.8 ± 1.3* (8.0 – 11.1)	12.68 ± 0.97 (11.41 – 13.59)	17.33 ± 1.77* (15.25 – 19.34)
	CV127 + imi	4	7.9 ± 1.3 (6.8 – 9.7)	14.24 ± 1.25 (12.42 – 15.20)	18.01 ± 1.04* (16.73 – 19.15)
	Std 1	1	7.7	11.69	14.36
	Std 2	1	7.6	11.45	13.11
Brasília	Isoline	4	8.3 ± 1.4 (6.9 – 10.2)	11.66 ± 0.38 (11.10 – 11.94)	16.31 ± 1.10 (15.05 – 17.32)
	CV127	4	8.9 ± 1.0 (7.6 – 9.9)	12.94 ± 0.65 (12.28 – 13.81)	19.60 ± 0.97* (18.27 – 20.55)
	CV127 + imi	4	8.5 ± 0.7 (7.8 – 9.2)	14.86 ± 2.84 (12.51 – 19.00)	16.89 ± 1.45 (15.59 – 18.61)
	Std 1	1	9.2	11.75	16.71
	Std 2	1	6.7	12.59	17.73
Santo Antônio de Posse	Isoline	4	8.4 ± 0.3 (8.0 – 8.7)	12.03 ± 1.42 (11.05 – 14.11)	15.77 ± 0.83 (14.91 – 16.73)
	CV127	4	7.5 ± 0.4 (7.1 – 8.0)	14.12 ± 1.48 (12.51 – 15.92)	18.11 ± 1.50* (16.48 – 20.05)
	CV127 + imi	4	7.7 ± 0.5 (7.0 – 8.2)	14.32 ± 1.53 (12.54 – 16.13)	18.32 ± 1.16* (17.49 – 20.01)
	Std 1	1	8.1	14.43	16.92
	Std 2	1	8.0	11.88	16.51

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-8. Fiber Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location	Treatment	N	Crude Fiber	Acid Detergent Fiber	Neutral Detergent Fiber
			Mean ± Standard Deviation (range) g/100 g dry weight		
Santo Antônio de Goiás	Isoline	4	8.1 ± 1.7 (6.9 – 10.6)	9.63 ± 0.95 (8.53 – 10.73)	14.84 ± 0.85 (13.58 – 15.44)
	CV127	4	7.6 ± 0.5 (7.1 – 8.3)	10.21 ± 0.56 (9.61 – 10.93)	14.66 ± 0.55 (14.02 – 15.35)
	CV127 + imi	4	9.1 ± 3.7 (6.8 – 14.7)	11.01 ± 0.73* (9.96 – 11.65)	16.17 ± 0.83* (15.17 – 16.98)
	Std 1	1	12.1	11.73	15.23
	Std 2	1	7.2	11.61	16.18
Teresina	Isoline	3	7.6 ± 0.8 (6.7 – 8.1)	10.29 ± 0.59 (9.61 – 10.70)	15.10 ± 0.97 (14.03 – 15.93)
	CV127	3	8.9 ± 0.7* (8.5 – 9.7)	10.58 ± 0.66 (9.88 – 11.19)	15.68 ± 1.07 (15.04 – 16.91)
	CV127 + imi	3	9.0 ± 0.1* (8.9 – 9.1)	13.00 ± 0.52* (12.41 – 13.40)	16.75 ± 1.64 (14.96 – 18.17)
	Std 1	1	7.8	9.94	15.59
	Std 2	1	7.4	12.08	12.26
Vilhena	Isoline	4	7.6 ± 1.0 (6.7 – 8.5)	10.94 ± 1.21 (9.26 – 12.05)	12.32 ± 1.80 (11.32 – 15.02)
	CV127	4	7.8 ± 0.4 (7.3 – 8.3)	8.92 ± 0.90* (8.09 – 9.78)	12.67 ± 0.63 (11.86 – 13.32)
	CV127 + imi	4	7.4 ± 0.9 (6.2 – 8.1)	8.57 ± 1.06* (7.35 – 9.81)	13.16 ± 0.49 (12.53 – 13.66)
	Std 1	1	8.4	8.89	12.72
	Std 2	1	7.6	12.59	12.70
Brasília	Isoline	4	8.1 ± 0.8 (7.2 – 8.9)	10.14 ± 1.23 (8.73 – 11.70)	14.30 ± 1.19 (12.67 – 15.50)
	CV127	4	7.8 ± 0.7 (7.2 – 8.5)	9.16 ± 0.63 (8.37 – 9.86)	14.32 ± 0.55 (13.73 – 15.05)
	CV127 + imi	4	7.3 ± 0.4 (6.7 – 7.6)	10.03 ± 0.48 (9.44 – 10.57)	14.85 ± 1.36 (13.43 – 16.68)
	Std 1	1	7.7	10.84	15.00
	Std 2	1	7.3	11.22	13.23

*Statistically significantly different from the isoline control at $P \leq 0.05$.

Table D-9. Amino Acid Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location/ Treatment	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
Santo Antônio de Goiás	Mean ± Standard Deviation (range) g/100 g dry weight								
Isoline	4.53 ± 0.33 (4.25 – 5.00)	1.55 ± 0.09 (1.46 – 1.68)	2.07 ± 0.13 (1.93 – 2.25)	7.47 ± 0.48 (7.20 – 8.19)	2.06 ± 0.10 (1.92 – 2.16)	1.59 ± 0.11 (1.49 – 1.74)	1.62 ± 0.08 (1.57 – 1.74)	0.53 ± 0.01 (0.51 – 0.53)	1.59 ± 0.17 (1.36 – 1.76)
CV127	4.50 ± 0.13 (4.33 – 4.64)	1.53 ± 0.04 (1.47 – 1.56)	2.04 ± 0.05 (1.97 – 2.09)	7.51 ± 0.29 (7.10 – 7.79)	1.91 ± 0.06 (1.82 – 1.95)	1.63 ± 0.05 (1.56 – 1.68)	1.58 ± 0.05 (1.52 – 1.63)	0.53 ± 0.01 (0.52 – 0.54)	1.60 ± 0.11 (1.44 – 1.70)
CV127 + imi	4.85 ± 0.34 (4.36 – 5.12)	1.63 ± 0.11 (1.47 – 1.72)	2.17 ± 0.14 (1.95 – 2.26)	8.01 ± 0.54 (7.22 – 8.42)	2.02 ± 0.13 (1.83– 2.11)	1.72 ± 0.10 (1.57 – 1.79)	1.67 ± 0.09 (1.54 – 1.74)	0.53 ± 0.01 (0.52 – 0.54)	1.68 ± 0.10 (1.53 – 1.74)
Std 1	4.72	1.55	2.16	7.68	1.99	1.71	1.67	0.54	1.63
Std 2	4.13	1.40	1.90	6.58	1.78	1.46	1.50	0.52	1.50
Uberaba									
Isoline	5.02 ± 0.16 (4.79 – 5.16)	1.63 ± 0.03 (1.60 – 1.66)	2.28 ± 0.08 (2.17 – 2.34)	8.17 ± 0.37 (7.63 – 8.46)	2.12 ± 0.06 (2.04 – 2.17)	1.78 ± 0.09 (1.65 – 1.86)	1.75 ± 0.05 (1.68 – 1.78)	0.54 ± 0.01 (0.53 – 0.56)	1.76 ± 0.04 (1.70 – 1.79)
CV127	4.36 ± 0.45* (3.70 – 4.66)	1.46 ± 0.14 (1.25 – 1.56)	1.98 ± 0.19* (1.71 – 2.12)	7.17 ± 0.73* (6.17 – 7.82)	1.81 ± 0.17* (1.65 – 1.96)	1.57 ± 0.14* (1.37 – 1.67)	1.56 ± 0.14* (1.36 – 1.67)	0.50 ± 0.01* (0.48 – 0.51)	1.59 ± 0.13 (1.42 – 1.71)
CV127 + imi	4.28 ± 0.37* (3.83 – 4.61)	1.46 ± 0.14 (1.30 – 1.61)	1.96 ± 0.19* (1.76 – 2.16)	7.01 ± 0.64* (6.20 – 7.56)	1.87 ± 0.13* (1.67 – 1.94)	1.55 ± 0.14* (1.39 – 1.71)	1.53 ± 0.14* (1.37 – 1.68)	0.51 ± 0.01* (0.50 – 0.52)	1.57 ± 0.16 (1.41 – 1.74)
Std 1	4.44	1.50	2.05	7.24	1.87	1.66	1.62	0.51	1.59
Std 2	4.64	1.52	2.14	7.52	1.97	1.68	1.68	0.49	1.67
Sete Lagoas									
Isoline	4.75 ± 0.61 (4.02 – 5.37)	1.59 ± 0.21 (1.34 – 1.80)	2.12 ± 0.24 (1.83 – 2.39)	7.77 ± 1.02 (6.59 – 8.92)	2.01 ± 0.24 (1.74 – 2.32)	1.66 ± 0.18 (1.45 – 1.87)	1.69 ± 0.20 (1.45 – 1.92)	0.52 ± 0.01 (0.51 – 0.53)	1.73 ± 0.22 (1.46 – 1.98)
CV127	4.44 ± 0.22 (4.20 – 4.65)	1.48 ± 0.07 (1.38 – 1.54)	1.96 ± 0.08 (1.87 – 2.03)	7.33 ± 0.31 (6.95 – 7.60)	1.91 ± 0.08 (1.85 – 2.01)	1.60 ± 0.06 (1.52 – 1.67)	1.59 ± 0.08 (1.48 – 1.66)	0.51 ± 0.02 (0.50 – 0.54)	1.59 ± 0.08 (1.47 – 1.66)
CV127 + imi	4.86 ± 0.33 (4.43 – 5.17)	1.62 ± 0.07 (1.57 – 1.70)	2.15 ± 0.12 (2.02 – 2.29)	8.04 ± 0.42 (7.53 – 8.45)	2.04 ± 0.09 (1.90 – 2.12)	1.72 ± 0.07 (1.64 – 1.81)	1.71 ± 0.04 (1.67 – 1.76)	0.51 ± 0.01 (0.50 – 0.52)	1.73 ± 0.05 (1.68 – 1.79)
Std 1	4.33	1.51	1.96	7.05	1.84	1.56	1.61	0.53	1.64
Std 2	4.14	1.43	1.90	6.83	1.77	1.50	1.52	0.50	1.63

Table D-9. continued.

Location/ Treatment	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
Londrina	Mean ± Standard Deviation (range) g/100 g dry weight								
Isoline	4.42 ± 0.09 (4.32 – 4.50)	1.53 ± 0.05 (1.48 – 1.59)	2.01 ± 0.06 (1.95 – 2.08)	7.35 ± 0.20 (7.13 – 7.59)	1.87 ± 0.04 (1.84 – 1.93)	1.60 ± 0.05 (1.55 – 1.66)	1.57 ± 0.04 (1.52 – 1.62)	0.54 ± 0 (0.54)	1.59 ± 0.04 (1.55 – 1.63)
CV127	4.23 ± 0.40 (3.67 – 4.52)	1.49 ± 0.15 (1.26 – 1.58)	1.97 ± 0.20 (1.70 – 2.18)	7.01 ± 0.66 (6.10 – 7.57)	1.82 ± 0.17 (1.56 – 1.93)	1.57 ± 0.16 (1.33 – 1.69)	1.55 ± 0.16 (1.32 – 1.64)	0.52 ± 0.01* (0.51 – 0.53)	1.59 ± 0.17 (1.34 – 1.68)
CV127 + imi	4.50 ± 0.15 (4.36 – 4.72)	1.55 ± 0.01 (1.54 – 1.56)	2.07 ± 0.07 (2.01 – 2.16)	7.48 ± 0.27 (7.19 – 7.85)	1.92 ± 0.05 (1.86 – 1.99)	1.65 ± 0.05 (1.60 – 1.72)	1.65 ± 0.04 (1.61 – 1.70)	0.52 ± 0.01* (0.52 – 0.54)	1.66 ± 0.05 (1.60 – 1.72)
Std 1	4.27	1.52	1.92	6.98	1.76	1.56	1.57	0.53	1.58
Std 2	4.43	1.51	2.00	7.25	1.86	1.58	1.59	0.52	1.61
Brasília									
Isoline	4.61 ± 0.20 (4.44 – 4.86)	1.55 ± 0.07 (1.48 – 1.63)	2.04 ± 0.08 (1.95 – 2.13)	7.51 ± 0.34 (7.21 – 7.92)	1.89 ± 0.10 (1.78 – 2.00)	1.63 ± 0.08 (1.54 – 1.71)	1.62 ± 0.07 (1.56 – 1.70)	0.57 ± 0.01 (0.55 – 0.58)	1.65 ± 0.05 (1.59 – 1.70)
CV127	4.51 ± 0.10 (4.42 – 4.65)	1.50 ± 0.02 (1.48 – 1.51)	2.02 ± 0.02 (1.99 – 2.04)	7.29 ± 0.16 (7.10 – 7.47)	1.74 ± 0.03 (1.69 – 1.77)	1.63 ± 0.02 (1.61 - 1.66)	1.59 ± 0.03 (1.56 – 1.63)	0.55 ± 0.01* (0.54 – 0.56)	1.56 ± 0.04 (1.52 – 1.61)
CV127 + imi	4.64 ± 0.10 (4.50 – 4.74)	1.54 ± 0.05 (1.49 – 1.60)	2.09 ± 0.05 (2.02 – 2.14)	7.49 ± 0.16 (7.30 – 7.69)	1.80 ± 0.05 (1.76 – 1.86)	1.68 ± 0.03 (1.64 – 1.71)	1.63 ± 0.04 (1.57 – 1.66)	0.55 ± 0.01* (0.54 – 0.56)	1.64 ± 0.06 (1.56 – 1.71)
Std 1	4.26	1.49	1.88	6.93	1.76	1.55	1.55	0.53	1.58
Std 2	3.94	1.36	1.78	6.16	1.58	1.41	1.39	0.56	1.40
Santo Antônio de Posse									
Isoline	4.54 ± 0.28 (4.32 – 4.94)	1.52 ± 0.07 (1.47 – 1.63)	2.02 ± 0.13 (1.92 – 2.20)	7.39 ± 0.42 (7.02 – 7.99)	1.94 ± 0.23 (1.81 – 2.28)	1.64 ± 0.11 (1.57 – 1.81)	1.62 ± 0.12 (1.54 – 1.79)	0.56 ± 0.01 (0.55 – 0.58)	1.64 ± 0.11 (1.54 – 1.79)
CV127	4.74 ± 0.51 (4.14 – 5.24)	1.54 ± 0.26 (1.27 – 1.70)	2.17 ± 0.27 (1.79 – 2.41)	7.52 ± 1.06 (6.33 – 8.46)	2.01 ± 0.28 (1.70 – 2.31)	1.70 ± 0.24 (1.45 – 1.94)	1.69 ± 0.21 (1.48 – 1.89)	0.53 ± 0.01* (0.52 – 0.53)	1.69 ± 0.27 (1.42 – 1.94)
CV127 + imi	4.54 ± 0.59 (3.66 – 4.91)	1.53 ± 0.19 (1.25 – 1.67)	2.01± 0.26 (1.63 – 2.19)	7.21 ± 0.96 (5.83 – 8.00)	1.86 ± 0.27 (1.49 – 2.11)	1.60 ± 0.20 (1.32 – 1.74)	1.58 ± 0.22 (1.27 – 1.73)	0.53 ± 0.01* (0.52 – 0.54)	1.58 ± 0.22 (1.26 – 1.76)
Std 1	4.17	1.46	1.88	6.66	1.64	1.50	1.50	0.53	1.46
Std 2	4.32	1.51	1.94	6.93	2.05	1.51	1.56	0.53	1.64

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-9. continued.

Location/ Treatment	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
Santo Antônio de Goiás	Mean ± Standard Deviation (range) g/100 g dry weight								
Isoline	0.60 ± 0.01 (0.60 – 0.61)	1.57 ± 0.15 (1.42 – 1.77)	2.86 ± 0.20 (2.68 – 3.14)	1.33 ± 0.10 (1.25 – 1.47)	2.02 ± 0.12 (1.92 – 2.20)	2.43 ± 0.16 (2.24 – 2.64)	0.88 ± 0.07 (0.79 – 0.96)	2.91 ± 0.30 (2.64 – 3.34)	0.77 ± 0.12 (0.65 – 0.89)
CV127	0.60 ± 0.01 (0.59 – 0.61)	1.57 ± 0.08 (1.46 – 1.63)	2.87 ± 0.14 (2.66 – 2.97)	1.31 ± 0.05 (1.25 – 1.36)	1.95 ± 0.10 (1.80 – 2.01)	2.45 ± 0.10 (2.30 – 2.52)	0.88 ± 0.02 (0.86 – 0.91)	2.93 ± 0.13 (2.75 – 3.04)	0.78 ± 0.08 (0.69 – 0.86)
CV127 + imi	0.61 ± 0.01 (0.60 – 0.62)	1.67 ± 0.13 (1.48 – 1.75)	3.02 ± 0.21 (2.70 – 3.13)	1.36 ± 0.09 (1.23 – 1.42)	2.07 ± 0.15 (1.86 – 2.19)	2.57 ± 0.18 (2.30 – 2.68)	0.88 ± 0.07 (0.78 – 0.94)	3.16 ± 0.16 (2.92 – 3.29)	0.79 ± 0.14 (0.61 – 0.94)
Std 1	0.61	1.64	2.97	1.39	2.04	2.54	0.84	2.97	0.82
Std 2	0.55	1.50	3.65	1.20	1.83	2.22	0.79	2.57	0.70
Uberaba									
Isoline	0.65 ± 0.02 (0.63 – 0.67)	1.76 ± 0.06 (1.67 – 1.80)	3.18 ± 0.10 (3.03 – 3.22)	1.46 ± 0.04 (1.40 – 1.49)	2.18 ± 0.06 (2.09 – 2.23)	2.70 ± 0.10 (2.55 – 2.78)	0.94 ± 0.02 (0.92 – 0.96)	3.28 ± 0.13 (3.10 – 3.35)	0.74 ± 0.12 (0.59 – 0.87)
CV127	0.60 ± 0.02* (0.59 – 0.63)	1.50 ± 0.13* (1.31 – 1.58)	2.71 ± 0.24* (2.37 – 2.88)	1.25 ± 0.12* (1.11 – 1.36)	1.87 ± 0.17* (1.64 – 2.00)	2.33 ± 0.19* (2.08 – 2.49)	0.81 ± 0.05* (0.74 – 0.87)	2.82 ± 0.20* (2.57 – 2.98)	0.69 ± 0.02 (0.67 – 0.71)
CV127 + imi	0.62 ± 0.02* (0.59 – 0.64)	1.52 ± 0.13* (1.38 – 1.68)	2.71 ± 0.24* (2.48 – 2.98)	1.23 ± 0.11* (1.11 – 1.33)	1.88 ± 0.16* (1.71 – 2.07)	2.36 ± 0.19* (2.14 – 2.57)	0.83 ± 0.09* (0.75 – 0.95)	2.87 ± 0.24* (2.62 – 3.15)	0.79 ± 0.16 (0.67 – 1.03)
Std 1	0.60	1.58	2.83	1.35	1.96	2.46	0.81	2.82	0.57
Std 2	0.55	1.71	3.03	1.38	2.09	2.54	0.86	2.94	0.82
Sete Lagoas									
Isoline	0.64 ± 0.01 (0.63 – 0.65)	1.67 ± 0.22 (1.43 – 1.94)	2.96 ± 0.38 (2.51 – 3.09)	1.35 ± 0.16 (1.17 – 1.55)	2.06 ± 0.26 (1.77 – 2.37)	2.50 ± 0.32 (2.12 – 2.85)	0.90 ± 0.10 (0.79 – 1.03)	3.19 ± 0.42 (2.75 – 3.69)	0.75 ± 0.08 (0.65 – 0.83)
CV127	0.63 ± 0.03 (0.61 – 0.68)	1.56 ± 0.06 (1.55 – 1.64)	2.75 ± 0.13 (2.57 – 2.87)	1.23 ± 0.06 (1.15 – 1.28)	1.92 ± 0.10 (1.78 – 2.01)	2.40 ± 0.11 (2.27 – 2.53)	0.84 ± 0.06 (0.79 – 0.91)	3.02 ± 0.21 (2.76 – 3.23)	0.75 ± 0.09 (0.63 – 0.83)
CV127 + imi	0.63 ± 0.01 (0.62 – 0.63)	1.69 ± 0.05 (1.63 – 1.74)	3.02 ± 0.13 (2.87 – 3.16)	1.35 ± 0.04 (1.32 – 1.39)	2.09 ± 0.08 (1.98 – 2.16)	2.57 ± 0.08 (2.46 – 2.65)	0.89 ± 0.04 (0.86 – 0.94	3.23 ± 0.10 (3.10 – 3.33)	0.79 ± 0.16 (0.70 – 1.02)
Std 1	0.65	1.60	2.75	1.32	1.92	2.38	0.82	2.86	0.94
Std 2	0.59	1.55	2.73	1.20	1.88	2.31	0.80	2.74	0.77

Table D-9. continued.

Location/ Treatment	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
Londrina	Mean ± Standard Deviation (range) g/100 g dry weight								
Isoline	0.64 ± 0.01 (0.63 – 0.64)	1.52 ± 0.05 (1.48 – 1.59)	2.72 ± 0.08 (2.64 – 2.82)	1.28 ± 0.03 (1.25 – 1.32)	1.85 ± 0.05 (1.79 – 1.92)	2.39 ± 0.05 (2.33 – 2.44)	0.80 ± 0.01 (0.79 – 0.81)	2.80 ± 0.08 (2.69 – 2.89)	0.72 ± 0.09 (0.63 – 0.83)
CV127	0.62 ± 0.01* (0.60 – 0.63)	1.51 ± 0.15 (1.28 – 1.59)	2.69 ± 0.26 (2.31 – 2.83)	1.27 ± 0.13 (1.08 – 1.35)	1.85 ± 0.18 (1.59 – 1.97)	2.35 ± 0.23 (2.00 – 2.49)	0.79 ± 0.08 (0.69 – 0.86)	2.76 ± 0.27 (2.35 – 2.92)	0.70 ± 0.07 (0.62 – 0.76)
CV127 + imi	0.62 ± 0.01* (0.62 – 0.63)	1.60 ± 0.03 (1.58 – 1.64)	2.88 ± 0.08 (2.82 – 2.99)	1.35 ± 0.02 (1.33 – 1.38)	1.98 ± 0.04 (1.95 – 2.03)	2.47 ± 0.06 (2.40 – 2.54)	0.85 ± 0.03 (0.83 – 0.89)	2.94 ± 0.05 (2.90 – 3.01)	0.73 ± 0.10 (0.60 – 0.81)
Std 1	0.63	1.48	2.59	1.27	1.78	2.31	0.75	2.63	0.72
Std 2	0.60	1.53	2.76	1.27	1.88	2.36	0.79	2.75	0.72
Brasília									
Isoline	0.64 ± 0.02 (0.62 – 0.66)	1.56 ± 0.06 (1.51 – 1.65)	2.82 ± 0.11 (2.72 – 2.96)	1.30 ± 0.05 (1.23 – 1.34)	1.92 ± 0.09 (1.82 – 2.03)	2.43 ± 0.10 (2.31 – 2.54)	0.86 ± 0.07 (0.78 – 0.94)	3.00 ± 0.18 (2.78 – 3.22)	0.75 ± 0.08 (0.67 – 0.84)
CV127	0.64 ± 0.02 (0.62 – 0.66)	1.57 ± 0.03 (1.54 – 1.61)	2.73 ± 0.04 (2.70 – 2.79)	1.25 ± 0.01 (1.24 – 1.26)	1.89 ± 0.04 (1.87 – 1.95)	2.31 ± 0.03 (2.28 – 2.35)	0.77 ± 0.03 (0.75 – 0.82)	2.87 ± 0.07 (2.82 – 2.97)	0.75 ± 0.13 (0.60 – 0.92)
CV127 + imi	0.63 ± 0.01 (0.63 – 0.64)	1.62 ± 0.05 (1.57 – 1.68)	2.80 ± 0.07 (2.71 – 2.89)	1.28 ± 0.04 (1.24 – 1.32)	1.96 ± 0.05 (1.90 – 2.01)	2.39 ± 0.06 (2.32 – 2.46)	0.84 ± 0.01 (0.83 – 0.84)	2.96 ± 0.04 (2.90 – 2.99)	0.75 ± 0.13 (0.64 – 0.94)
Std 1	0.63	1.49	2.60	1.22	1.77	2.30	0.81	2.65	0.66
Std 2	0.60	1.38	2.45	1.12	1.64	2.08	0.72	2.34	0.77
Santo Antônio de Posse									
Isoline	0.62 ± 0.01 (0.61 – 0.63)	1.59 ± 0.09 (1.51 – 1.71)	2.81 ± 0.14 (2.73 – 3.01)	1.33 ± 0.06 (1.29 – 1.42)	1.93 ± 0.09 (1.87 – 2.06)	2.45 ± 0.18 (2.29 – 2.70)	0.87 ± 0.04 (0.83 – 0.93)	3.03 ± 0.18 (2.84 – 3.28)	0.66 ± 0.07 (0.59 – 0.72)
CV127	0.61 ± 0.02 (0.59 – 0.63)	1.64 ± 0.25 (1.36 – 1.88)	2.93 ± 0.44 (2.45 – 3.36)	1.32 ± 0.17 (1.15 – 1.50)	2.00 ± 0.30 (1.66 – 2.29)	2.58 ± 0.37 (2.17 – 2.93)	0.91 ± 0.14 (0.77 – 1.04)	3.06 ± 0.45 (2.56 – 3.51)	0.67 ± 0.07 (0.61 – 0.77)
CV127 + imi	0.60 ± 0.01 (0.59 – 0.61)	1.56 ± 0.22 (1.24 – 1.70)	2.78 ± 0.37 (2.24 – 3.04)	1.28 ± 0.16 (1.06 – 1.40)	1.89 ± 0.26 (1.52 – 2.08)	2.40 ± 0.33 (1.93 – 2.66)	0.85 ± 0.13 (0.67 – 0.97)	2.90 ± 0.39 (2.36 – 3.20)	0.73 ± 0.12 (0.63 – 0.91)
Std 1	0.60	1.39	2.51	1.22	1.69	2.18	0.74	2.49	0.64
Std 2	0.56	1.61	2.76	1.32	1.97	2.31	0.76	2.75	1.16

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-10. Amino Acid Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location/ Treatment	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
	Mean ± Standard Deviation (range) g/100 g dry weight								
Santo Antônio de Goiás									
Isoline	4.50 ± 0.17 (4.26 – 4.66)	1.46 ± 0.05 (1.41 – 1.50)	1.95 ± 0.09 (1.83 – 2.05)	7.30 ± 0.16 (7.06 – 7.41)	1.85 ± 0.09 (1.76 – 1.95)	1.62 ± 0.06 (1.55 – 1.68)	1.69 ± 0.02 (1.67 – 1.71)	0.54 ± 0.01 (0.53 – 0.55)	1.77 ± 0.05 (1.72 – 1.83)
CV127	4.48 ± 0.20 (4.23 – 4.68)	1.47 ± 0.11 (1.35 – 1.62)	1.92 ± 0.06 (1.85 – 1.99)	7.18 ± 0.28 (6.84 – 7.52)	1.86 ± 0.09 (1.80 – 1.99)	1.59 ± 0.08 (1.50 – 1.69)	1.67 ± 0.16 (1.52 – 1.90)	0.55 ± 0.01* (0.55 – 0.56)	1.72 ± 0.09 (1.63 – 1.84)
CV127 + imi	4.50 ± 0.11 (4.41 – 4.65)	1.50 ± 0.12 (1.39 – 1.67)	1.93 ± 0.05 (1.86 – 1.97)	7.23 ± 0.19 (7.00 – 7.46)	1.67 ± 0.38 (1.11– 1.88)	1.65 ± 0.13 (1.53 – 1.83)	1.69 ± 0.13 (1.54 – 1.86)	0.55 ± 0.01* (0.54 – 0.56)	1.73 ± 0.11 (1.56 – 1.79)
Std 1	4.77	1.51	2.05	7.54	1.87	1.72	1.74	0.53	1.83
Std 2	4.93	1.55	2.21	8.02	2.05	1.72	1.79	0.54	1.87
Teresina									
Isoline	4.43 ± 0.20 (4.20 – 4.57)	1.40 ± 0.05 (1.36 – 1.45)	1.90 ± 0.05 (1.85 – 1.95)	7.00 ± 0.28 (6.68 – 7.22)	1.80 ± 0.09 (1.70 – 1.86)	1.53 ± 0.03 (1.50 – 1.55)	1.51 ± 0.06 (1.45 – 1.56)	0.44 ± 0.01 (0.43 – 0.44)	1.41 ± 0.03 (1.37 – 1.43)
CV127	4.05 ± 0.14* (3.89 – 4.15)	1.17 ± 0.03* (1.15 – 1.21)	1.75 ± 0.05* (1.70 – 1.80)	6.35 ± 0.16* (6.20 – 6.51)	1.73 ± 0.08 (1.64 – 1.79)	1.42 ± 0.02* (1.41 – 1.44)	1.45 ± 0.06 (1.39 – 1.51)	0.46 ± 0.04 (0.43 – 0.51)	1.51 ± 0.02* (1.50 – 1.53)
CV127 + imi	4.38 ± 0.17 (4.19 – 4.52)	1.20 ± 0.03* (1.17 – 1.23)	1.74 ± 0.09* (1.65 – 1.82)	6.46 ± 0.27* (6.17 – 6.69)	1.75 ± 0.14 (1.62 – 1.89)	1.44 ± 0.04* (1.40 – 1.47)	1.66 ± 0.18 (1.46 – 1.82)	0.47 ± 0.02 (0.46 – 0.50)	1.54 ± 0.02* (1.51 – 1.55)
Std 1	5.06	1.34	1.91	8.55	2.05	1.60	1.58	0.45	1.38
Std 2	4.03	1.31	1.78	6.47	1.69	1.41	1.41	0.44	1.31

Table D-10. continued.

Location/ Treatment	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
	Mean ± Standard Deviation (range) g/100 g dry weight								
Vilhena									
Isoline	4.20 ± 0.23 (3.91 – 4.41)	1.40 ± 0.10 (1.29 – 1.50)	1.85 ± 0.09 (1.73 – 1.93)	6.82 ± 0.35 (6.40 – 7.18)	1.77 ± 0.07 (1.70 – 1.85)	1.52 ± 0.09 (1.44 – 1.64)	1.48 ± 0.04 (1.43 – 1.51)	0.54 ± 0.01 (0.53 – 0.54)	1.58 ± 0.05 (1.50 – 1.61)
CV127	4.12 ± 0.16 (3.91 – 4.28)	1.37 ± 0.06 (1.31 – 1.44)	1.78 ± 0.06 (1.71 – 1.86)	6.73 ± 0.33 (6.34 – 7.11)	1.79 ± 0.06 (1.72 – 1.85)	1.42 ± 0.08 (1.34 – 1.52)	1.69 ± 0.09* (1.58 – 1.79)	0.55 ± 0.01 (0.53 – 0.56)	1.66 ± 0.06 (1.61 – 1.74)
CV127 + imi	4.27 ± 0.25 (4.01 – 4.59)	1.39 ± 0.05 (1.31 – 1.43)	1.83 ± 0.10 (1.71 – 1.95)	6.83 ± 0.29 (6.55 – 7.22)	1.84 ± 0.04 (1.79 – 1.88)	1.45 ± 0.16 (1.34 – 1.68)	1.66 ± 0.05* (1.58 – 1.70)	0.55 ± 0.01 (0.54 – 0.56)	1.62 ± 0.06 (1.53 – 1.66)
Std 1	4.66	1.47	2.00	7.51	2.03	1.61	1.57	0.59	1.65
Std 2	4.32	1.42	1.90	6.86	1.81	1.55	1.54	0.56	1.63
Brasília									
Isoline	3.63 ± 0.11 (3.50 – 3.73)	1.25 ± 0.03 (1.21 – 1.28)	1.61 ± 0.05 (1.54 – 1.66)	5.90 ± 0.16 (5.72 – 6.07)	1.56 ± 0.04 (1.50 – 1.60)	1.33 ± 0.05 (1.28 – 1.39)	1.37 ± 0.07 (1.29 – 1.45)	0.51 ± 0.01 (0.50 – 0.51)	1.45 ± 0.09 (1.35 – 1.54)
CV127	3.93 ± 0.41 (3.32 – 4.18)	1.33 ± 0.12 (1.14 – 1.40)	1.76 ± 0.13* (1.56 – 1.84)	6.29 ± 0.54 (5.49 – 6.63)	1.68 ± 0.10* (1.56 – 1.80)	1.45 ± 0.14 (1.24 - 1.55)	1.50 ± 0.12 (1.33 – 1.59)	0.51 ± 0.01 (0.50 – 0.51)	1.64 ± 0.12* (1.49 – 1.76)
CV127 + imi	4.03 ± 0.15 (3.85 – 4.17)	1.36 ± 0.06 (1.29 – 1.43)	1.79 ± 0.04* (1.74 – 1.82)	6.53 ± 0.08* (6.45 – 6.63)	1.69 ± 0.03* (1.65 – 1.72)	1.47 ± 0.06 (1.40 – 1.53)	1.49 ± 0.10 (1.38 – 1.61)	0.51 ± 0.01 (0.50 – 0.51)	1.56 ± 0.13 (1.41 – 1.73)
Std 1	4.11	1.38	1.84	6.57	1.75	1.53	1.58	0.51	1.72
Std 2	3.70	1.23	1.67	5.90	1.60	1.35	1.41	0.54	1.53

Table D-10. continued

Location/ Treatment	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
	Mean ± Standard Deviation (range) g/100 g dry weight								
Santo Antônio de Goiás									
Isoline	0.63 ± 0.02 (0.62 – 0.66)	1.54 ± 0.05 (1.50 – 1.62)	2.79 ± 0.06 (2.72 – 2.86)	1.30 ± 0.04 (1.24 – 1.33)	1.89 ± 0.05 (1.82 – 1.94)	2.38 ± 0.06 (2.31 – 2.46)	1.29 ± 0.02 (1.27 – 1.32)	3.20 ± 0.11 (3.11 – 3.34)	0.66 ± 0.07 (0.57 – 0.74)
CV127	0.66 ± 0.01* (0.65 – 0.66)	1.60 ± 0.16 (1.46 – 1.83)	2.66 ± 0.15 (2.45 – 2.79)	1.22 ± 0.09 (1.12 – 1.33)	1.93 ± 0.16 (1.78 – 2.16)	2.43 ± 0.22 (2.27 – 2.76)	1.23 ± 0.18 (0.99 – 1.38)	3.10 ± 0.10 (2.98 – 3.22)	0.63 ± 0.09 (0.50 – 0.70)
CV127 + imi	0.66 ± 0.01* (0.65 – 0.66)	1.65 ± 0.22 (1.45 – 1.97)	2.75 ± 0.10 (2.66 – 2.85)	1.23 ± 0.11 (1.10 – 1.38)	1.84 ± 0.06 (1.78 – 1.90)	2.43 ± 0.13 (2.34 – 2.62)	1.21 ± 0.27 (0.91 – 1.52)	3.11 ± 0.03 (3.07 – 3.15)	0.62 ± 0.12 (0.51 – 0.76)
Std 1	0.62	1.57	2.92	1.39	1.96	2.45	1.29	3.45	0.61
Std 2	0.60	1.65	3.15	1.45	2.12	2.58	1.34	3.34	0.65
Teresina									
Isoline	0.54 ± 0.01 (0.53 – 0.55)	1.55 ± 0.04 (1.51 – 1.58)	2.53 ± 0.07 (2.46 – 2.60)	1.25 ± 0.03 (1.22 – 1.28)	1.82 ± 0.06 (1.77 – 1.88)	2.26 ± 0.08 (2.18 – 2.33)	0.82 ± 0.02 (0.80 – 0.83)	2.93 ± 0.11 (2.80 – 3.00)	0.61 ± 0.01 (0.60 – 0.62)
CV127	0.56 ± 0.05 (0.51 – 0.61)	1.46 ± 0.03 (1.43 – 1.48)	2.49 ± 0.04 (2.46 – 2.54)	1.09 ± 0.03* (1.05 – 1.11)	1.76 ± 0.02 (1.74 – 1.78)	2.16 ± 0.04 (2.12 – 2.20)	1.27 ± 0.08* (1.18 – 1.34)	2.62 ± 0.11* (2.51 – 2.73)	0.60 ± 0.04 (0.56 – 0.63)
CV127 + imi	0.58 ± 0.01 (0.58 – 0.60)	1.45 ± 0.07 (1.37 – 1.51)	2.50 ± 0.06 (2.44 – 2.55)	1.11 ± 0.03* (1.09 – 1.14)	1.77 ± 0.07 (1.69 – 1.81)	2.13 ± 0.13 (1.99 – 2.25)	1.18 ± 0.10* (1.10 – 1.29)	2.81 ± 0.09 (2.71 – 2.88)	0.61 ± 0.08 (0.55 – 0.70)
Std 1	0.56	1.35	2.85	1.24	1.90	2.29	0.83	2.85	0.63
Std 2	0.53	1.47	2.39	1.16	1.71	2.09	0.73	2.65	0.64

Table D-10. continued.

Location/ Treatment	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
	Mean ± Standard Deviation (range) g/100 g dry weight								
Vilhena									
Isoline	0.62 ± 0.02 (0.60 – 0.64)	1.49 ± 0.07 (1.43 – 1.58)	2.59 ± 0.09 (2.49 – 2.67)	1.17 ± 0.04 (1.13 – 1.22)	1.78 ± 0.06 (1.70 – 1.85)	2.32 ± 0.14 (2.16 – 2.47)	1.17 ± 0.08 (1.07 – 1.26)	2.80 ± 0.11 (2.66 – 2.90)	0.63 ± 0.05 (0.60 – 0.70)
CV127	0.64 ± 0.01 (0.63 – 0.66)	1.20 ± 0.11* (1.11 – 1.35)	2.69 ± 0.14 (2.54 – 2.86)	1.08 ± 0.04* (1.05 – 1.14)	1.78 ± 0.09 (1.68 – 1.90)	2.21 ± 0.06 (2.13 – 2.28)	1.85 ± 0.32* (1.40 – 2.09)	2.61 ± 0.08* (2.50 – 2.69)	0.69 ± 0.08 (0.60 – 0.76)
CV127 + imi	0.64 ± 0.02 (0.62 – 0.66)	1.21 ± 0.19* (1.08 – 1.48)	2.59 ± 0.03 (2.56 – 2.62)	1.09 ± 0.05* (1.02 – 1.14)	1.77 ± 0.06 (1.70 – 1.85)	2.27 ± 0.11 (2.17 – 2.41)	1.80 ± 0.43* (1.17 – 2.13)	2.68 ± 0.14 (2.53 – 2.86)	0.72 ± 0.11 (0.58 – 0.84)
Std 1	0.69	1.57	2.77	1.18	1.93	2.51	1.14	3.24	0.73
Std 2	0.62	1.53	2.72	1.19	1.90	2.36	1.23	2.84	0.72
Brasília									
Isoline	0.58 ± 0.01 (0.58 – 0.59)	1.29 ± 0.06 (1.22 – 1.35)	2.31 ± 0.09 (2.19 – 2.41)	1.03 ± 0.06 (0.98 – 1.11)	1.58 ± 0.05 (1.52 – 1.63)	2.03 ± 0.04 (1.97 – 2.06)	1.08 ± 0.03 (1.04 – 1.12)	2.45 ± 0.06 (2.37 – 2.50)	0.65 ± 0.08 (0.53 – 0.71)
CV127	0.59 ± 0.01 (0.58 – 0.60)	1.40 ± 0.20 (1.10 – 1.55)	2.55 ± 0.17* (2.30 – 2.69)	1.10 ± 0.06 (1.01 – 1.16)	1.71 ± 0.11* (1.55 – 1.78)	2.34 ± 0.18* (2.23 – 2.61)	1.43 ± 0.49 (0.93 – 2.11)	2.71 ± 0.18* (2.44 – 2.85)	0.66 ± 0.10 (0.54 – 0.79)
CV127 + imi	0.58 ± 0.01 (0.58 – 0.59)	1.41 ± 0.08 (1.34 – 1.52)	2.58 ± 0.10* (2.47 – 2.69)	1.12 ± 0.06 (1.06 – 1.21)	1.74 ± 0.07* (1.65 – 1.80)	2.21 ± 0.06* (2.12 – 2.27)	1.22 ± 0.19 (0.96 – 1.39)	2.74 ± 0.14* (2.54 – 2.84)	0.73 ± 0.09 (0.59 – 0.79)
Std 1	0.59	1.53	2.63	1.25	1.79	2.28	1.17	2.72	0.56
Std 2	0.57	1.37	2.43	1.14	1.64	2.03	1.05	2.49	0.69

*Statistically significantly different from the isoline control at $P \leq 0.05$.

Table D-11. Fatty Acid Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location/ Treatment	C14:0 Myristic	C16:0 Palmitic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C20:0 Arachidic	C20:1 Eicosenoic	C22:0 Behenic
Santo Antônio de Goiás	Mean ± Standard Deviation (range) g/100 g DW								
Isoline	<0.02 (nd^ – 0.02)	2.36 ± 0.03 (2.31 – 2.40)	0.75 ± 0.09 (0.69 – 0.88)	4.70 ± 0.21 (4.42 – 4.93)	11.39 ± 0.13 (11.23 – 11.55)	1.40 ± 0.07 (1.30 – 1.46)	0.08 ± 0.01 (0.08 – 0.09)	0.03 ± 0.01 (0.02 – 0.03)	0.10 ± 0.01 (0..09 – 0.10)
CV127	0.02 ± 0 (0.02)	2.54 ± 0.07* (2.45 – 2.61)	0.76 ± 0.02 (0.74 – 0.79)	5.43 ± 0.14* (5.27 – 5.61)	11.85 ± 0.18* (11.64 – 12.05)	1.38 ± 0.06 (1.32 – 1.45)	0.08 ± 0.01 (0.07 – 0.09)	0.03 ± 0.01 (0.02 – 0.04)	0.10 ± 0.01 (0.09 – 0.11)
CV127 + imi	<0.02 (nd – 0.02)	2.44 ± 0.04 (2.40 – 2.48)	0.71 ± 0.01 (0.70 – 0.73)	5.97 ± 0.57* (5.40 – 6.57)	11.02 ± 0.17* (10.86 – 11.26)	1.25 ± 0.07* (1.21 – 1.35)	0.07 ± 0.01 (0.07 – 0.09)	0.04 ± 0* (0.04)	0.11 ± 0.01 (0.10 – 0.11)
Std 1	0.02	2.08	0.66	4.06	10.75	1.56	0.06	0.02	0.08
Std 2	0.02	2.21	0.70	4.94	12.71	1.65	0.07	0.02	0.09
Uberaba									
Isoline	0.02 ± 0 (0.02)	2.21 ± 0.13 (2.08 – 2.39)	0.76 ± 0.02 (0.74 – 0.80)	4.66 ± 0.19 (4.49 – 4.90)	11.07 ± 0.67 (10.68 – 12.06)	1.37 ± 0.08 (1.31 – 1.48)	0.08 ± 0.01 (0.08 – 0.09)	0.03 ± 0.01 (0.02 – 0.03)	0.10 ± 0.01 (0.10 – 0.11)
CV127	0.02 ± 0 (0.02)	2.43 ± 0.07* (2.35 – 2.53)	0.84 ± 0.03* (0.81 – 0.87)	5.23 ± 0.14* (5.06 – 5.35)	11.23 ± 0.22* (11.03 – 11.53)	1.27 ± 0.02* (1.25 – 1.29)	0.09 ± 0* (0.09)	0.02 ± 0.01 (0.02 – 0.03)	0.11 ± 0 (0.11)
CV127 + imi	<0.02 (nd – 0.02)	2.47± 0.05* (2.41 – 2.53)	0.84 ± 0.01* (0.83 – 0.85)	5.50 ± 0.08* (5.39 – 5.59)	11.26 ± 0.26* (10.95 – 11.57)	1.24 ± 0.03* (1.21 – 1.28)	0.09 ± 0* (0.09)	0.02 ± 0* (0.02)	0.10 ± 0.01 (0.10 – 0.11)
Std 1	0.02	2.18	0.76	3.99	11.35	1.65	0.06	0.03	0.08
Std 2	nd	2.19	0.76	5.07	13.09	1.68	0.07	0.03	0.09
Sete Lagoas									
Isoline	<0.02 (nd – 0.02)	2.23 ± 0.04 (2.20– 2.28)	0.89 ± 0.04 (0.84 – 0.93)	5.02 ± 0.09 (4.93 – 5.11)	11.01 ± 0.18 (10.85 – 11.26)	1.27 ± 0.04 (1.23 – 1.32)	0.09 ± 0.01 (0.08 – 0.10)	0.03 ± 0.01 (0.02 – 0.04)	0.11 ± 0.01 (0.10 – 0.12)
CV127	<0.02 (nd – 0.02)	2.36 ± 0.10* (2.26 – 2.47)	0.93 ± 0.02 (0.89 – 0.94)	5.56 ± 0.08* (5.44 – 5.62)	11.14 ± 0.34 (10.77 – 11.53)	1.23 ± 0.04 (1.19 – 1.27)	0.09 ± 0 (0.09)	0.02 ± 0* (0.02)	0.10 ± 0.01 (0.09 – 0.11)
CV127 + imi	<0.02 (nd – 0.02)	2.42 ± 0.03* (2.39– 2.45)	0.91 ± 0.02 (0.89 – 0.92)	6.03 ± 0.12* (5.86 – 6.15)	11.29 ± 0.30 (11.05 – 11.73)	1.21 ± 0.07 (1.16 – 1.31)	0.09 ± 0 (0.09)	0.02 ± 0.01 (0.02 – 0.03)	0.11 ± 0 (0.11)
Std 1	nd	2.28	0.84	4.28	11.39	1.54	0.08	0.03	0.08
Std 2	nd	2.34	0.84	5.14	12.94	1.52	0.07	0.03	0.09

Table D-11. continued.

Location/ Treatment	C14:0 Myristic	C16:0 Palmitic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C20:0 Arachidic	C20:1 Eicosenoic	C22:0 Behenic
Londrina	Mean ± Standard Deviation (range) g/100 g DW								
Isoline	<0.02 (nd – 0.02)	2.58 ± 0.05 (2.52 – 2.64)	0.79 ± 0.02 (0.77 – 0.81)	5.17 ± 0.18 (4.95 – 5.32)	11.86 ± 0.12 (11.73 – 11.98)	1.48 ± 0.06 (1.41 – 1.53)	0.09 ± 0 (0.09)	0.03 ± 0.01 (0.02 – 0.04)	0.12 ± 0.01 (0.11 – 0.13)
CV127	0.02 ± 0 (0.02)	2.61 ± 0.03 (2.57 – 2.63)	0.78 ± 0.01 (0.77 – 0.78)	5.41 ± 0.19 (5.18 – 5.63)	11.87 ± 0.14 (11.66 – 11.96)	1.37 ± 0.03* (1.33 – 1.41)	0.09 ± 0 (0.09)	0.03 ± 0.01 (0.02 – 0.04)	0.10 ± 0.01* (0.10 – 0.11)
CV127 + imi	0.02 ± 0 (0.02)	2.62 ± 0.03 (2.58 – 2.64)	0.80 ± 0.01 (0.78 – 0.82)	5.50 ± 0.19* (5.24 – 5.67)	11.91 ± 0.12 (11.76 – 12.06)	1.36 ± 0.02* (1.35 – 1.39)	0.09 ± 0 (0.09)	0.03 ± 0.01 (0.02 – 0.04)	0.10 ± 0.01* (0.09 – 0.11)
Std 1	nd	2.49	0.79	4.22	12.21	1.85	0.07	0.02	0.09
Std 2	nd	2.42	0.81	5.41	13.22	1.63	0.07	0.03	0.10
Brasília									
Isoline	0.02 ± 0 (0.02)	2.37 ± 0.06 (2.29 – 2.41)	0.86 ± 0.04 (0.81 – 0.89)	4.69 ± 0.24 (4.43 – 4.97)	10.18 ± 0.22 (9.97 – 10.38)	1.32 ± 0.02 (1.31 – 1.35)	0.09 ± 0.01 (0.09 – 0.10)	0.04 ± 0.01 (0.03 – 0.04)	0.11 ± 0.01 (0.10 – 0.12)
CV127	0.02 ± 0 (0.02)	2.41 ± 0.08 (2.29 – 2.47)	0.84 ± 0.01 (0.82 – 0.85)	5.10 ± 0.20 (4.96 – 5.38)	10.25 ± 0.42 (9.63 – 10.52)	1.25 ± 0.06* (1.17 – 1.31)	0.09 ± 0.01 (0.08 – 0.10)	0.03 ± 0.01 (0.02 – 0.04)	0.10 ± 0.01 (0.09 – 0.12)
CV127 + imi	0.02 ± 0 (0.02)	2.41 ± 0.07 (2.36 – 2.51)	0.80 ± 0.03* (0.77 – 0.82)	4.76 ± 0.35 (4.44 – 5.25)	10.26 ± 0.30 (10.09 – 10.71)	1.26 ± 0.01 (1.25 – 1.28)	0.08 ± 0.01* (0.08 – 0.09)	0.02 ± 0* (0.02)	0.10 ± 0.01 (0.10 – 0.11)
Std 1	0.02	2.45	0.85	4.20	11.51	1.69	0.09	0.04	0.11
Std 2	0.02	2.42	0.86	4.82	12.63	1.69	0.09	0.02	0.09
Santo Antônio de Posse									
Isoline	0.02 ± 0 (0.02)	2.38 ± 0.05 (2.34 – 2.44)	0.85 ± 0.01 (0.84 – 0.86)	4.79 ± 0.07 (4.74 – 4.89)	10.84 ± 0.22 (10.64 – 11.15)	1.32 ± 0.02 (1.30 – 1.34)	0.10 ± 0.01 (0.09 – 0.10)	0.03 ± 0.01 (0.02 – 0.04)	0.12 ± 0 (0.12)
CV127	0.02 ± 0 (0.02)	2.61± 0.08* (2.57 – 2.73)	0.90 ± 0.04* (0.88 – 0.97)	5.35 ± 0.20* (5.23 – 5.65)	11.69 ± 0.49* (11.40 – 12.43)	1.31 ± 0.07 (1.26 – 1.41)	0.09 ± 0.01 (0.09 – 0.11)	0.04 ± 0.01 (0.02 – 0.04)	0.11 ± 0.01 (0.11 – 0.12)
CV127 + imi	0.02 ± 0 (0.02)	2.63 ± 0.11* (2.48 – 2.73)	0.90 ± 0.03* (0.87 – 0.94)	5.45 ± 0.19* (5.19 – 5.62)	11.89 ± 0.33* (11.59 – 12.28)	1.32 ± 0.02 (1.29 – 1.34)	0.09 ± 0.01 (0.09 – 0.10)	0.04 ± 0* (0.04)	0.12 ± 0.01 (0.11 – 0.13)
Std 1	0.02	2.38	0.78	4.05	11.72	1.65	0.08	0.03	0.09
Std 2	0.02	2.34	0.80	4.83	13.01	1.61	0.07	0.04	0.09

*Statistically significantly different from the isoline control at $P < 0.05$. ^ nd = not detected

Table D-12. Fatty Acid Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location/ Treatment	C14:0 Myristic	C16:0 Palmitic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C20:0 Arachidic	C22:0 Behenic	C24:0 Tetracosanoic
	Mean ± Standard Deviation (range) g/100 g DW								
Santo Antônio de Goiás									
Isoline	0.02 ± 0 (0.02) N=2	1.83 ± 0.05 (1.79 – 1.89)	0.85 ± 0.05 (0.80 – 0.91)	4.43 ± 0.07 (4.33 – 4.49)	8.71 ± 0.24 (8.48 – 9.04)	1.23 ± 0.06 (1.18 – 1.30)	0.08 ± 0.01 (0.06 – 0.09)	0.09 ± 0.01 (0.09 – 0.10)	0.03 ± 0 (0.03)
CV127	0.02 ± 0 (0.02)	1.91 ± 0.04* (1.85 – 1.95)	0.89 ± 0.03 (0.86 – 0.94)	4.85 ± 0.07* (4.77 – 4.91)	9.05 ± 0.36 (8.79 – 9.57)	1.30 ± 0.06 (1.23 – 1.38)	0.08 ± 0.01 (0.08 – 0.09)	0.10 ± 0.01 (0.09 – 0.11)	0.03 ± 0 (0.03)
CV127 + imi	0.02 ± 0 (0.02) N=3	1.96 ± 0.02* (1.93 – 1.98)	0.91 ± 0.04 (0.87 – 0.95)	5.20 ± 0.10* (4.98 – 5.20)	9.09 ± 0.20 (8.83 – 9.31)	1.30 ± 0.03 (1.27 – 1.34)	0.09 ± 0.01 (0.08 – 0.10)	0.10 ± 0.01 (0.09 – 0.10)	0.03 ± 0 (0.03)
Std 1	0.01	1.80	0.83	3.87	8.10	1.37	0.07	0.08	0.01
Std 2	NA	1.77	0.89	4.82	9.44	1.27	0.08	0.10	0.03
Teresina									
Isoline	0.02 ± 0 (0.02) N=2	2.37 ± 0.14 (2.21 – 2.48)	0.80 ± 0.05 (0.74 – 0.85)	6.45 ± 0.99 (5.88 – 7.59)	11.27 ± 1.32 (9.74 – 12.05)	1.09 ± 0.12 (0.96 – 1.19)	0.09 ± 0.02 (0.08 – 0.11)	0.15 ± 0.02 (0.14 – 0.17)	0.07 ± 0.02 (0.05 – 0.09)
CV127	0.02 ± 0 (0.02)	2.58 ± 0.11 (2.48 – 2.70)	0.87 ± 0.01 (0.85 – 0.88)	6.86 ± 0.78 (6.07 – 7.62)	11.34 ± 1.16 (10.16 – 12.47)	1.02 ± 0.11 (0.92 – 1.13)	0.10 ± 0.01 (0.10 – 0.11)	0.14 ± 0.01 (0.13 – 0.15)	0.08 ± 0.01 (0.06 – 0.09)
CV127 + imi	NA	2.33 ± 0.23 (1.56 – 2.31)	0.89 ± 0.05* (0.84 – 0.93)	8.42 ± 1.12* (7.21 – 9.42)	8.75 ± 2.12 (6.91 – 11.07)	0.84 ± 0.14* (0.74 – 1.00)	0.11 ± 0.01 (0.10 – 0.12)	0.16 ± 0.02 (0.14 – 0.17)	0.10 ± 0.03 (0.06 – 0.12)
Std 1	NA	2.14	0.70	5.48	11.49	1.26	0.06	0.11	0.04
Std 2	0.02	2.09	0.76	7.04	12.44	1.19	0.06	0.12	0.08

Table D-12. continued.

Location/ Treatment	C14:0 Myristic	C16:0 Palmitic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C20:0 Arachidic	C22:0 Behenic	C24:0 Tetracosanoic
	Mean ± Standard Deviation (range) g/100 g DW								
Vilhena									
Isoline	NA	2.24 ± 0.05 (2.18 – 2.28)	0.87 ± 0.01 (0.86 – 0.88)	5.09 ± 0.08 (4.99 – 5.18)	10.28 ± 0.22 (10.00 – 10.54)	1.38 ± 0.03 (1.33 – 1.39)	0.08± 0.01 (0.08 – 0.09)	0.11 ± 0.01 (0.10 – 0.12)	0.04 ± 0 (0.04)
CV127	0.02 ± 0 (0.02) N=2	2.18 ± 0.04 (2.14 – 2.22)	0.91 ± 0.01* (0.90 – 0.91)	5.57 ± 0.04* (5.53 – 5.60)	10.06 ± 0.32 (9.74 – 10.35)	1.31 ± 0.04* (1.27 – 1.34)	0.09 ± 0 (0.09)	0.11 ± 0.01 (0.10 – 0.12)	0.04 ± 0 (0.04)
CV127 + imi	0.02 N=1	2.12 ± 0.06* (2.03 – 2.16)	0.90 ± 0.01* (0.90 – 0.91)	5.59 ± 0.06* (5.50 – 5.63)	9.72 ± 0.24* (9.41 – 9.97)	1.30 ± 0.03* (1.26 – 1.32)	0.09 ± 0.01 (0.08 – 0.10)	0.10 ± 0.01 (0.10 – 0.12)	0.04 ± 0 (0.04)
Std 1	0.02	1.88	0.91	4.52	8.51	1.33	0.09	0.09	0.03
Std 2	NA	2.21	0.81	4.70	11.88	1.64	0.06	0.09	0.04
Brasília									
Isoline	0.02 N=1	1.97 ± 0.03 (1.94 – 2.00)	0.72 ± 0.02 (0.69 – 0.73)	4.20 ± 0.06 (4.13 – 4.25)	9.55 ± 0.14 (9.47 – 9.70)	1.52 ± 0.03 (1.48 – 1.54)	0.07 ± 0.01 (0.07 – 0.08)	0.08 ± 0.01 (0.08 – 0.09)	0.02 ± 0.01 (0.02 – 0.03)
CV127	0.02 ± 0 (0.02)	1.93 ± 0.02 (1.91 – 1.96)	0.73 ± 0.01 (0.72 – 0.74)	4.41 ± 0.09* (4.29 – 4.48)	9.35 ± 0.06 (9.28 – 9.39)	1.54 ± 0.03 (1.51 – 1.58)	0.08 ± 0 (0.08)	0.08 ± 0 (0.08)	0.03 ± 0.01 (0.02 – 0.03)
CV127 + imi	0.02 ± 0 (0.02) N=3	2.00 ± 0.07 (1.90 – 2.05)	0.75 ± 0.01* (0.74 – 0.76)	4.52 ± 0.05* (4.48 – 4.58)	9.56 ± 0.27 (9.19 – 9.82)	1.56 ± 0.05 (1.51 – 1.61)	0.07 ± 0.01 (0.05 – 0.08)	0.08 ± 0 (0.08)	0.02 ± 0.01 (0.02 – 0.03)
Std 1	0.02	2.10	0.78	3.97	10.10	1.68	0.05	0.08	0.02
Std 2	NA	1.97	0.74	4.24	10.61	1.67	0.05	0.08	0.03

NA=Not Available

*Statistically significantly different from the isoline control at $P \leq 0.05$.

Table D-13. Mineral Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location	Treatment	N	Calcium	Iron	Phosphorus	Magnesium	Potassium
			Mean ± Standard Deviation (range) mg/100 g dry weight				
Santo Antônio de Goiás	Isoline	4	258 ± 30 (222 – 296)	8.45 ± 1.48 (6.40 – 9.85)	618 ± 20 (594 – 643)	252 ± 20 (222 – 265)	1878 ± 35 (1837 – 1912)
	CV127	4	269 ± 24 (238 – 290)	8.18 ± 0.44 (7.68 – 8.69)	686 ± 60 (617 – 759)	290 ± 10* (281 – 304)	1938 ± 46 (1908 – 2006)
	CV127 + imi	4	230 ± 16* (214 – 247)	7.78 ± 0.68 (7.20 – 8.58)	618 ± 42 (588 – 680)	285 ± 13* (274 – 303)	1844 ± 63 (1778 – 1918)
	Std 1	1	216	9.07	580	264	2044
	Std 2	1	278	10.35	559	308	1772
Uberaba	Isoline	4	266 ± 19 (238 – 280)	6.84 ± 1.25 (6.01 – 8.69)	557 ± 19 (541 – 584)	226 ± 25 (204 – 260)	1857± 68 (1782 – 1935)
	CV127	4	294 ± 23 (274 – 327)	5.96 ± 0.35 (5.56 – 6.42)	552 ± 45 (515 – 611)	231 ± 9 (218 – 240)	1803 ± 57 (1720 – 1850)
	CV127 + imi	4	296 ± 17* (276 – 318)	5.96 ± 0.19 (5.79 – 6.22)	576 ± 32 (546 – 611)	241 ± 12 (227 – 257)	1797 ± 37 (1751 – 1833)
	Std 1	1	256	6.54	541	225	1960
	Std 2	1	279	7.35	527	257	1822
Sete Lagoas	Isoline	4	237 ± 4 (232 – 241)	7.82 ± 0.14 (7.71 – 8.01)	703 ± 64 (666 – 799)	230 ± 7 (219 – 235)	1905 ± 81 (1785 – 1959)
	CV127	4	249 ± 13 (231 – 261)	6.97 ± 0.17 (6.84 – 7.22)	728 ± 14 (712 – 745)	236 ± 5 (229 – 240)	1783 ± 21* (1756 – 1806)
	CV127 + imi	4	240 ± 8 (231 – 248)	7.09 ± 0.22 (6.94 – 7.40)	723 ± 19 (712 – 752)	247 ± 7 (240 – 256)	1791 ± 104* (1703 – 1907)
	Std 1	1	205	7.23	674	240	2020
	Std 2	1	301	8.24	719	273	1945
Londrina	Isoline	4	236 ± 16 (221 – 257)	9.76 ± 0.47 (9.41 – 10.43)	740 ± 53 (677 – 805)	249 ± 6 (240 – 255)	1977 ± 56 (1902 - 2035)
	CV127	4	284 ± 33* (242 – 322)	10.27 ± 0.78 (9.18 – 10.91)	725 ± 76 (618 – 799)	306 ± 26* (268 – 326)	2109 ± 94* (1969 – 2164)
	CV127 + imi	4	266 ± 15* (251 – 280)	9.92 ± 0.47 (9.41 – 10.48)	691 ± 32 (644 – 717)	289 ± 16* (267 – 304)	2040 ± 35* (1989 – 2065)
	Std 1	1	221	9.50	729	255	2048
	Std 2	1	244	9.46	742	297	2011
Brasília	Isoline	4	314 ± 11 (305 – 330)	9.02 ± 0.47 (8.64 – 9.71)	786 ± 34 (763 – 834)	262 ± 5 (256 – 266)	1902 ± 29 (1874– 1936)
	CV127	4	276 ± 14* (265 – 295)	7.18 ± 0.59* (6.67 – 8.02)	675 ± 20* (655 – 703)	267 ± 1 (266 - 269)	1815 ± 33 (1775 – 1850)
	CV127 + imi	4	275 ± 9* (265 – 284)	7.27 ± 0.43* (6.78 – 7.76)	694 ± 21* (679 – 724)	267 ± 4 (264 – 273)	1848 ± 11 (1833 – 1857)
	Std 1	1	246	8.22	733	257	1911
	Std 2	1	313	9.54	875	306	1933

Table D-13. continued.

Location	Treatment	N	Calcium	Iron	Phosphorus	Magnesium	Potassium
			Mean ± Standard Deviation (range) mg/100 g dry weight				
Santo Antônio de Posse	Isoline	4	296 ± 12 (281 – 309)	9.15 ± 0.16 (8.93 – 9.28)	722 ± 54 (672 – 789)	256 ± 5 (248 – 259)	2050 ± 26 (2013 – 2071)
	CV127	4	291 ± 23 (261 – 312)	8.78 ± 0.28 (8.47 – 9.13)	694 ± 59 (619 – 751)	265 ± 12 (251 – 281)	1936 ± 132 (1822 – 2100)
	CV127 + imi	4	288 ± 14 (271 – 305)	8.46 ± 0.39 (8.03 – 8.97)	701 ± 48 (655 – 760)	266 ± 4 (261 – 269)	1964± 119 (1794 – 2069)
	Std 1	1	209	8.04	620	258	2103
	Std 2	1	286	9.25	745	283	1964

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-14. Mineral Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location	Treatment	N	Calcium	Iron	Phosphorus	Magnesium	Potassium
			Mean ± Standard Deviation (range) mg/100 g dry weight				
Santo Antônio de Goiás	Isoline	4	235 ± 14 (223 – 252)	8.67 ± 0.44 (8.24 – 9.24)	831 ± 14 (817 – 845)	240 ± 5 (235 – 245)	1643 ± 37 (1593 – 1676)
	CV127	4	267 ± 13* (255 – 279)	9.63 ± 1.08 (8.64 – 10.93)	739 ± 21* (719 – 769)	268 ± 9* (258 – 279)	1794 ± 18* (1775 – 1810)
	CV127 + imi	4	256 ± 9* (244 – 264)	9.03 ± 0.25 (8.70 – 9.26)	706 ± 41* (654 – 743)	275 ± 10* (268 – 289)	1760 ± 118* (1599 – 1875)
	Std 1	1	192	8.68	793	230	1631
	Std 2	1	213	9.98	808	255	1595
Teresina	Isoline	3	354 ± 8 (345 – 359)	8.44 ± 0.41 (7.98 – 8.77)	758 ± 20 (735 – 771)	258 ± 7 (252 – 266)	1983 ± 76 (1910 – 2061)
	CV127	3	377 ± 48 (332 – 427)	8.44 ± 0.32 (8.23 – 8.81)	711 ± 63 (672 – 784)	277 ± 14 (261 – 288)	2018 ± 56 (1970 – 2079)
	CV127 + imi	3	448 ± 61* (377 – 484)	8.45 ± 0.49 (7.90 – 8.81)	797 ± 70 (733 – 871)	296 ± 14* (280 – 308)	1933 ± 95 (1832 – 2021)
	Std 1	1	329	7.93	734	264	2033
	Std 2	1	323	8.27	703	279	1911

Table D-14. continued.

Location	Treatment	N	Calcium	Iron	Phosphorus	Magnesium	Potassium
			Mean ± Standard Deviation (range) mg/100 g dry weight				
Vilhena	Isoline	4	234 ± 5 (230 – 242)	9.90 ± 0.84 (8.96 – 10.90)	704 ± 52 (655 – 752)	243 ± 12 (231 – 258)	1693 ± 32 (1666 – 1739)
	CV127	4	273 ± 13* (257 – 287)	12.10 ± 0.99* (10.70 – 13.00)	708 ± 35 (679 – 759)	287 ± 18* (270 – 309)	1821 ± 101* (1713 – 1945)
	CV127 + imi	4	275 ± 17* (260 – 297)	10.33 ± 0.99 (9.00 – 11.40)	753 ± 14 (741 – 770)	304 ± 6* (296 – 309)	1894 ± 22* (1875 – 1926)
	Std 1	1	231	14.13	790	246	1815
	Std 2	1	224	12.23	614	293	1630
Brasília	Isoline	4	188 ± 11 (172 – 194)	9.47 ± 0.26 (9.23 – 9.80)	778 ± 47 (708 – 809)	218 ± 7 (211 – 227)	1715 ± 80 (1645– 1831)
	CV127	4	205 ± 17 (190 – 228)	9.01 ± 0.49 (8.60 – 9.70)	707 ± 38 (654 – 736)	276 ± 3* (271 - 279)	1911 ± 16* (1894 – 1930)
	CV127 + imi	4	206 ± 11 (198 – 223)	8.63 ± 0.60* (7.80 – 9.20)	690 ± 90 (580 – 799)	277 ± 9* (270 – 289)	1887 ± 96* (1796 – 2001)
	Std 1	1	167	9.43	754	223	1708
	Std 2	1	195	10.60	755	253	1738

*Statistically significantly different from the isoline control at $p \leq 0.05$.

Table D-15. Selected Vitamin Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location/ Treatment	α-tocopherol (mg/100 g DW)	β-tocopherol (mg/100 g DW)	γ-tocopherol (mg/100 g DW)	δ-tocopherol (mg/100 g DW)	Total Tocopherol (mg/100 g DW)	Vitamin E (IU/100 g DW)	Vitamin E (mg/100 g DW)	Vitamin B1 (mg/100 g DW)	Folic Acid (µg/100 gDW)
Santo Antônio de Goiás	Mean ± Standard Deviation (range)								
Isoline	2.87 ± 0.26 (2.62 – 3.19)	0.40 ± 0.21 (0.20 – 0.61)	18.31 ± 1.69 (17.19 – 20.83)	7.04± 0.71 (6.01 – 7.53)	27.94 ± 3.24 (23.73 – 31.54)	6 ± 1 (6 – 7)	5.71 ± 0.33 (5.22 – 5.96)	0.61 ± 0.12 (0.48 – 0.74)	362 ± 44 (320 – 424)
CV127	3.05 ± 0.15 (2.86 – 3.22)	0.72 ± 0.12* (0.58 – 0.86)	16.70 ± 1.07* (15.19 – 17.69)	7.64 ± 0.44 (7.11 – 8.15)	28.11 ± 1.48 (26.10 – 29.61)	6 ± 0 (6)	5.60 ± 0.25 (5.37 – 5.84)	0.51 ± 0.10 (0.40 – 0.63)	290 ± 13* (276 – 304)
CV127 + imi	2.81 ± 0.28 (2.43 – 3.08)	0.69 ± 0.12* (0.58 – 0.81)	15.88 ± 1.45* (14.55 – 17.64)	7.21 ± 0.43 (6.62 – 7.65)	26.53 ± 1.62 (25.13 – 26.97)	6 ± 1 (5 – 6)	5.21 ± 0.44 (4.68 – 5.53)	0.48 ± 0.15 (0.34 – 0.67)	299 ± 15* (282 – 316)
Std 1	3.29	0.85	14.22	6.31	24.66	6	5.51	0.66	277
Std 2	2.45	0.12	20.88	7.42	30.86	6	5.40	0.66	350
Uberaba									
Isoline	2.82 ± 0.25 (2.54 – 3.13)	0.59 ± 0.20 (0.34 – 0.82)	16.51 ± 1.09 (15.48 – 18.04)	6.35 ± 0.38 (6.08 – 6.90)	26.31 ± 1.61 (25.05 – 28.58)	6 ± 1 (5 – 6)	5.17 ± 0.20 (4.91 – 5.40)	0.70 ± 0.17 (0.44 – 0.79)	431 ± 23 (402 – 456)
CV127	3.18 ± 0.07* (3.11 – 3.25)	0.93 ± 0.09* (0.83 – 1.04)	15.48 ± 0.56 (14.83 – 16.11)	6.98 ± 0.22* (6.81 – 7.30)	26.57 ± 0.65 (25.60 – 26.96)	6 ± 0* (6)	5.61 ± 0.13* (5.41 – 5.70)	0.46 ± 0.05* (0.40 – 0.52)	276 ± 30* (235 – 305)
CV127 + imi	3.30 ± 0.14* (3.21 – 3.51)	0.98 ± 0.17* (0.88 – 1.22)	16.49 ± 0.49 (16.06 – 17.03)	7.38 ± 0.09* (7.27 – 7.47)	28.16 ± 0.53 (27.66 – 28.90)	7 ± 1* (6 – 7)	5.88 ± 0.18* (5.77 – 6.15)	0.47 ± 0.08* (0.37 – 0.54)	303 ± 28* (277 – 338)
Std 1	2.98	0.71	12.68	5.31	21.68	5	4.95	0.45	381
Std 2	2.53	0.55	17.89	6.06	27.04	6	5.18	0.42	403
Sete Lagoas									
Isoline	2.53 ± 0.05 (2.48 – 2.59)	0.49 ± 0.07 (0.40 – 0.57)	16.20 ± 0.36 (15.90 – 16.71)	6.64 ± 0.21 (6.40 – 6.85)	25.81 ± 0.39 (25.26 – 26.16)	5 ± 0 (5)	4.92 ± 0.08 (4.81 – 4.97)	0.62 ± 0.14 (0.49 – 0.78)	367 ± 14 (352 – 379)
CV127	3.04 ± 0.09* (2.93 – 3.12)	0.90 ± 0.20* (0.69 – 1.17)	15.98 ± 0.78 (14.82 – 16.49)	6.83 ± 0.23 (6.64 – 7.16)	26.57 ± 1.04 (25.06 – 27.29)	6 ± 0* (6)	5.49 ± 0.18 (5.26 – 5.65)	0.50 ± 0.03 (0.47 – 0.54)	284 ± 43* (242 – 327)
CV127 + imi	2.95 ± 0.21* (2.49 – 3.23)	0.81 ± 0.09* (0.68 – 0.88)	15.95 ± 0.65 (15.33 – 16.68)	6.86 ± 0.47 (6.37 – 7.50)	26.50 ± 1.34 (25.18 – 28.26)	6 ± 0* (6)	5.39 ± 0.31 (5.12 – 5.80)	0.59 ± 0.13 (0.49 – 0.78)	297 ± 27* (282 – 337)
Std 1	3.27	0.47	14.50	6.04	24.28	6	5.44	0.63	317
Std 2	2.48	0.49	17.83	6.47	27.05	6	5.11	0.49	205

Table D-15. continued.

Location/ Treatment	α-tocopherol (mg/100 g DW)	β-tocopherol (mg/100 g DW)	γ-tocopherol (mg/100 g DW)	δ-tocopherol (mg/100 g DW)	Total Tocopherol (mg/100 g DW)	Vitamin E (IU/100 g DW)	Vitamin E (mg/100 g DW)	Vitamin B1 (mg/100 g DW)	Folic Acid (µg/100 g DW)
Londrina	Mean ± Standard Deviation (range)								
Isoline	3.42 ± 0.19 (3.23 – 3.66)	0.93 ± 0.08 (0.83 – 1.01)	16.31 ± 0.24 (16.01 – 16.33)	6.93 ± 0.50 (6.22 – 7.38)	27.51 ± 0.46 (26.87 – 27.95)	7 ± 1 (6 – 7)	5.94 ± 0.15 (5.78 – 6.12)	0.63 ± 0.04 (0.58 – 0.68)	266 ± 37 (234 – 319)
CV127	3.68 ± 0.23 (3.46 – 3.91)	1.01 ± 0.16 (0.81 – 1.20)	17.26 ± 0.56 (16.43 – 17.58)	6.81 ± 0.14 (6.64 – 6.93)	28.54 ± 0.37* (28.11 – 28.99)	7 ± 0 (7)	6.27 ± 0.27* (5.97 – 6.54)	0.55 ± 0.04 (0.51 – 0.58)	260 ± 23 (229 – 279)
CV127 + imi	3.91 ± 0.23* (3.64 – 4.20)	0.97 ± 0.10 (0.85 – 1.08)	17.79 ± 0.76 (17.18 – 18.84)	7.37 ± 0.42 (7.11 – 7.99)	30.03 ± 1.15* (28.79 – 31.12)	7 ± 1 (7 – 8)	6.66 ± 0.31* (6.28 – 7.01)	0.50 ± 0.04 (0.45 – 0.55)	260 ± 30 (234 – 302)
Std 1	3.89	0.99	13.99	6.26	25.15	7	6.13	0.71	228
Std 2	3.39	0.73	20.02	6.48	31.03	7	6.51	0.53	247
Brasília									
Isoline	2.93 ± 0.40 (2.33 – 3.21)	0.70 ± 0.10 (0.55 – 0.77)	13.73 ± 2.06 (11.62 – 16.03)	4.78 ± 0.28 (4.41 – 5.08)	22.0 ± 2.55 (19.26 – 24.61)	6 ± 1 (5 – 6)	5.00 ± 0.63 (4.11 – 5.48)	0.63 ± 0.17 (0.48 – 0.86)	244 ± 32 (216 – 290)
CV127	3.24 ± 0.20* (3.07 – 3.52)	1.03 ± 0.22* (0.81 – 1.32)	13.39 ± 1.02 (12.50 – 14.86)	5.49 ± 0.93* (4.85 – 6.87)	23.08 ± 2.36* (21.59 – 26.57)	6 ± 1* (6 – 7)	5.38 ± 0.41 (5.05 – 5.97)	0.46 ± 0.05* (0.40 – 0.52)	234 ± 32 (201 – 274)
CV127 + imi	3.47 ± 0.21* (3.16 – 3.62)	1.05 ± 0.15* (0.89 – 1.19)	13.59 ± 1.05 (12.31 – 14.86)	5.42 ± 0.36* (4.96 – 5.78)	23.57 ± 1.39* (21.95 – 25.35)	6 ± 1* (6 – 7)	5.65 ± 0.35* (5.13 – 5.92)	0.48 ± 0.05* (0.45 – 0.55)	196 ± 9* (183 – 205)
Std 1	3.21	0.91	13.44	5.65	23.20	6	5.34	0.71	314
Std 2	2.74	0.67	16.81	5.51	25.73	6	5.27	0.56	254
Santo Antônio de Posse									
Isoline	3.65 ± 0.55 (3.20 – 4.44)	0.49 ± 0.03 (0.45 – 0.52)	18.04 ± 0.85 (17.00 – 19.00)	5.32 ± 0.35 (5.04 – 5.84)	27.51 ± 1.60 (25.95 – 29.74)	7 ± 1 (6 – 8)	6.29 ± 0.65 (5.70 – 7.21)	0.70 ± 0.10 (0.59 – 0.81)	310 ± 28 (272 – 333)
CV127	4.48 ± 0.26* (4.29 – 4.86)	0.82 ± 0.11* (0.68 – 0.94)	16.14 ± 0.86* (15.29 – 17.08)	5.18 ± 0.32 (4.95 – 5.66)	26.56 ± 0.75 (25.44 – 27.01)	8 ± 1* (7– 8)	6.93 ± 0.27* (6.69 – 7.30)	0.62 ± 0.06 (0.54 – 0.67)	258 ± 17* (235 – 272)
CV127 + imi	4.48 ± 0.23* (4.20 – 4.69)	0.93 ± 0.14* (0.82 – 1.13)	15.20 ± 1.48* (13.84 – 17.16)	5.12 ± 0.27 (4.90 – 5.49)	25.74 ± 1.91 (24.04 – 27.89)	8 ± 1* (7 – 8)	6.69 ± 0.32 (6.42 – 7.16)	0.60 ± 0.02 (0.57 – 0.62)	266 ± 8* (260 – 277)
Std 1	4.61	1.08	15.31	6.03	27.00	8	7.05	0.62	291
Std 2	3.80	0.80	17.77	5.76	28.14	7	6.48	0.58	227

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-16. Selected Vitamin Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location/ Treatment	α-tocopherol (mg/100 g DW)	β-tocopherol (mg/100 g DW)	γ-tocopherol (mg/100 g DW)	δ-tocopherol (mg/100 g DW)	Total tocopherol (mg/100 g DW)	Vitamin E (IU/100 g DW)	Vitamin E (mg/100 g DW)	Vitamin B1 (mg/100 g DW)	Folic Acid (µg/100 g DW)
	Mean ± Standard Deviation (range)								
Santo Antônio de Goiás									
Isoline	2.50 ± 0.36 (2.20 – 3.00)	0.81 ± 0.16 (0.63 – 1.03)	14.32 ± 0.47 (13.79 – 14.88)	7.84 ± 0.28 (7.58 – 8.12)	25.49 ± 0.44 (25.05 – 25.90)	5 ± 1 (5 – 6)	4.75 ± 0.37 (4.42 – 5.26)	0.64 ± 0.13 (0.52 – 0.80)	444 ± 19 (428 – 462)
CV127	2.81 ± 0.27 (2.51 – 3.17)	1.12 ± 0.17* (0.99 – 1.35)	15.04 ± 0.39* (14.77 – 15.61)	8.97 ± 0.26* (8.71 – 9.32)	27.93 ± 0.26* (27.71 – 28.27)	6 ± 1 (5 – 6)	5.25 ± 0.30* (4.90 – 5.64)	0.66 ± 0.05 (0.61 – 0.72)	443 ± 12 (432 – 460)
CV127 + imi	2.53 ± 0.18 (2.37 – 2.73)	0.79 ± 0.16 (0.68 – 1.02)	14.17 ± 0.32 (13.80 – 14.46)	7.95 ± 0.34 (7.75 – 8.46)	25.44 ± 0.86 (24.63 – 26.66)	5 ± 1 (5 – 6)	4.75 ± 0.23 (4.52 – 5.05)	0.58 ± 0.02 (0.55 – 0.60)	452 ± 15 (430 – 464)
Std 1	2.67	1.24	11.65	7.51	23.07	5	4.67	0.71	401
Std 2	2.53	0.66	15.44	7.18	25.81	5	4.88	0.59	438
Teresina									
Isoline	6.61 ± 1.42 (5.38 – 8.17)	0.69 ± 0.13 (0.54 – 0.77)	19.24 ± 2.17 (17.38 – 21.63)	5.05 ± 0.65 (4.52 – 5.77)	31.58 ± 2.56 (29.48 – 34.43)	10 ± 2 (9 – 12)	9.46 ± 1.34 (8.12 – 10.79)	0.43 ± 0.06 (0.37 – 0.48)	291 ± 36 (249 – 315)
CV127	6.85 ± 0.12 (6.71 – 6.94)	0.79 ± 0.05 (0.73 – 0.82)	19.67 ± 1.93 (17.46 – 21.05)	5.79 ± 1.00 (4.67 – 6.58)	33.10 ± 2.95 (29.77 – 35.38)	10 ± 1 (10 – 11)	9.80 ± 0.28 (9.53 – 10.09)	0.40 ± 0.07 (0.35 – 0.48)	348 ± 47 (302 – 396)
CV127 + imi	7.84 ± 1.38 (6.47 – 9.23)	0.94 ± 0.11* (0.81 – 1.00)	15.66 ± 3.13 (12.28 – 18.46)	4.57 ± 1.18 (3.31 – 5.66)	29.00 ± 2.87 (25.82 – 31.40)	11 ± 1 (10 – 12)	10.27 ± 0.98 (9.26 – 11.21)	0.37 ± 0.15 (0.28 – 0.55)	367 ± 26* (352 – 397)
Std 1	7.51	1.34	14.62	5.61	29.09	11	9.92	0.41	261
Std 2	5.23	0.53	22.27	5.16	33.19	9	8.46	0.35	267

Table D-16. continued.

Location/ Treatment	α-tocopherol (mg/100 g DW)	β-tocopherol (mg/100 g DW)	γ-tocopherol (mg/100 g DW)	δ-tocopherol (mg/100 g DW)	Total tocopherol (mg/100 g DW)	Vitamin E (IU/100 g)	Vitamin E (mg/100 g DW)	Vitamin B1 (mg/100 g DW)	Folic Acid (µg/100 g DW)
	Mean ± Standard Deviation (range)								
Vilhena									
Isoline	2.77 ± 0.36 (2.37 – 3.16)	0.98 ± 0.10 (0.90 – 1.12)	17.30 ± 1.26 (16.32 – 19.14)	8.67 ± 0.93 (7.73 – 9.72)	29.72 ± 2.40 (27.40 – 32.93)	6 ± 1 (5 – 7)	5.47 ± 0.51 (4.93 – 6.11)	0.43 ± 0.06 (0.34 – 0.47)	320 ± 17 (302 – 339)
CV127	3.21 ± 0.14* (3.09 – 3.40)	0.96 ± 0.14 (0.76 – 1.09)	15.88 ± 0.89 (14.99 – 17.11)	8.75 ± 0.76 (7.63 – 9.31)	28.80 ± 1.72 (26.72 – 30.91)	6 ± 1 (6 – 7)	5.72 ± 0.28 (5.48 – 6.11)	0.49 ± 0.08 (0.40 – 0.60)	311 ± 7 (303 – 320)
CV127 + imi	3.17 ± 0.25 (2.79 – 3.32)	0.94 ± 0.26 (0.68 – 1.26)	15.45 ± 1.10* (14.34 – 16.59)	8.70 ± 1.06 (7.51 – 9.77)	28.25 ± 2.24 (25.90 – 30.27)	6 ± 0 (6)	5.61 ± 0.33 (5.16 – 5.89)	0.56 ± 0.06 (0.50 – 0.62)	337 ± 12 (320 – 349)
Std 1	3.43	1.04	12.63	7.04	24.14	6	5.50	0.34	329
Std 2	2.69	0.66	20.93	8.94	33.23	6	5.81	0.31	285
Brasília									
Isoline	1.80 ± 0.08 (1.74 – 1.92)	0.85 ± 0.21 (0.69 – 1.15)	15.51 ± 1.09 (14.18 – 16.85)	8.38 ± 0.26 (8.13 – 8.73)	26.54 ± 1.06 (25.02 – 27.46)	5 ± 1 (4 – 5)	4.23 ± 0.20 (3.94 – 4.40)	0.55 ± 0.07 (0.47 – 0.64)	389 ± 46 (323 – 427)
CV127	2.09 ± 0.14* (1.96 – 2.26)	1.23 ± 0.27 (0.95 – 1.49)	14.55 ± 0.86 (13.91 – 15.81)	10.49 ± 1.02* (9.62 – 11.72)	28.34 ± 1.73 (26.96 – 30.82)	5 ± 0 (5)	4.50 ± 0.20 (4.35 – 4.80)	0.63 ± 0.05 (0.57 – 0.70)	346 ± 14 (326 – 355)
CV127 + imi	2.20 ± 0.16* (1.96 – 2.33)	1.18 ± 0.24 (0.85 – 1.37)	14.74 ± 0.60 (14.21 – 15.59)	10.49 ± 0.63* (9.86 – 11.35)	27.36 ± 2.98 (23.38 – 30.55)	5 ± 0 (5)	4.62 ± 0.20* (4.35 – 4.84)	0.65 ± 0.07 (0.59 – 0.75)	338 ± 13* (324 – 354)
Std 1	2.07	0.65	11.98	6.86	21.56	4	3.95	0.53	309
Std 2	2.16	0.89	15.94	7.63	26.61	5	4.64	0.60	294

*Statistically significantly different from the isoline control at $P \leq 0.05$.

Table D-17. Isoflavone Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location/ Treatment	Daidzin	Malonyl-Daidzin	Daidzein	Glycitin	Malonyl-glycitin	Glycitein	Genistin	Malonyl-genistin	Genistein
Santo Antônio de Goiás	Mean ± Standard Deviation (range) mg/100 g dry weight								
Isoline	13.2 ± 2.8 (9.3 – 15.9)	54.9 ± 3.5 (51.7 – 59.9)	1.1 ± 0.4 (0.8 – 1.6)	4.6 ± 1.0 (3.9 – 6.1)	14.0 ± 1.6 (11.9 – 15.8)	<0.8 (nd^ – 0.9)	16.2 ± 3.0 (11.7 – 18.0)	85.7 ± 4.0 (82.1 – 91.1)	1.1 ± 0.4 (0.7 – 1.7)
CV127	11.7 ± 0.5 (11.0 – 12.3)	40.3 ± 1.9* (38.5 – 42.7)	1.0 ± 0.3 (0.6 – 1.3)	5.0 ± 0.4 (4.5 – 5.3)	14.0 ± 0. 9 (12.8 – 14.7)	<0.8 (nd – 0.8)	15.4 ± 0.8 (14.5 – 16.4)	69.9 ± 2.6* (68.2 – 73.8)	0.6 ± 0.1* (0.5 – 0.7)
CV127 + imi	11.2 ± 0.3 (10.9 – 11.6)	35.8 ± 1.9* (34.1 – 37.7)	1.1 ± 0. 1 (1.1 – 1.2)	4.9 ± 0.5 (4.3 – 5.4)	12.5 ± 1.4 (11.0 – 14.3)	0.7 ± 0 (0.7)	15.1 ± 0.3 (14.8 – 15.5)	62.9 ± 2.8* (59.5 – 65.5)	0.7 ± 0.1* (0.7 – 0.8)
Std 1	14.2	60.1	1.3	9.4	26.3	0.6	18.1	103.4	0.6
Std 2	11.5	55.5	1.7	12.7	36.2	1.6	15.1	87.1	0.6
Uberaba									
Isoline	12.1 ± 2.4 (9.3 – 15.0)	58.2 ± 7.7 (50.0 – 66.6)	1.1 ± 0.3 (0.7 – 1.5)	5.2 ± 0.4 (4.8 – 5.6)	17.5 ± 2.9 (13.6 – 20.3)	<1 (nd – 1.1)	13.1 ± 2.2 (11.2 – 16.2)	86.0 ± 9.1 (75.4 – 93.6)	0.9 ± 0.4 (0.6 – 1.5)
CV127	10.9 ± 0.7 (9.9 – 11.3)	46.3 ± 2.8* (42.5 – 49.1)	1.1 ± 0.08 (1.0 – 1.2)	5.8 ± 0.1 (5.6 – 5.9)	17.6 ± 1.5 (15.8 – 19.2)	0.8 ± 0. 1 (0.7 – 0.9)	13.1 ± 1.1 (11.6 – 14.1)	80.5 ± 4.1 (75.8 – 85.7)	0.7 ± 0.2 (0.5 – 0.9)
CV127 + imi	11.4 ± 1.8 (9.0 – 13.2)	48.6 ± 6.8* (42.0 – 58.0)	1.2 ± 0.2 (1.0 – 1.4)	6.3 ± 0.9 (5.2 – 7.3)	18.1 ± 3.0 (14.4 – 21.5)	0.7 ± 0.1 (0.5 – 0.8)	13.9 ± 0.7 (13.2 – 14.5)	82.7 ± 5.5 (78.7 – 90.8)	0.8 ± 0.2 (0.7 – 1.1)
Std 1	12.2	70.5	1.7	10.3	34.5	1.4	15.6	132.7	1.2
Std 2	12.6	65.0	0.8	12.6	34.6	1.5	16.2	115.9	1.5
Sete Lagoas									
Isoline	15.7 ± 1.4 (14.3 – 17.2)	52.4 ± 5.9 (47.3 – 59.3)	2.0 ± 0.4 (1.5 – 2.4)	5.1 ± 1.0 (3.8 – 5.9)	13.6 ± 2.2 (10.4 – 15.1)	<0.7 (nd – 0.7)	17.7 ± 1.6 (16.0 – 19.4)	83.0 ± 7.5 (76.1 – 92.2)	2.2 ± 0.4 (1.7 – 2.7)
CV127	11.7 ± 1.5* (10.2 – 13.3)	37.2 ± 6.9 (29.7 – 45.3)	1.5 ± 0.4 (0.9 – 1.8)	5.9 ± 0.9 (5.3 – 7.2)	15.3 ± 2.0 (13.6 – 18.1)	0.8 ± 0.3* (0.4 – 1.0)	14.6 ± 2.9 (11.9 – 18.7)	61.7 ± 13.7* (49.6 – 81.3)	1.9 ± 0.1 (1.8 – 2.0)
CV127 + imi	12.5 ± 1.6* (11.3 – 14.7)	38.6 ± 5.6 (34.0 – 46.3)	1.7 ± 0.3 (1.2 – 1.9)	5.7 ± 1.0 (4.5 – 6.7)	14.5 ± 2.8 (10.7 – 17.4)	0.5 ± 0.3* (0.3 – 0.9)	15.7 ± 1.3 (14.3 – 17.3)	64.6 ± 4.0* (61.6 – 70.4)	2.0 ± 0.2 (1.7 – 2.1)
Std 1	15.3	57.0	2.8	12.1	31.9	1.1	21.0	100.3	1.9
Std 2	15.2	53.9	2.7	15.3	39.7	1.8	24.3	97.0	3.2

Table D-17. continued.

Location/ Treatment	Daidzin	Malonyl-Daidzin	Daidzein	Glycitin	Malonyl-glycitin	Glycitein	Genistin	Malonyl-genistin	Genistein
Londrina	Mean ± Standard Deviation (range) mg/100 g dry weight								
Isoline	19.6 ± 1.4 (18.5 – 21.7)	74.5 ± 6.0 (70.8 – 83.4)	2.0 ± 0.4 (1.5 – 2.3)	5.5 ± 0.2 (5.2 – 5.7)	15.9 ± 0.9 (15.2 – 17.2)	<0.9 (nd – 1.0)	22.9 ± 1.5 (21.8 – 25.0)	112.6 ± 9.7 (105.5 – 126.9)	1.4 ± 0.1 (1.3 – 1.5)
CV127	13.1 ± 1.2* (12.2 – 14.9)	45.8 ± 3.7* (41.8 – 50.8)	1.5 ± 0.5* (0.9 – 1.7)	4.9 ± 0.6 (4.1 – 5.5)	12.9 ± 1.4* (11.1 – 14.5)	<0.7 (nd – 0.8)	20.5 ± 3.3 (17.8 – 25.3)	94.7 ± 11.5 (86.1 – 110.8)	1.6 ± 0.3 (1.3 – 1.9)
CV127 + imi	12.6 ± 0.6* (12.1 – 13.4)	41.1 ± 3.1* (37.8 – 45.2)	1.6 ± 0.4* (1.0 – 1.8)	5.7 ± 1.0 (4.2 – 6.2)	14.4 ± 2.7 (10.4 – 16.3)	0.7 ± 0.3 (0.3 – 0.9)	17.7 ± 2.5* (16.0 – 21.4)	79.4 ± 13.1* (67.5 – 97.9)	1.7 ± 0.1 (1.5 – 1.8)
Std 1	18.3	72.7	2.7	10.4	29.5	1.3	26.7	130.7	1.9
Std 2	19.3	70.8	2.9	11.5	28.5	1.5	30.7	128.7	2.9
Brasília									
Isoline	14.2 ± 1.6 (12.5 – 15.9)	57.0 ± 8.8 (46.0 – 67.3)	2.5 ± 0.4 (2.2 – 3.0)	5.9 ± 0.9 (5.2 – 7.0)	18.1 ± 3.1 (15.5 – 22.7)	0.9 ± 0.1 (0.8 – 1.0)	14.2 ± 1.9 (11.7 – 15.8)	78.5 ± 11.8 (61.7. – 89.5)	1.6 ± 0.2 (1.3 – 1.8)
CV127	10.8 ± 0.7* (10.2 – 11.6)	39.1 ± 4.9* (33.7 – 43.6)	2.0 ± 0.3 (1.6 – 2.4)	5.9 ± 1.0 (5.2 – 7.3)	15.2 ± 2.3 (13.8 – 18.6)	0.8 ± 0.1 (0.7 – 0.8)	12.5 ± 2.0 (9.5 – 13.6)	59.5 ± 10.0* (45.7 – 69.5)	1.4 ± 0.4 (0.9 – 1.7)
CV127 + imi	11.7 ± 0.3* (11.5 – 12.2)	39.1 ± 2.5* (36.4 – 42.2)	2.2 ± 0.3 (1.9 – 2.5)	6.4 ± 0.3 (6.1 – 6.7)	16.7 ± 1.2 (15.3 – 18.3)	0.9 ± 0.1 (0.8 – 0.9)	11.6 ± 1.7 (10.3 – 14.1)	54.2 ± 8.2* (48.5 – 66.4)	1.7 ± 0.3 (1.4 – 1.9)
Std 1	14.4	67.3	1.7	11.7	33.8	1.4	20.1	109.6	1.2
Std 2	15.1	70.7	1.2	12.8	34.8	0.6	20.4	118.7	1.1
Santo Antônio de Posse									
Isoline	12.5 ± 1.4 (10.6 – 13.7)	38.8 ± 5.7 (33.4 – 43.8)	1.6 ± 0.5 (0.9 – 2.0)	5.8 ± 0.6 (5.2 – 6.4)	15.6 ± 0.5 (15.2 – 16.2)	0.7 ± 0.2 (0.4 – 0.9)	12.5 ± 2.7 (9.2 – 15.5)	59.7 ± 11.3 (46.7 – 72.1)	1.3 ± 0.2 (1.2 – 1.6)
CV127	11.2 ± 0.5 (10.8 – 11.8)	31.0 ± 1.4* (29.9 – 33.0)	1.2 ± 0.1 (1.0 – 1.3)	5.9 ± 0.9 (5.1 – 7.1)	13.2 ± 1.4* (12.0 – 14.9)	0.6 ± 0.2 (0.3 – 0.7)	12.7 ± 1.6 (11.1 – 14.8)	54.5 ± 7.2 (46.7 – 63.7)	1.3 ± 0.2 (1.1 – 1.6)
CV127 + imi	11.1 ± 1.6 (9.6 – 13.4)	31.5 ± 4.9* (27.2 – 38.4)	1.3 ± 0.2 (1.1 – 1.6)	5.1 ± 1.2 (3.9 – 6.5)	11.9 ± 2.4* (8.9 – 14.5)	0.7 ± 0.2 (0.3 – 0.8)	14.4 ± 2.4 (12.3 – 17.5)	60.2 ± 10.6 (49.4 – 73.7)	1.4 ± 0.1 (1.2 – 1.5)
Std 1	15.2	54.9	2.4	14.0	38.8	0.7	23.9	102.7	1.9
Std 2	20.6	68.1	2.9	22.9	50.7	1.8	27.6	106.6	3.1

*Statistically significantly different from the isoline control at $P < 0.05$.
^nd = not detected

Table D-18. Isoflavone Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location/ Treatment	Daidzin	Malonyl-Daidzin	Daidzein	Glycitin	Malonyl-glycitin	Glycitein	Genistin	Malonyl-genistin	Genistein
	Mean ± Standard Deviation (range) mg/100 g dry weight								
Santo Antônio de Goiás									
Isoline	14.2 ± 1.4 (12.2 – 15.3)	91.4 ± 2.9 (87.2 – 94.0)	0.8 ± 0.1 (0.7 – 0.9)	3.7 ± 0.5 (3.1 – 4.2)	13.3 ± 0.3 (13.0 – 13.8)	0.6 ± 0.2 (0.3 – 0.8)	17.2 ± 3.7 (12.0 – 20.8)	116.6 ± 9.0 (107.8 – 126.8)	nd
CV127	9.2 ± 0.5* (8.6 – 9.8)	56.5 ± 3.8* (51.7 – 59.8)	<0.5* (nd – 0.5)	3.3 ± 0.5 (2.7 – 3.9)	11.2 ± 1.8 (8.8 – 13.1)	<0.8 (nd^ – 0.8)	10.0 ± 1.9* (8.4 – 12.1)	84.2 ± 1.8* (82.3 – 86.6)	nd
CV127 + imi	9.3 ± 0.5* (8.7 – 9.9)	58.6 ± 3.0* (56.1 – 62.3)	0.6 ± 0* (0.5 – 0.6)	3.6 ± 0.5 (3.0 – 4.2)	11.8 ± 2.3 (9.6 – 14.2)	nd	11.8 ± 0.6* (11.2 – 12.4)	83.6 ± 3.6* (78.5 – 86.6)	nd
Std 1	13.9	94.1	0.7	7.5	26.9	0.7	17.7	119.6	nd
Std 2	10.4	71.5	0.8	6.6	23.4	1.8	16.0	108.1	nd
Teresina									
Isoline	6.2 ± 1.4 (4.9 – 7.7)	16.8 ± 3.7 (13.9 – 20.9)	0.7 ± 0.2 (0.4 – 0.8)	2.8 ± 1.2 (1.6 – 3.9)	7.7 ± 3.0 (4.7 – 10.7)	1.5 ± 0.3 (1.1 – 1.7)	5.6 ± 1.6 (3.8 – 6.8)	30.0 ± 8.0 (21.7 – 37.7)	0.5 ± 0.2 (0.3 – 0.7)
CV127	6.9 ± 0.8 (6.3 – 7.8)	20.0 ± 4.2 (15.3 – 23.4)	0.7 ± 0.1 (0.6 – 0.8)	4.0 ± 0.8 (3.1 – 4.5)	10.2 ± 2.4 (7.5 – 12.0)	< 0.8 (nd – 0.8)	6.7 ± 2.5 (4.1 – 9.1)	31.2 ± 9.3 (22.1 – 40.6)	0.5 ± 0.2 (0.4 – 0.8)
CV127 + imi	5.7 ± 1.5 (4.0 – 6.7)	16.7 ± 6.4 (9.7 – 22.3)	<1.0 (nd – 1.2)	3.3 ± 1.2 (1.9 – 4.2)	7.8 ± 3.0 (4.4 – 10.2)	<0.4* (nd – 0.4)	4.0 ± 2.0 (2.2 – 6.2)	17.0 ± 9.0 (8.9 – 26.6)	< 0.4 (nd – 0.4)
Std 1	9.4	28.0	1.8	9.6	20.6	1.4	15.9	65.1	1.9
Std 2	8.0	22.1	1.0	10.8	23.4	1.4	10.2	43.8	1.4

Table D-18. continued.

Location/ Treatment	Daidzin	Malonyl-Daidzin	Daidzein	Glycitin	Malonyl-glycitin	Glycitein	Genistin	Malonyl-genistin	Genistein
	Mean ± Standard Deviation (range) mg/100 g dry weight								
Vilhena									
Isoline	14.5 ± 1.7 (12.1 – 15.8)	61.1 ± 7.3 (51.9 – 67.0)	1.3 ± 0.2 (1.0 – 1.5)	3.9 ± 0.6 (3.1 – 4.6)	11.0 ± 1.4 (9.6 – 12.7)	<0.9 (nd – 0.9)	17.3 ± 0.7 (16.7 – 18.3)	101.6 ± 10.6 (87.9 – 111.2)	0.7 ± 0.2 (0.5 – 0.9)
CV127	9.7 ± 1.0* (8.7 – 10.7)	40.1 ± 4.5* (35.3 – 45.8)	0.8 ± 0.1* (0.7 – 0.9)	4.0 ± 0.4 (3.7 – 4.4)	11.8 ± 1.6 (10.6 – 14.1)	< 0.7 (nd – 0.7)	14.6 ± 2.2* (12.3 – 17.4)	71.6 ± 9.1* (62.1 – 82.8)	0.6 ± 0.1 (0.5 – 0.8)
CV127 + imi	9.8 ± 0.6* (9.0 – 10.5)	40.7 ± 3.2* (38.1 – 45.4)	0.9 ± 0* (0.9 – 1.0)	4.1 ± 0.7 (3.6 – 5.1)	10.7 ± 0.5 (10.2 – 11.2)	<0.4* (nd – 0.5)	14.0 ± 1.1* (12.6 – 15.3)	70.7 ± 5.1* (65.1 – 75.4)	0.6 ± 0.1 (0.5 – 0.6)
Std 1	15.7	73.9	1.2	7.5	22.2	0.8	14.9	102.2	0.5
Std 2	15.5	63.4	2.0	11.0	26.2	1.5	20.4	118.0	1.1
Brasília									
Isoline	29.6 ± 0.5 (29.1 – 30.2)	196.1 ± 6.3 (191.1 – 205.0)	1.0 ± 0.2 (0.8 – 1.2)	5.5 ± 0.5 (5.2 – 6.2)	18.4 ± 1.6 (16.9 – 20.0)	1.0 ± 0.2 (0.6 – 1.1)	34.4 ± 7.2 (28.1 – 40.6)	224.4 ± 3.4 (220.9 – 227.3)	<0.4 (nd – 0.4)
CV127	19.6 ± 1.2* (18.6 – 21.2)	131.7 ± 5.4* (125.2 – 137.5)	0.6 ± 0.1* (0.5 – 0.7)	4.4 ± 0.6* (3.8 – 5.2)	14.9 ± 2.0* (12.8 – 17.2)	<0.8 (nd – 0.8)	27.8 ± 4.6 (22.9 – 32.0)	194.8 ± 3.3* (191.7 – 198.2)	< 0.3 (nd – 0.3)
CV127 + imi	20.6 ± 0.6* (19.7 – 21.1)	134.5 ± 1.1* (133.8 – 136.2)	0.7 ± 0.1* (0.5 – 0.8)	4.6 ± 0.5* (4.2 – 5.3)	14.2 ± 1.4* (12.4 – 15.9)	<0.6 (nd – 0.6)	30.8 ± 5.2 (23.2 – 34.3)	200.4 ± 8.9* (192.9 – 210.8)	<0.4 (nd – 0.4)
Std 1	26.9	157.5	1.4	8.0	28.0	1.2	29.0	216.7	0.3
Std 2	25.1	156.5	1.0	10.5	30.2	1.4	35.7	217.5	0.4

*Statistically significantly different from the isoline control at $P \leq 0.05$.
^nd = not detected

Table D-19. Phospholipid Composition of Grain of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location	Treatment	N	Phosphatidyl ethanolamine	Phosphatidic acid	Phosphatidyl inositol	Phosphatidyl choline
			Mean ± Standard Deviation (range) mg/g fat			
Santo Antônio de Goiás	Isoline	4	107.1 ± 4.1 (101.0 – 109.4)	3.8 ± 1.3 (2.0 – 5.1)	11.7 ± 0.5 (11.1 – 12.1)	31.6 ± 0.9 (30.4 – 32.7)
	CV127	4	102.3 ± 5.5 (96.1 – 107.6)	2.8 ± 0.3 (2.5 – 3.1)	10.1 ± 0.4 (9.7 – 10.6)	29.5 ± 1.9 (27.7 – 32.1)
	CV127 + imi	4	103.3 ± 4.7 (96.8 – 107.3)	3.1 ± 0.1 (2.9 – 3.2)	10.1 ± 0.5 (9.7 – 10.7)	30.7 ± 1.2 (29.5 – 32.3)
	Std 1	1	104.5	3.7	12.3	32.6
	Std 2	1	91.3	5.6	11.5	30.4
Uberaba	Isoline	4	114.5 ± 16.1 (92.2 – 127.9)	2.3 ± 0.5 (1.8 – 2.9)	12.2 ± 1.3 (10.5 – 13.5)	33.5 ± 5.4 (25.9 – 38.5)
	CV127	4	98.5 ± 7.9 (93.5 – 110.3)	1.0 ± 0.1 (0.8 – 1.1)	10.0 ± 0.9 (8.9 – 11.0)	30.4 ± 2.5 (28.6 – 34.1)
	CV127 + imi	4	103.5 ± 3.6* (101.0 – 108.9)	1.4 ± 0.4 (1.0 – 1.9)	9.8 ± 1.0* (8.3 – 10.4)	30.1 ± 1.0 (28.8 – 30.7)
	Std 1	1	108.7	2.1	12.2	38.2
	Std 2	1	103.4	3.4	12.9	27.0
Sete Lagoas	Isoline	4	97.9 ± 2.6 (94.6 – 100.9)	5.7 ± 1.0 (4.6 – 6.9)	12.0 ± 0.5 (11.5 – 12.4)	26.9 ± 1.1 (25.4 – 27.8)
	CV127	4	93.6 ± 3.5 (90.1 – 98.4)	3.7 ± 0.5* (3.2 – 4.3)	9.8 ± 0.8* (9.3 – 10.9)	25.9 ± 0.9 (24.8 – 26.7)
	CV127 + imi	4	78.5 ± 21.7* (57.9 – 99.2)	2.8 ± 0.4* (2.5 – 3.2)	8.2 ± 2.3* (6.1 – 10.7)	21.3 ± 5.3* (15.9 – 26.7)
	Std 1	1	113.7	4.8	13.1	33.2
	Std 2	1	79.4	7.5	11.4	25.6
Londrina	Isoline	4	96.3 ± 9.0 (89.7 – 109.6)	2.5 ± 0.5 (2.0 – 3.2)	10.4 ± 1.3 (9.2 – 12.2)	25.8 ± 0.8 (24.9 – 26.7)
	CV127	4	94.9 ± 5.3 (90.2 – 100.7)	2.9 ± 0.5 (2.4 – 3.5)	10.0 ± 0.7 (9.2 – 10.9)	28.7 ± 1.8 (26.7 – 30.3)
	CV127 + imi	4	91.1 ± 0.3 (90.9 – 91.5)	3.2 ± 0.2 (3.1 – 3.4)	10.6 ± 0.7 (9.7 – 11.2)	27.0 ± 0.3 (26.6 – 27.4)
	Std 1	1	100.8	2.3	12.6	31.3
	Std 2	1	94.4	3.5	12.1	29.4
Brasília	Isoline	4	95.5 ± 5.1 (91.7 – 102.9)	4.6 ± 0.7 (4.0 – 5.5)	11.7 ± 1.0 (10.7 – 13.0)	28.8 ± 1.6 (27.6 – 31.1)
	CV127	4	92.4 ± 4.8* (88.5 – 98.6)	3.0 ± 0.7 (2.3 – 3.7)	9.5 ± 1.2* (7.9 – 10.7)	27.2 ± 1.1 (25.8 – 28.6)
	CV127 + imi	4	93.6 ± 7.3* (84.8 – 102.6)	3.5 ± 1.3 (1.6 – 4.7)	10.4 ± 0.5* (9.9 – 10.9)	27.5 ± 2.5 (25.1 – 30.6)
	Std 1	1	97.4	2.5	11.6	30.6
	Std 2	1	98.1	2.9	11.4	31.8
Santo Antônio de Posse	Isoline	4	100.1 ± 6.9 (95.0 – 110.0)	5.2 ± 0.6 (4.4 – 5.9)	12.9 ± 1.0 (11.8 – 14.2)	29.0 ± 2.1 (27.3 – 31.9)
	CV127	4	74.0 ± 16.3* (51.0 – 87.6)	3.8 ± 0.6* (3.3 – 4.6)	9.3 ± 1.7* (6.8 – 10.6)	20.6 ± 4.2 (14.9 – 23.9)
	CV127 + imi	4	75.2 ± 18.2* (49.4 – 88.5)	3.6 ± 0.8* (2.7 – 4.4)	8.8 ± 1.8* (6.2 – 10.0)	21.9 ± 5.5 (13.9 – 25.8)
	Std 1	1	101.4	4.6	13.5	32.0
	Std 2	1	84.9	6.0	12.6	26.8

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-20. Phospholipid Composition of Grain of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location	Treatment	N	Phosphatidyl ethanolamine	Phosphatidic acid	Phosphatidyl inositol	Phosphatidyl choline
			Mean ± Standard Deviation (range) mg/g fat			
Santo Antônio de Goiás	Isoline	4	136.5 ± 20.7 (113.8 – 156.8)	0.9 ± 0.2 (0.7 – 1.1)	12.1 ± 2.3 (10.1 – 14.8)	39.5 ± 5.0 (34.7 – 45.0)
	CV127	4	135.6 ± 14.6 (120.6 – 155.5)	1.0 ± 0.2 (0.7 – 1.1)	11.3 ± 1.3 (10.2 – 13.2)	37.4 ± 1.8 (35.4 – 39.4)
	CV127 + imi	4	126.1 ± 9.9 (115.9 – 139.5)	0.9 ± 0.2 (0.6 – 1.1)	10.6 ± 0.4 (10.1 – 11.0)	38.1 ± 1.8 (35.8 – 40.2)
	Std 1	1	132.2	0.9	11.7	39.1
	Std 2	1	133.7	1.4	12.4	42.0
Teresina	Isoline	3	68.6 ± 3.7 (65.7 – 72.7)	5.6 ± 1.0 (4.9 – 6.7)	8.8 ± 0.1 (8.7 – 8.9)	21.6 ± 1.5 (20.6 – 23.4)
	CV127	3	63.6 ± 1.8 (61.7 – 65.2)	5.0 ± 0.9 (4.3 – 6.1)	8.7 ± 0.8 (8.1 – 9.6)	21.2 ± 1.8 (19.8 – 23.2)
	CV127 + imi	3	61.8 ± 14.0 (52.1 – 77.8)	8.1 ± 1.6* (7.0 – 9.9)	8.7 ± 0.9 (8.0 – 9.8)	19.2 ± 2.8 (17.5 – 22.4)
	Std 1	1	70.1	5.3	10.6	24.3
	Std 2	1	51.1	7.2	8.1	17.0
Vilhena	Isoline	4	98.0 ± 6.4 (88.9 – 103.8)	2.2 ± 0.2 (1.8 – 2.3)	9.6 ± 0.7 (8.6 – 10.1)	30.9 ± 1.9 (28.6 – 33.1)
	CV127	4	102.7 ± 4.9 (96.0 – 107.8)	2.4 ± 0.6 (2.0 – 3.2)	9.8 ± 1.0 (9.2 – 11.3)	31.5 ± 1.5 (30.2 – 33.5)
	CV127 + imi	4	107.0 ± 10.5 (98.1 – 120.7)	2.1 ± 0.2 (1.9 – 2.4)	9.6 ± 0.9 (8.4 – 10.2)	32.0 ± 0.5 (31.3 – 32.4)
	Std 1	1	116.2	2.8	12.1	39.0
	Std 2	1	86.6	4.7	10.6	30.4
Brasília	Isoline	4	124.2 ± 13.1 (114.7 – 142.7)	0.8 ± 0.1 (0.7 – 0.9)	10.6 ± 0.8 (9.7 – 11.7)	36.8 ± 3.1 (32.9 – 40.4)
	CV127	4	123.4 ± 10.7 (112.4 – 135.8)	0.8 ± 0.1 (0.6 – 0.9)	10.0 ± 0.3 (9.7 – 10.4)	36.1 ± 2.1 (33.7 – 38.2)
	CV127 + imi	4	117.9 ± 6.2 (110.2 – 124.8)	0.8 ± 0.2 (0.5 – 0.9)	9.8 ± 0.5 (9.2 – 10.3)	37.5 ± 1.7 (35.0 – 39.0)
	Std 1	1	107.2	0.9	10.8	37.2
	Std 2	1	106.7	0.8	10.5	38.5

*Statistically significantly different from the isoline control at $P \leq 0.05$.

Table D-21. Antinutrient Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Six Locations in Brazil in the 2006/07 Season

Location	Treatment	N	Rafffinose (g/100 g dry wt.)	Stachyose (g/100 g dry wt.)	Trypsin Inhib. (TIU/mg dry wt.)	Urease (ΔpH)	Lectin (HU/mg dry wt.)	Phytate (mg/g dry wt.)
			Mean ± Standard Deviation (range)					
Santo Antônio de Goiás	Isoline	4	1.1 ± 0.1 (1.0 – 1.2)	4.0 ± 0.2 (3.8 – 4.2)	11.45 ± 1.66 (9.28 – 12.95)	2.05 ± 0.05 (2.01 – 2.12)	2.35 ± 0.68 (1.36 – 2.79)	2.85 ± 0.86 (2.12 – 4.01)
	CV127	4	1.1 ± 0.2 (1.0 – 1.3)	3.8 ± 0.1 (3.6 – 3.9)	15.56 ± 0.73* (14.98 – 16.58)	2.10 ± 0.04 (2.05 – 2.13)	2.27 ± 0.62 (1.54 – 3.01)	2.89 ± 0.70 (1.87 – 3.41)
	CV127 + imi	4	1.1 ± 0.1 (1.0 – 1.2)	3.6 ± 0.1* (3.5 – 3.8)	13.29 ± 1.20* (12.95 – 14.73)	2.12 ± 0.03 (2.08 – 2.15)	2.60 ± 0.81 (1.57 – 3.49)	3.16 ± 1.44 (2.06 – 5.27)
	Std 1	1	1.2	4.1	6.11	2.05	3.53	1.47
	Std 2	1	1.0	3.7	9.59	2.10	1.52	2.65
Uberaba	Isoline	4	1.1 ± 0.1 (1.0 – 1.1)	4.1 ± 0.1 (3.9 – 4.1)	7.92 ± 3.36 (6.03 – 12.95)	2.05 ± 0.05 (2.01 – 2.12)	2.28 ± 0.74 (1.75 – 3.35)	2.31 ± 0.63 (1.89 – 3.23)
	CV127	4	1.2 ± 0.1 (1.1 – 1.3)	3.8 ± 0.2 (3.6 – 4.0)	10.15 ± 1.41 (8.69 – 12.01)	2.09 ± 0.04 (2.05 – 2.13)	2.01 ± 0.49* (1.46 – 2.57)	2.47 ± 0.86 (1.25 – 3.22)
	CV127 + imi	4	1.2 ± 0.1 (1.1 – 1.3)	3.9 ± 0.2 (3.7 – 4.1)	9.72 ± 0.91 (8.97 – 10.98)	2.02 ± 0.14 (1.81 – 2.13)	1.37 ± 0.16* (1.27 – 1.56)	2.10 ± 1.05 (0.93 – 3.36)
	Std 1	1	1.1	4.6	5.40	2.07	1.38	2.19
	Std 2	1	1.0	3.7	5.03	2.06	1.58	2.02
Sete Lagoas	Isoline	4	1.4 ± 0.1 (1.3 – 1.5)	3.2 ± 0.1 (3.0 – 3.2)	11.71 ± 0.36 (11.30 – 12.14)	1.64 ± 0.25 (1.27 – 1.80)	2.65 ± 0.41 (2.20 – 3.15)	3.19 ± 1.59 (1.43 – 4.94)
	CV127	4	1.7 ± 0.1* (1.6 – 1.8)	3.1 ± 0.2 (2.9 – 3.4)	12.65 ± 2.60 (10.28 – 16.08)	1.21 ± 0.49* (0.67 – 1.74)	2.45 ± 0.94 (1.68 – 3.70)	3.02 ± 0.71 (2.38 – 3.89)
	CV127 + imi	4	1.5 ± 0.2* (1.3 – 1.7)	3.2 ± 0.1 (3.1 – 3.2)	11.62 ± 0.80 (10.44 – 12.17)	1.47 ± 0.40* (0.90 – 1.79)	2.83 ± 0.34 (2.42 – 3.25)	2.22 ± 0.49 (1.60 – 2.77)
	Std 1	1	1.6	3.7	10.73	1.43	2.13	2.28
	Std 2	1	1.0	3.1	11.06	1.65	1.68	2.05

Table D-21. continued.

Location	Treatment	N	Rafffinose (g/100 g dry wt.)	Stachyose (g/100 g dry wt.)	Trypsin Inhib. (TIU/ mg dry wt.)	Urease (ΔpH)	Lectin (HU/mg dry wt.)	Phytate (mg/g dry wt.)
			Mean ± Standard Deviation (range)					
Londrina	Isoline	4	1.1 ± 0.1 (1.0 – 1.2)	3.8 ± 0.2 (3.6 – 4.0)	14.58 ± 1.82 (12.08 – 16.40)	1.74 ± 0.13 (1.66 – 1.93)	1.74 ± 0.26 (1.52 – 2.10)	3.56 ± 1.43 (2.22 – 5.37)
	CV127	4	1.3 ± 0.1 (1.2 – 1.3)	3.7 ± 0.1 (3.6 – 3.7)	13.14 ± 1.63 (11.23 – 15.21)	1.68 ± 0.15 (1.55 – 1.89)	2.19 ± 0.76 (31.86 – 65.25)	2.64 ± 0.91 (1.45 – 3.51)
	CV127 + imi	4	1.2 ± 0.1* (1.1 – 1.3)	3.8 ± 0.1 (3.7 – 3.9)	12.92 ± 1.66 (11.32 – 15.13)	1.78 ± 0.25 (1.60 – 2.15)	2.52 ± 0.74 (1.49 – 3.24)	1.72 ± 0.80 (0.71 – 2.50)
	Std 1	1	1.2	4.1	13.43	1.78	1.85	3.65
	Std 2	1	0.8	3.6	17.40	1.83	2.17	2.38
Brasília	Isoline	4	1.0 ± 0.1 (0.9 – 1.0)	3.6 ± 0.2 (3.3 – 3.8)	15.74 ± 0.65 (14.94 – 16.39)	2.09 ± 0.01 (2.09 – 2.10)	2.21 ± 0.85 (1.35 – 3.20)	3.22 ± 1.98 (1.73 – 6.09)
	CV127	4	1.2 ± 0.1* (1.2 – 1.3)	3.6 ± 0.2 (3.3 – 3.8)	11.08 ± 0.82 (10.48 – 12.25)	2.06 ± 0.11 (1.90 – 2.14)	2.97 ± 0.43 (2.59 – 3.47)	2.86 ± 1.33 (1.45 – 4.52)
	CV127 + imi	4	1.2 ± 0.1* (1.1 – 1.2)	3.6 ± 0.1 (3.5 – 3.7)	11.64 ± 0.75 (10.54 – 12.25)	2.17 ± 0.04 (2.13 – 2.21)	1.81 ± 0.70 (1.44 – 2.85)	2.96 ± 0.66 (2.42 – 3.91)
	Std 1	1	1.0	4.2	14.54	2.15	1.60	3.99
	Std 2	1	0.9	3.8	19.64	2.16	2.48	7.39
Santo Antônio de Posse	Isoline	4	1.2 ± 0.1 (1.1 – 1.3)	3.7 ± 0.1 (3.5 – 3.8)	12.37 ± 0.63 (11.66 – 13.10)	1.99 ± 0.03 (1.96 – 2.02)	2.20 ± 0.73 (1.60 – 3.16)	2.58 ± 0.67 (1.80 – 3.31)
	CV127	4	1.7 ± 0.1* (1.6 – 1.7)	3.4 ± 0.1* (3.3 – 3.5)	11.73 ± 1.73 (10.34 – 14.24)	1.96 ± 0.16 (1.79 – 2.18)	1.49 ± 0.05*Z (1.43 – 1.54)	2.62 ± 0.90 (1.53 – 3.39)
	CV127 + imi	4	1.5 ± 0.2* (1.2 – 1.7)	3.4 ± 0.1* (3.3 – 3.4)	12.88 ± 1.17 (11.76 – 14.49)	1.91 ± 0.02 (1.88 – 1.94)	1.87 ± 0.84* (1.36 – 3.12)	3.08 ± 0.97 (1.75 – 3.87)
	Std 1	1	1.3	4.1	10.88	1.96	2.35	2.09
	Std 2	1	1.0	3.3	13.58	1.94	2.13	2.53

*Statistically significantly different from the isoline control at $P < 0.05$.

Table D-22. Antinutrient Composition of Grain on a Dry-Weight Basis of CV127 Soybean Treatments (CV127 and CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Grown at Four Locations in Brazil in the 2007 Season

Location	Treatment	N	Raffinose (g/100 g dry wt.)	Stachyose (g/100 g dry wt.)	Trypsin Inhib. (TIU/mg dry wt.)	Urease (ΔpH)	Lectin (HU/mg dry wt.)	Phytate (mg/g dry wt.)
			Mean ± Standard Deviation (range)					
Santo Antônio de Goiás	Isoline	4	1.4 ± 0.1 (1.2 – 1.5)	4.2 ± 0.1 (4.1 – 4.2)	13.38 ± 1.15 (11.88 – 14.68)	1.81 ± 0.09 (1.71 – 1.92)	1.31 ± 0.03 (1.28 – 1.34)	2.70 ± 0.85 (1.89 – 3.72)
	CV127	4	1.2 ± 0.1* (1.1 – 1.2)	3.9 ± 0.1* (3.7 – 4.0)	13.84 ± 0.52 (13.05 – 14.13)	1.78 ± 0.10 (1.69 – 1.91)	0.94 ± 0.46 (0.33 – 1.29)	4.29 ± 1.05* (3.06 – 5.62)
	CV127 + imi	4	1.2 ± 0.1* (1.1 – 1.3)	3.9 ± 0.1* (3.8 – 4.0)	13.27 ± 0.67 (12.46 – 14.05)	1.84 ± 0.09 (1.75 – 1.95)	0.57 ± 0.49* (0.22 – 1.29)	3.81 ± 0.74 (2.75 – 4.32)
	Std 1	1	1.4	4.8	13.62	1.92	0.67	5.04
	Std 2	1	1.2	4.0	16.76	1.74	1.66	2.52
Teresina	Isoline	3	1.4 ± 0.1 (1.3 – 1.4)	3.4 ± 0.3 (3.0 – 3.6)	10.02 ± 1.33 (8.48 – 10.82)	2.04 ± 0.02 (2.03 – 2.06)	1.46 ± 0.22 (1.33 – 1.72)	4.57 ± 1.83 (2.50 – 6.00)
	CV127	3	1.3 ± 0 (1.3)	3.2 ± 0.2 (3.0 – 3.4)	9.64 ± 1.47 (8.16 – 11.09)	1.95 ± 0.02* (1.94 – 1.97)	1.71 ± 0 (1.71)	3.13 ± 0.39 (2.90 – 3.58)
	CV127 + imi	3	1.5 ± 0.3 (1.2 – 1.7)	2.6 ± 0.3* (2.4 – 3.0)	10.31 ± 2.53 (7.82 – 12.87)	1.96 ± 0.02* (1.94 – 1.97)	2.29 ± 0.99 (1.71 – 3.43)	3.09 ± 0.41 (2.63 – 3.38)
	Std 1	1	1.5	3.8	9.84	1.98	1.34	2.48
	Std 2	1	1.4	3.1	12.56	2.02	2.67	4.73

Table D-22. continued.

Location	Treatment	N	Raffinose (g/100 g dry wt.)	Stachyose (g/100 g dry wt.)	Trypsin Inhib. (TIU/mg dry wt.)	Urease (ΔpH)	Lectin (HU/mg dry wt.)	Phytate (mg/g dry wt.)
			Mean ± Standard Deviation (range)					
Vilhena	Isoline	4	1.3 ± 0.2 (1.2 – 1.5)	4.0 ± 0.3 (3.7 – 4.3)	14.25 ± 2.52 (12.44 – 17.97)	0.40 ± 0.15 (0.28 – 0.61)	1.50 ± 0.43 (0.85 – 1.71)	3.23 ± 0.55 (2.81 – 4.01)
	CV127	4	1.2 ± 0.1 (1.1 – 1.3)	3.8 ± 0.3 (3.4 – 4.0)	17.30 ± 0.99* (16.28 – 18.20)	0.67 ± 0.25 (0.41– 0.91)	0.17 ± 0* (0.17)	3.92 ± 1.17 (2.66 – 5.49)
	CV127 + imi	4	1.2 ± 0.1 (1.1 – 1.3)	3.9 ± 0.1 (3.7 – 4.0)	17.49 ± 0.36* (17.22 – 18.03)	0.65 ± 0.17 (0.49 – 0.87)	0.13 ± 0.03* (0.11 – 0.17)	4.57 ± 0.31* (4.12 – 4.77)
	Std 1	1	1.2	4.4	14.61	1.16	1.71	4.79
	Std 2	1	1.4	3.7	16.66	0.27	1.71	2.37
Brasília	Isoline	4	1.1 ± 0.1 (1.0 – 1.1)	4.4 ± 0.2 (4.2 – 4.6)	14.21 ± 0.39 (13.90 – 14.73)	2.00 ± 0.02 (1.98 – 2.03)	2.47 ± 1.13 (1.29 – 3.43)	3.25 ± 0.90 (2.19 – 4.19)
	CV127	4	1.0 ± 0.1 (0.9 – 1.1)	4.0 ± 0.1* (3.9 – 4.2)	13.08 ± 0.48 (12.45 – 13.57)	1.98 ± 0.02 (1.96 – 2.00)	0.75 ± 0.54* (0.26 – 1.37)	4.31 ± 1.80 (2.57 – 6.22)
	CV127 + imi	4	1.0 ± 0.1 (0.9 – 1.1)	3.9 ± 0.1* (3.8 – 4.1)	13.26 ± 1.35 (11.35 – 14.44)	1.98 ± 0.05 (1.93 – 2.03)	1.03 ± 0.84* (0.34 – 2.06)	3.59 ± 0.33 (3.17 – 3.98)
	Std 1	1	1.1	4.8	14.91	1.98	1.72	5.44
	Std 2	1	1.3	4.3	13.18	2.02	1.72	5.14

*Statistically significantly different from the isoline control at $P \leq 0.05$.

Table D-23. Proximate Composition of Forage of CV127 Soybean (CV127 + Imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2), Grown at Six Locations in Brazil in the 2007/2008 Season

Location	Treatment	N	Moisture (g/100 g FW)	Ash (g/100 g DW)	Fat (g/100 g DW)	Protein (g/100 g DW)	Carbohydrates (g/100 g DW)	Calories (kcal/100 g DW)
			Mean ± Standard Deviation (range)					
Santo Antônio de Goiás	Isoline	3	82.0 ± 1.0 (81.2 – 83.1)	9.1 ± 0.9 (8.2 – 9.9)	2.3 ± 0.6 (1.7 – 2.7)	17.9 ± 0.4 (17.6 – 18.3)	70.8 ± 1.0 (69.8 – 71.8)	375 ± 8 (367 – 383
	CV127 + imi	3	81.8 ± 0.8 (81.1 – 82.7)	9.3 ± 1.1 (8.3 – 10.4)	3.0 ± 0.2 (2.7 – 3.1)	17.9 ± 1.3 (16.5 – 19.0)	69.7 ± 0.9 (68.8 – 70.5)	380 ± 4 (376 – 383)
	Std 1	3	82.9 ± 0.7 (82.2 – 83.5)	10.9 ± 0.3* (10.6 – 11.2)	2.7 ± 0.3 (2.5 – 3.1)	21.3 ± 3.2* (17.6 – 23.1)	65.1 ± 3.6* (62.4 – 69.2)	370 ± 2* (368 – 371)
	Std 2	3	82.0 ± 1.3 (80.9 – 83.5)	10.2 ± 0.4 (9.7 – 10.5)	2.6 ± 0.4 (2.3 – 3.0)	19.3 ± 1.4 (17.9 – 20.7)	68.2 ± 2.1 (66.1 – 70.2)	375 ± 3 (372 – 377)
Uberaba	Isoline	3	79.8 ± 0.8 (79.0 – 80.6)	7.9 ± 0.7 (7.1 – 8.5)	2.5 ± 0.3* (2.1 – 2.7)	18.4 ± 0.2 (18.3 – 18.6)	71.3 ± 0.6 (70.8 – 71.9)	381 ± 5 (376 – 386)
	CV127 + imi	3	79.1 ± 0.3 (78.8 – 79.3)	7.3 ± 0.3 (7.0 – 7.5)	3.1 ± 0.3 (2.9 – 3.4)	17.5 ± 1.1 (16.6 – 18.7)	72.0 ± 1.4 (70.3 – 72.9)	385 ± 2 (383 – 387)
	Std 1	3	80.0 ± 1.7 (78.4 – 81.8)	8.4 ± 0.9* (7.6 – 9.3)	2.5 ± 0.2* (2.3 – 2.7)	18.6 ± 0.3 (18.3 – 18.9)	70.5 ± 0.6 (69.8 – 70.9)	378 ± 2 (376 – 380)
	Std 2	3	80.1 ± 0.7 (79.3 – 80.5)	8.4 ± 0.1 (8.3 – 8.5)	2.3 ± 0.2* (2.1 – 2.5)	18.8 ± 0.5* (18.4 – 19.3)	70.6 ± 0.5 (70.0 – 70.9)	376 ± 5* (372 – 382)
Sete Lagoas	Isoline	3	80.8 ± 0.7 (80.2 – 81.6)	8.1 ± 0.4 (7.6 – 8.3)	2.4 ± 0.3* (2.1 – 2.7)	17.9 ± 0.5 (17.5 – 18.5)	71.9 ± 0.8 (71.2 – 72.7)	380 ± 1 (379 – 381)
	CV127 + imi	3	79.4 ± 1.1 (78.4 – 80.5)	8.6 ± 0.2 (8.4 – 8.8)	1.9 ± 0.1 (1.9 – 2.0)	17.3 ± 0.4 (16.9 – 17.7)	72.2 ± 0.4 (71.8 – 72.6)	376 ± 2 (375 – 379)
	Std 1	3	81.7 ± 2.3 (79.2 – 83.7)	8.5 ± 0.4 (8.0 – 8.7)	2.2 ± 0.3 (1.9 – 2.4)	19.3 ± 0.6 (18.6 – 19.7)	70.0 ± 0.7 (69.3 – 70.7)	378 ± 4 (373 – 380)
	Std 2	3	77.9 ± 4.6 (73.1 – 82.2)	8.3 ± 1.3 (6.8 – 9.1)	2.2 ± 0.2 (1.9 – 2.3)	19.4 ± 2.1 (17.0 – 20.7)	70.1 ± 2.9 (68.4 – 73.5)	379 ± 6 (375 – 386)

Table D-23. continued

Location	Treatment	N	Moisture (g/100 g FW)	Ash (g/100 g DW)	Fat (g/100 g DW)	Protein (g/100 g DW)	Carbohydrates (g/100 g DW)	Calories (kcal/100 g DW)
			Mean ± Standard Deviation (range)					
Londrina	Isoline	3	80.7 ± 0.7 (80.1 – 81.4)	8.2 ± 0.5 (7.8 – 8.8)	2.0 ± 0.3 (1.7 – 2.2)	16.2 ± 0.9 (15.1 – 16.9)	71.3 ± 1.6 (70.1 – 73.1)	374 ± 3 (371 – 376)
	CV127 + imi	3	81.2 ± 1.1 (80.3 – 82.4)	8.8 ± 1.1 (7.6 – 9.8)	2.3 ± 0.5 (1.9 – 2.8)	17.0 ± 2.2 (15.3 – 19.5)	73.4 ± 2.7 (70.3 – 75.6)	377 ± 8 (371 – 386)
	Std 1	3	81.8 ± 0.6 (81.3 – 82.5)	8.4 ± 1.8 (6.4 – 9.7)	2.2 ± 0.2 (2.0 – 2.4)	17.5 ± 1.4 (15.9 – 18.7)	72.5 ± 3.5 (68.4 – 74.9)	377 ± 5 (372 – 381)
	Std 2	3	82.4 ± 1.1 (81.7 – 83.7)	9.1 ± 0.2 (8.9 – 9.3)	2.1 ± 0.1 (2.0 – 2.2)	17.8 ± 0.5 (17.3 – 18.3)	71.5 ± 0.5 (71.0 – 71.8)	377 ± 3 (374 – 380)
Brasilia	Isoline	3	81.9 ± 1.1 (80.9 – 83.1)	9.2 ± 1.1 (8.1 – 10.3)	3.4 ± 0.2 (3.2 – 3.5)	18.8 ± 1.0 (17.6 – 19.5)	68.5 ± 2.2 (66.9 – 71.0)	381 ± 2 (379 – 383)
	CV127 + imi	3	81.4 ± 1.1 (80.7 – 82.6)	9.3 ± 1.2 (8.6 – 10.7)	3.3 ± 0.1 (3.2 – 3.4)	17.4 ± 1.4 (16.6 – 19.0)	70.0 ± 2.9 (66.7 – 71.9)	377 ± 3 (374 – 380)
	Std 1	3	82.1 ± 0.3 (81.8 – 82.3)	8.7 ± 0.6 (8.1 – 9.3)	3.4 ± 0.1 (3.4 – 3.5)	19.3 ± 1.8 (17.6 – 21.1)	68.6 ± 2.4 (65.9 – 70.6)	383 ± 3* (380 – 385)
	Std 2	3	81.9 ± 0.5 (81.4 – 82.4)	9.0 ± 0.3 (8.7 – 9.2)	3.8 ± 0.3* (3.6 – 4.1)	20.5 ± 1.6* (19.5 – 22.4)	66.8 ± 1.8 (64.8 – 68.3)	387 ± 1* (386 – 387)
Santo Antônio de Posse	Isoline	3	83.3 ± 0.9 (82.7 – 84.3)	8.6 ± 0.4 (8.2 – 8.9)	2.2 ± 0.1 (2.2 – 2.3)	16.9 ± 0.4 (16.5 – 17.3)	72.4 ± 0.5 (71.8 – 72.8)	378 ± 3 (376 – 382)
	CV127 + imi	3	83.6 ± 1.5 (82.5 – 85.3)	8.6 ± 0.3 (8.4 – 8.9)	2.1 ± 0.4 (1.6 – 2.4)	16.7 ± 1.7 (15.3 – 18.6)	72.5 ± 1.6 (70.9 – 74.1)	377 ± 4 (374 – 381)
	Std 1	3	85.0 ± 0.3 (84.8 – 85.3)	9.2 ± 0.3 (8.9 – 9.4)	2.3 ± 0.2 (2.2 – 2.5)	18.1 ± 0.3 (17.8 – 18.3)	70.4 ± 0.3* (70.1 – 70.7)	374 ± 1 (373 – 375)
	Std 2	3	84.0 ± 0.3 (83.7 – 84.2)	8.9 ± 0.2 (8.8 – 9.1)	1.9 ± 0.3 (1.7 – 2.2)	18.6 ± 0.8 (17.7 – 19.1)	70.4 ± 0.7* (69.8 – 71.2)	375 ± 2 (373 – 377)

*Statistically significantly different from CV127 + imi at $P < 0.05$.

Table D-24. Fiber Composition of Forage of CV127 Soybean (CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2), Grown at Six Locations in Brazil in the 2007/2008 Season

Location	Treatment	N	Crude Fiber	ADF	NDF
			Mean ± Standard Deviation (range) g/100 g DW		
Santo Antônio de Goiás	Isoline	3	29.0 ± 0.9 (28.4 – 30.0)	34.87 ± 0.81 (34.04 – 35.65)	44.36 ± 3.25 (40.61 – 46.31)
	CV127 + imi	3	29.7 ± 0.6 (29.1 – 30.3)	33.66 ± 0.27 (33.39 – 33.92)	41.32 ± 1.27 (40.07 – 42.60)
	Std 1	3^	26.9 ± 2.6* (25.1 – 29.9)	32.33 ± 1.92 (30.97 – 33.69)	42.56 ± 3.79 (39.88 – 45.24)
	Std 2	3^	28.8 ± 1.3 (27.9 – 30.3)	33.45 ± 0.28 (33.25 – 33.65)	44.67 ± 0.92 (44.02 – 45.32)
Uberaba	Isoline	3	30.4 ± 1.4 (30.3 – 31.9)	36.93 ± 1.42 (35.56 – 38.39)	46.40 ± 1.89 (44.88 – 48.52)
	CV127 + imi	3	29.6 ± 1.1 (28.6 – 30.7)	35.44 ± 1.68 (33.50 – 36.47)	46.30 ± 1.93 (44.19 – 47.96)
	Std 1	3	30.1 ± 1.1 (29.4 – 31.4)	36.72 ± 1.23 (35.51 – 37.97)	44.66 ± 1.70 (42.94 – 46.33)
	Std 2	3	30.5 ± 0.6 (29.9 – 31.0)	36.05 ± 1.31 (34.65 – 37.25)	44.88 ± 0.23 (44.67 – 45.13)
Sete Lagoas	Isoline	3	28.7 ± 1.1 (27.5 – 29.7)	35.40 ± 1.15 (34.33 – 36.62)	44.77 ± 1.84 (42.69 – 46.20)
	CV127 + imi	3	28.7 ± 0.7 (27.9 – 29.1)	35.03 ± 1.24 (34.13 – 36.45)	44.56 ± 1.75 (43.11 – 46.50)
	Std 1	3	27.0 ± 0.2* (26.8 – 27.2)	35.76 ± 1.28 (34.29 – 36.66)	43.74 ± 1.71 (42.11 – 45.52)
	Std 2	3	28.8 ± 0.6 (28.3 – 29.5)	35.34 ± 0.73 (34.91 – 36.18)	43.84 ± 1.73 (42.13 – 45.58)
Londrina	Isoline	3	29.4 ± 1.6 (27.6 – 30.4)	35.91 ± 2.70 (34.34 – 39.03)	44.70 ± 3.37 (42.21 – 48.54)
	CV127 + imi	3	30.2 ± 0.9 (29.2 – 30.8)	37.26 ± 1.09 (36.08 – 38.23)	45.31 ± 0.40 (44.84 – 45.56)
	Std 1	3	29.1 ± 2.5 (26.3 – 30.6)	36.71 ± 3.93 (32.31 – 39.88)	44.78 ± 4.85 (39.33 – 48.61)
	Std 2	3	29.7 ± 1.0 (28.6 – 30.5)	36.83 ± 2.03 (35.48 – 39.17)	45.44 ± 1.79 (43.79 – 47.34)
Brasilia	Isoline	3	28.3 ± 0.4 (27.9 – 28.6)	35.08 ± 2.85 (31.79 – 36.82)	42.49 ± 2.77 (39.71 – 45.25)
	CV127 + imi	3	28.8 ± 0.6 (28.2 – 29.3)	35.97 ± 1.13 (34.75 – 36.97)	43.97 ± 0.53 (43.38 – 44.40)
	Std 1	3	27.6 ± 0.6 (26.9 – 28.1)	31.00 ± 2.81* (28.94 – 34.20)	41.68 ± 1.80 (39.68 – 43.18)
	Std 2	3	27.8 ± 1.2 (27.0 – 29.1)	32.17 ± 1.97 (30.43 – 34.30)	40.28 ± 1.21* (38.93 – 41.28)

Table D-24. continued

Location	Treatment	N	Crude Fiber	ADF	NDF
			Mean ± Standard Deviation (range) g/100 g DW		
Santo Antônio de Posse	Isoline	3	32.6 ± 0.7 (31.9 – 33.2)	41.23 ± 1.50 (39.83 – 42.81)	48.99 ± 1.96 (46.99 – 50.90)
	CV127 + imi	3	31.9 ± 1.2 (30.6 – 32.7)	41.07 ± 1.31 (39.62 – 42.16)	50.69 ± 2.18 (48.49 – 52.85)
	Std 1	3	31.8 ± 0.4 (31.6 – 32.3)	40.45 ± 2.25 (37.86 – 41.82)	48.66 ± 2.29 (46.16 – 50.67)
	Std 2	3	32.2 ± 1.3 (30.8 – 33.1)	41.33 ± 3.00 (38.67 – 44.58)	48.95 ± 2.88 (47.26 – 52.28)

*Statistically significantly different from CV127 + imi at $P < 0.05$.
^ N= 2 for ADF and NDF.

Table D-25. Proximate and Fiber Content of Soybean Forage (% of Dry Matter) as Reported in OECD (2001).

Nutrient	Soybean Forage Content (% of Dry Matter)
Moisture	74 - 79
Protein	11.2 - 17.3
Fat	3.1 - 5.1
Ash	8.8 - 10.5
NDF	34 - 40
ADF	32 - 38

Appendix E

Methods and Results of Seed Germination, Dormancy and Seed Quality Studies of CV127 Soybean

Summary

Soybean (*Glycine max* L. Merrill) plants have been developed that are tolerant to the imidazolinone class of agricultural herbicides and are referred to as CV127 soybean. Seed quality and germination parameters are important characteristics used in comparisons of agronomic performance among different varieties of a crop species and seed dormancy is an important biological characteristic used to assess the weediness potential of different plant species. Therefore, seed quality (measured by a tetrazolium staining method), seed germination and dormancy (measured as hard seed) parameters of CV127 soybean plants (grown with either imidazolinone herbicide or conventional herbicide treatment for weed control) were compared to the isoline control and two conventional soybean varieties to confirm that CV127 soybean is equivalent to the isoline control and other conventional soybean varieties with respect to these biological characteristics. Seed samples from all treatments were harvested from field trials conducted in six different locations in Brazil during the 2007 short growing season. The results of seed quality, germination and dormancy tests demonstrated that there were no statistically significant differences across field trial locations in seed quality parameters, seed germination or seed dormancy between CV127 soybean and the isoline control or the conventional soybean varieties that were also included in the tests. These results demonstrate that the seed quality, germination and dormancy of CV127 soybeans are not different from that of the isoline control or other conventional soybean varieties. Furthermore, these results support the conclusion that CV127 soybean does not pose any greater weediness potential or any different environmental impact than conventional soybean varieties.

Introduction

Assessments of seed germination, dormancy and quality were compared between CV127 soybean, the isoline control and two conventional soybean varieties. Seed germination and quality are important agronomic characteristics used in comparisons of different varieties within a crop species, and seed dormancy or "hard seed" in plants is an important characteristic often associated with plants that are weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003). An assessment of seed dormancy is often used to assess the weediness potential of different plant species (Baker, 1974). Therefore, seed quality and germination parameters of CV127 soybeans were compared to the isoline control as well as to two conventional soybean varieties [Monsoy 8001 and Coodetec 217 (CD 217)] to confirm that CV127 soybean is equivalent to conventional soybean varieties with respect to these biological characteristics. Also, even though soybeans do not typically display seed dormancy characteristics (OECD, 2000), the potential for seed dormancy in CV127 soybeans was assessed and compared to that of the isoline control as well as the two conventional soybean varieties. There are scientifically recognized tests that are used in assessing seed quality and germination characteristics. These include tetrazolium staining for seed quality and the standard germination test (SGT). Seed dormancy was assessed by hard seed

determinations. The purpose of these studies was to confirm that CV127 soybean does not pose any greater weediness potential or any different environmental impact than conventional soybean varieties.

Materials and Methods

Test, Control and Reference Seeds. The soybean seed materials used in this study were produced at six different field trial locations (Uberaba, MG; Sete Lagoas, MG; Santo Antônio de Goiás, GO; Brasília, DF; Vilhena, RO; Teresina, PI) within Brazil in the regulatory field trials of the 2007 short season that are described in Appendix F of this petition, and are listed below. Test, control and reference seeds tested included the following:

1. ***Test Seed. (Treatments T1 and T2)***
   The test seed was harvested from CV127 plants grown in the regulatory field trials in Brazil in the 2007 short-season. The test seed was harvested from two different treatments, T1 and T2. T1 seed was harvested from CV127 plants treated with an imidazolinone herbicide (imazapyr, 70 g ai/ha), whereas T2 seed was harvested from CV127 plants treated with conventional herbicide (bentazon + acifluorfen-sodium, commercial name Volt®, 570 ai g/ha). Grain production of treatment T1 was compromised in Uberaba and Sete Lagoas due to misapplication of imidazolinone herbicide, and data were not recorded for this treatment at these sites.

2. ***Near-Isogenic (Isoline) Control Seed. (Treatment T3)***
   The isoline control seed was harvested from plants grown in the regulatory field trials in Brazil in the 2007 short-season. The isoline control seed was harvested from the near-isogenic (null segregant) soybean that is genetically similar to, and derived from, imidazolinone-tolerant CV127 soybean by breeding, but which does not contain the imidazolinone-tolerance trait. The isoline control is included to provide germination characteristic values to which the test seed can be compared. T3 plants were treated with the conventional herbicide Volt® as described above.

3. ***Reference Seed. (Treatments T4 and T5)***
   The reference seeds were obtained from two different conventional soybean varieties that are typical commercial varieties grown in Brazil and these were cultivated and harvested in the same 2007 field trials as the CV127 seed. The reference soybean varieties were Monsoy 8001 (T4) and Coodetec 217 (T5). These reference seeds will provide a range of background values for dormancy and germination characteristics that are common to soybean. T4 and T5 plants were treated with the conventional herbicide Volt® as described above.

Prior to the initiation of the tests, the identities of the test, isoline control, and reference seed materials were verified by testing for the presence or absence of the *csr1-2* transgene by PCR analyses, and by chain-of-custody records to verify identities of the isoline control and conventional soybean varieties.

Seed Quality. Tetrazolium testing for determining seed viability was performed following the procedures described by França Neto *et al*. (1998). The tetrazolium dye reacts with

dehydrogenase enzymes in living tissues to form a red pigment, thereby distinguishing living from nonliving tissues. Two sub-samples of 50 seed each were tested for all treatments (T1, T2, T3, T4, and T5) harvested from each of the six field trial locations. Initially, seed were kept overnight in a moist germination paper towel at 25ºC for 16 hours in a saturated environment with a relative humidity of 100%. After conditioning, the seeds were placed in plastic cups, and covered with a 0.075% tetrazolium solution. The cups were incubated in an oven at 40ºC for 150 minutes. After staining, the seeds were rinsed with tap water several times to stop the staining reaction. Seeds were kept submerged in water to avoid dehydration. Individual seeds were examined with a magnifying lens (6X) under fluorescent light. Seed were dissected longitudinally through the midsection of the embryonic axis with a single-edge razor blade. After the seed was sectioned, the seed halves were separated and the seed coat was removed to expose the outer surface of the cotyledons. The inner and outer surfaces of the cotyledons were observed to identify seed defects that are revealed by the staining procedure and attributed to either mechanical, weathering, or stink bug damage for each sample. Special care was applied during the evaluation of the radicle-hypocotyl axis and the vascular region on the cotyledons, which are structures of major importance for the viability of the seed. After the seed was sectioned, the internal surfaces of live cotyledons are normally white, due to the lack of diffusion of the tetrazolium solution to the inner tissues of the cotyledons, and viability was measured accordingly.

Seeds were rated for each type of damage (mechanical, weathering, or stink bug) according to the following scale (Franca Neto *et al*., 1998; AOSA, 2007):

Rating	Description
1	very high vigor, no seed damage, viable
2	high vigor, only minor seed damage, viable
3	medium vigor, low seed damage, viable
4	low vigor, moderate seed damage, viable
5	very low vigor, high seed damage, viable
6	nonviable, some living tissue but with moderate damage that is lethal
7	nonviable, some living tissue but with extensive damage that is lethal
8	dead, no living tissue

Seed Germination. Two hundred seed per experimental unit [four sub-samples of 50 seed each derived from each treatment (T1, T2, T3, T4, and T5) harvested from each of six field trial locations] were tested for germination, following the procedures prescribed by the Rules for Testing Seeds (AOSA, 2007). To assess seed germination, each sub-sample of 50 seed was placed in rolled, moist paper toweling and incubated at 100% humidity in an incubator at 20/30ºC with the lower temperature maintained for 16 hours and the higher temperature for 8 hours. Germination was assessed at five and eight days after planting. Seeds that failed to germinate in this test were evaluated for developmental abnormalities and hard or firm seed coat characteristics to determine the cause of lack of germination. Hard seed did not imbibe during the test period, whereas firm seed imbibed water and were swollen but did not germinate during the test period. The hard or firm seed coat characteristic is related to seed dormancy in soybean. Any seeds that did not germinate and did not fall into the abnormal and hard or firm seed coat categories were designated as dead seeds. Hard seed was determined at the final reading of the

germination test and the percentage hard seed was added to the germination percentage, based on the fact that hard-seeded soybean is considered viable (Potts *et al*., 1978).

Seed Dormancy. Seeds that failed to germinate in the germination study and were classified as hard seed as described above were evaluated as dormant seeds.

Statistical Methods. In all field locations, five treatments were replicated four times in a randomized complete block design. Tukey's test (Steel and Torrie, 1980) was used to compare means of sources of variation determined by ANOVA to have a significant effect on each of the measured traits.

Results

Seed Quality and Seed Germination. In the analysis of variance (ANOVA) of data collected for traits associated with tetrazolium staining and seed germination (Table E-1), location ("Location" in Table E-1) had a statistically significant ($P<0.01$) effect on all the traits except for firm seed (which was zero for all treatments in all locations). Given the vast geographical and environmental differences among the trial locations, significant differences among locations were expected. In contrast to location, and despite genetic differences between the CV127 lines and the commercial varieties, there was no statistically significant treatment ("Treatment" in Table E-1) effect for any of the traits.

In general, seed damage (due to mechanical, weathering, and stink bugs), seed viability, and seed germination levels were very good for seed of all treatments produced at all field trial locations with the exception of seeds produced in Teresina (Table E-2). Seeds harvested from Teresina had the highest amount of seed damage (from weathering and stink bugs) and the highest percent abnormal and dead seed. In addition, seed produced in Teresina had the lowest percent seed viability and germination. It is likely that the quality of seed produced in Teresina was negatively affected by the high temperatures and large amounts of rainfall that commonly occur at this location during the production season.

A statistically significant interaction between treatment and location ("L x T" in Table E-1), was observed for all variables except stink bug damage and percent abnormal seedlings. Differences among treatments were observed for mechanical damage and percent hard seeds at Santo Antônio de Goiás; for viability, germination, and percent dead seeds at Vilhena; for viability and percent hard seeds at Teresina; for percent dead seeds at Sete Lagoas; and for percent hard seeds at Brasilia (Table E-4). Differences among T1, T2, and T3, were significant for mechanical damage at Santo Antônio de Goiás, for viability and percent hard seeds at Teresina, for percent dead seeds at Sete Lagoas, and for percent hard seeds at Brasilia (Table E-4).

Seed Dormancy. Seed dormancy in soybeans can be expressed as hard seed. Based on the germination test, the percentages of hard seed of each treatment, as a mean across locations, were not significantly different (Table E-1). The treatments means ranged from 0.9% to 0.04% across the six locations (Table E-3). Based on the location by treatment interaction (Table E-4), statistically significant differences among CV127 treatments and/or the isoline (T1, T2, and/or T3) for percent hard seed were evident at Brasilia, where the T1 treatment was higher than either

treatments T2 or T3 and at Teresina, where the T3 treatment (isoline) was higher than the T1 or T2 (CV127) treatments. In either case, the values are low enough to pose no environmental risk. These results were consistent with the fact that soybean typically does not display dormancy characteristics.

Conclusions

The results of seed quality (seed damage and viability), seed germination (percent germination, abnormal, dead, hard, or firm seeds), and dormancy tests (percent hard seeds) demonstrated that there were statistically significant differences among location means for all measured traits ("Location" in Table E-1), but there were no statistically significant differences among treatment means for any of the measured traits ("Treatment" in Table E-1). The difference among locations was expected given the environmental differences among the locations. However, lack of significant treatment differences demonstrates that the seed quality, germination and dormancy characteristics of CV127 soybean are not different from that of the isoline control or other conventional soybean varieties. These results support the conclusion that CV127 does not pose any greater weediness potential or any different environmental impact than conventional soybean varieties.

Table E-1. Mean Squares and Significance of Sources of Variation Considered in the Statistical Model for Traits Assessed in the Tetrazolium and Germination Tests.

Sources of Variation	Traits Assessed (%) [1]								
	Tetrazolium Test[2]				Germination Test				
	Dmec (6-8)	Dweath (6-8)	Dstink (6-8)	Viab (1-5)	Germ	Abnor	Dead	Hard	Firm
Location (L)	19.82**	2362.23**	126.36**	3007.83**	6494.27**	835.06**	2838.46**	7.23**	-
Treatment (T)	24.60	178.12	21.59	77.34	80.08	34.96	87.12	3.61	-
L x T	6.20*	153.10**	9.45 ns	110.58**	102.64**	15.30 ns	91.27**	3.93**	-
Mean	3.21	4.91	3.28	88.61	84.96	9.36	5.77	0.40	0
CV%	54.81	117.45	75.98	5.42	6.77	33.74	87.87	163.91	-

[1]Dmec: mechanical damage; Dweath: weathering damage; Dstink: damage by stink bugs; Viab: seed viability; Germ: seed germination; Abnor: abnormal seedling; Dead: dead seed; Firm: firm seed; Hard: hard seed.
[2]Seeds receiving a rating of 6 to 8 are considered nonviable.
*,** = significant at P<0.05 and P<0.01, respectively. ns = not significant

Table E-2. Means of Each Location Across Treatments for Traits Assessed in the Tetrazolium and Germination Tests.

Locations	Traits Assessed [1, 4]								
	Tetrazolium Test[2]				Germination Test				
	Dmec[3] (6-8)	Dweath (6-8)	Dstink (6-8)	Viab (1-5)	Germ	Abnor	Dead	Hard[3]	Firm
Uberaba	3.13 a[4]	0.19 b	1.19 c	95.31 a	93.88 a	5.91 bc	0.22 b	0.00 a	0.00
Sete Lagoas	2.63 a	0.63 b	0.13 c	96.63 a	95.38 a	4.09 c	0.53 b	0.00 a	0.00
Santo Antônio de Goiás	4.40 a	0.20 b	5.40 ab	90.00 a	89.15 a	9.40 b	1.45 b	0.73 a	0.00
Brasília	4.40 a	0.30 b	2.05 c	93.25 a	91.65 a	7.80 bc	0.55 b	0.08 a	0.00
Vilhena	2.30 a	0.85 b	3.15 bc	93.70 a	92.55 a	6.05 bc	1.40 b	0.03 a	0.00
Teresina	2.12 a	29.12 a	7.35 a	61.71 b	45.06 b	23.23 a	32.29 a	1.59 a	0.00

[1] Dmec: mechanical damage (%); Dweath: weathering damage (%); Dstink: damage by stink bugs (%); Viab: seed viability (%); Germ: seed germination (%); Abnor: abnormal seedling; Dead: dead seed; Firm: firm seed; Hard: hard seed.
[2]Seeds receiving a rating of 6 to 8 are considered nonviable.
[3] Differences among location means for mechanical damage and hard seed were not detected by Tukey's test.
[4]Means in a column followed by the same letter do not differ statistically significantly at the 5% level (Tukey's test).

Table E-3. Mean of Each Treatment Across Locations for the Traits Assessed in the Tetrazolium and Germination Tests.

Traits Assessed [1]									
Trt	Tetrazolium Test[2]				Germination Test				
	Dmec (6-8)	Dweath (6-8)	Dstink (6-8)	Viab (1-5)	Germ	Abnor	Dead	Hard	Firm
T1	2.40[3]	11.60	3.67	82.67	80.73	8.80	11.13	0.23	0.00
T2	3.17	2.65	3.09	91.00	88.48	8.22	3.30	0.04	0.00
T3	4.91	2.48	1.83	90.78	87.43	9.13	3.43	0.72	0.00
T4	2.71	2.79	4.63	89.83	85.13	9.48	5.40	0.04	0.00
T5	2.63	7.33	3.29	86.75	81.71	10.90	7.40	0.90	0.00

[1]Dmec: mechanical damage (%); Dweath: weathering damage (%); Dstink: damage by stink bugs (%); Viab: seed viability (%); Germ: seed germination (%); Abnor: abnormal seedling; Dead: dead seed; Firm: firm seed; Hard: hard seed.

[2]Seeds receiving a rating of 6 to 8 are considered nonviable. The cause of the nonviability of these seeds was further assessed and the results are reported here.

Table E-4. Means of Each Combination of Location and Treatment for the Traits Assessed in the Tetrazolium and Germination Tests.

Traits Assessed[1, 4]										
		Tetrazolium Test[2]				Germination Test				
Location	Trt	Dmec (6-8)	Dweath (6-8)	Dstink (6-8)	Viab (1-5)	Germ	Abnor	Dead	Hard	Firm
Uberaba	T1	*[3]	*	*	*	*	*	*	*	*
	T2	3.25 a[4]	0.50 a	0.50	95.50 a	93.50 a	6.25	0.25 a	0.00 a	0.00 a
	T3	4.50 a	0.25 a	0.75	94.50 a	93.25 a	6.50	0.25 a	0.00 a	0.00 a
	T4	1.50 a	0.00 a	1.75	96.25 a	95.00 a	4.75	0.25 a	0.00 a	0.00 a
	T5	3.25 a	0.00 a	1.75	95.00 a	93.75 a	6.12	0.13 a	0.00 a	0.00 a
Sete Lagoas	T1	*[3]	*	*	*	*	*	*	*	*
	T2	2.00 a	1.25 a	0.00	96.75 a	96.75 a	3.25	0.00 b	0.00 a	0.00 a
	T3	3.00 a	0.25 a	0.00	96.75 a	96.00 a	3.75	0.25 ab	0.00 a	0.00 a
	T4	2.75 a	0.50 a	0.50	96.25 a	94.50 a	4.12	1.38 a	0.00 a	0.00 a
	T5	2.75 a	0.50 a	0.00	96.75 a	94.25 a	5.25	0.50 ab	0.00 a	0.00 a
Santo Antônio de Goiás	T1	3.00 b	0.75 a	4.75	91.50 a	91.00 a	7.75	1.25 a	0.25 b	0.00 a
	T2	5.75 ab	0.00 a	6.00	88.25 a	88.25 a	10.50	1.25 a	0.25 b	0.00 a
	T3	7.75 a	0.00 a	2.75	89.25 a	86.50 a	12.25	1.25 a	0.13 b	0.00 a
	T4	2.75 b	0.00 a	5.25	92.00 a	91.50 a	7.38	1.13 a	0.25 b	0.00 a
	T5	2.75 b	0.25 a	8.25	89.00 a	88.50 a	9.13	2.38 a	2.75 a	0.00 a
Brasília	T1	3.50 a	0.75 a	2.50	93.25 a	93.00 a	6.25	0.75 a	0.38 a	0.00 a
	T2	4.00 a	0.00 a	1.75	94.25 a	92.25 a	7.50	0.25 a	0.00 b	0.00 a
	T3	7.50 a	0.50 a	1.75	90.25 a	88.75 a	10.50	0.75 a	0.00 b	0.00 a
	T4	3.50 a	0.00 a	2.00	94.50 a	92.25 a	7.38	0.38 a	0.00 b	0.00 a
	T5	3.50 a	0.25 a	2.25	94.00 a	92.00 a	7.38	0.63 a	0.00 b	0.00 a
Vilhena	T1	2.00 a	1.50 a	2.25	94.25 ab	94.75 a	4.50	0.75 ab	0.13 a	0.00 a
	T2	1.00 a	1.00 a	2.75	95.25 ab	94.25 a	4.25	1.50 ab	0.00 a	0.00 a
	T3	3.00 a	0.00 a	1.00	96.00 a	94.75 a	5.00	0.25 b	0.00 a	0.00 a
	T4	2.00 a	0.00 a	7.75	90.25 b	90.25 ab	7.25	2.50 a	0.00 a	0.00 a
	T5	3.50 a	1.75 a	2.00	92.75 ab	88.75 b	9.25	2.00 ab	0.00 a	0.00 a
Teresina	T1	0.67 a	54.00 a	5.67	41.33 b	32.00 a	19.33	52.00 a	0.17 b	0.00 a
	T2	3.00 a	16.67 a	9.00	71.00 ab	58.33 a	20.67	21.00 a	0.00 b	0.00 a
	T3	3.33 a	17.67 a	5.67	73.67 a	58.00 a	19.33	22.67 a	5.33 a	0.00 a
	T4	3.75 a	16.25 a	10.50	69.75 ab	47.25 a	26.00	26.75 a	0.00 b	0.00 a
	T5	0.00 a	41.25 a	5.50	53.00 ab	33.00 a	28.25	38.75 a	2.63 ab	0.00 a

[1] Dmec: mechanical damage (%); Dweath: weathering damage (%); Dstink: damage by stink bugs (%); Viab: seed viability (%); Germ: seed germination (%); Abnor: abnormal seedling; Dead: dead seed; Firm: firm seed; Hard: hard seed.
[2]Seeds receiving a rating of 6 to 8 are considered nonviable.
[3] Seed were not harvested from this treatment at these field sites because of a misapplication of imazapyr herbicide.
[4] Means in a column followed by the same letter do not differ statistically significantly at the 5% level (Tukey's test).

Appendix F

Materials and Methods and Results from the Agronomic, Phenotypic, and Ecological Interaction Evaluations of CV127 Soybean

Introduction

Evaluations of the agronomic, phenotypic and ecological interactions of CV127 soybeans were conducted at a total of thirteen field trial locations in Brazil, seven in the 2006/2007 growing season and six in the 2007 short growing season. In all field trials, the CV127 soybeans were compared to a near-isogenic, null segregant soybean (referred to as the isoline control) that does not contain the *csr1-2* gene cassette and to two other conventional soybean varieties commonly cultivated in Brazil. The field trial sites were representative of the different regions within Brazil where soybean is commercially cultivated and each trial location consisted of four replications of the two CV127 treatments, isoline control and two conventional soybean varieties that were organized in a randomized block design. The methodology for the trials conducted in the two different seasons was similar. The methodology used in the 2006/2007 trials is presented here and, for the 2007 season, only differences in the methodology from the 2006/2007 season are noted. The complete results from the evaluations of both growing seasons are also presented.

Field observations included seed germination rate, seedling vigor, days to reach key developmental stages, plant height, and grain yield, as well as susceptibility to and interactions with diseases and insects. In addition, the impact of cultivating CV127 soybeans on factors relevant to the nitrogen fixing symbiosis with *Bradyrhizobium japonicum* was assessed.

The treatments included in the field trials used for phenotypic, agronomic, and ecological evaluations are listed below:

Treatment	Genotype	Herbicide application
Treatment 1 (T1)	CV127	Imazapyr (70 g ai/ha)
Treatment 2 (T2)	CV127	Volt® (570 g ai/ha)
Treatment 3 (T3)	Isoline control	Volt® (570 g ai/ha)
Treatment 4 (T4)	Monsoy 8001	Volt® (570 g ai/ha)
Treatment 5 (T5)	Coodetec 217 (CD 217)	Volt® (570 g ai/ha)

Results of these studies demonstrated that, except for herbicide tolerance, CV127 soybean is phenotypically, phenologically and agronomically equivalent to the isoline control and other conventional soybean varieties. Further, these studies showed no different biological effect of CV127 compared to the isoline control soybeans with respect to interaction with *Bradyrhizobium japonicum* and nitrogen fixation capacity or interaction with various diseases or insects. Therefore, these results reinforce the conclusion that the cultivation of CV127 soybeans poses no different plant pest or weediness potential and will have no different environmental impact than the cultivation of conventional soybean varieties.

The methods and results presented in this Appendix are organized as follows:

1.0. Agronomic and phenotypic evaluations in the a) 2006/2007 and b) 2007 growing seasons
2.0. Disease susceptibility in the a) 2006/2007 and b) 2007 growing seasons
3.0. Insect interactions in the a) 2006/2007 and b) 2007 growing seasons
4.0. Nitrogen fixation characteristics in the a) 2006/2007 and b) 2007 growing seasons

1.0 Agronomic and Phenotypic Evaluations

a) 2006/2007 growing season.

Materials and Methods

Field Locations. The experiments were planted during the 2006/2007 growing season at seven experimental stations in Brazil. One station is owned and operated by BASF S.A. and six by Embrapa. The stations are located in regions that are representative of areas of commercial soybean production and areas in which the soybean trial entries are adapted. All locations had a Certificate of Quality in Biosafety (CQB), an established infrastructure, laboratory facilities and field equipment, and personnel experienced in agricultural research and trained in biosafety (Table F-1).

Table F-1. Experimental Station Locations with Experiment Codes, 2006/2007.

Research Station	City, State	Location (Experiment) Code
BASF Experimental Station	Santo Antônio de Posse, SP	EEA (011)
Embrapa SNT	Ponta Grossa, PR	SNT (008)
Embrapa Soybean	Londrina, PR	CNPSO (010)
Embrapa / EPAMIG	Uberaba, MG	CTTP (012)
Embrapa Horticulture	Brasília, DF	CNPH (016)
Embrapa Rice and Beans	Santo Antônio de Goiás, GO	CNPAF (014)
Embrapa Corn and Sorghum	Sete Lagoas, MG	CNPMS (013)

The predominant soil type, the tillage system (conventional disc tillage or no tillage), and the cropping history varied by location (Table F-2). All but one location (CNPMS) had been used for crop production within the previous two years.

Table F-2. A Description of the Soybean Trial Field Locations, 2006/2007.

Location	Soil type	Tillage system	Cropping history			
			Summer 2004/05	Short Season 2005	Summer 2005/06	Short Season 2006
SNT	Muddy sand	Conventional	Soybean	Oats	Soybean	Oats
CNPSo	Clay	Conventional	Soybean	Oats	Soybean	Oats
EEA	Clay sand	Conventional	Soybean	Fallow	Corn and soybean	Potato
CTTP	Sandy	No till	Soybean	Fallow	Soybean	Fallow
CNPMS	Clay	Conventional	Fallow	Fallow	Fallow	Fallow
CNPAF	Clay sand	Conventional	Soybean	Soybean	Fallow	Fallow
CNPH	Clay	Conventional	Soybean	Fallow	Fallow	Fallow

Rainfall and temperature were recorded at each location for the period from planting to harvest (Table F-3). While irrigation was required to supplement rainfall at each location, the quantity of water and frequency of application was dependent on the quantity and distribution of rainfall. In general, the amount of precipitation was below average at EEA, near average at CNPH, CNPAF, SNT, CTTP and CNPSo, and above average at CNPMS. Temperatures were near average at all locations, except at SNT and CTTP where they were below and above average, respectively.

Table F-3. Rainfall Amount (R) and Average Monthly Temperature (T) at Each of the Soybean Field Trial Locations During the Conduct of the Trials, 2006/2007.

Location	October 2006		November 2006		December 2006		January 2007		February 2007		March 2007		April 2007	
	R	T	R	T	R	T	R	T	R	T	R	T	R	T
	mm	˚C	mm	˚C	mm	˚C	mm	˚C	mm	˚C	mm	˚C	mm	˚C
SNT	91	18.1	172	22.1	155	24.1	210	21.0	207	20.9	228	21.4	86	18.7
CNPSo	61	22.8	130	22.1	188	22.8	300	22.2	161	22.6	196	22.7	-	-
EEA	41	22.7	125	22.4	201	24.0	241	22.3	81	24.4	89	24.1	-	-
CTTP	-	-	178	24.5	178	24.5	413	25.5	198	25.8	105	25.9	-	-
CNPMS	-	-	202	22.2	337	24.1	390	24.2	491	22.7	92	24.6	-	-
CNPAF	-	-	203	22.7	295	22.4	211	22.7	196	22.2	49	24.1	-	-
CNPH	-	-	161	22.6	192	22.7	309	22.8	203	22.4	132	25.0	-	-

***Field, and Plot Design*.** In all field locations, five treatments (T1-T5, Table F-6) were replicated four times in a randomized complete block design. Each plot consisted of six 8 m-long rows with 0.5 m spacing between rows of the same plot and 1.0 m spacing between rows of adjacent plots. Soil, plant, and insect samples were collected from (or between) the outer (rows 1 and 6) and inner (rows 2 and 5) border rows of each plot, while phenotypic, phenologic, and agronomic measurements were determined from plants within the center two rows (rows 3 and 4).

Land and Crop Management. The land and crop management practices at each location, including the management of pests, pathogens, and weeds, were as recommended for each specific region in which the field trials were located.

Pre-plant:
All land used for the trials has a long history of use for the production of crops. Fields were prepared for planting by adding fertilizer (nitrogen, phosphorous, and/or potassium) based on soil analyses and local recommendations, and disc-tilled to ensure a uniform seedbed.

At-planting:
Seeds were planted by hand at a rate of 13-15 seeds/m (depending on local recommendations) or approximately 260,000 seeds per ha between 10 October and 21 November 2006 (Table F-4). Heavy rain following the initial planting at Ponta Grossa negatively impacted the germination of all entries and prompted the need to replant at this location. The replanted seed of the test and isoline control were from an F_3 family that is related to, but not genetically identical to, the F_3 family used in all other test locations.

Table F-4. Planting and Harvest Dates for Each Field Trial Location in Brazil, 2006/2007.

Location	Site code	Planting date (2006)	Harvest date (2007)
Santo Antônio de Posse, SP	EEA	Oct. 25	Mar. 16
Ponta Grossa, PR	SNT	Oct. 10	Poor germination, replanted
		Oct. 31†	Destroyed prior to harvest, Apr. 20
Londrina, PR	CNPSO	Oct. 18	Mar. 14
Uberaba, MG	CTTP	Nov. 21	Mar. 29
Brasília, DF	CNPH	Nov. 16	Mar. 29
Santo Antônio de Goiás, GO	CNPAF	Nov. 7	Mar. 15
Sete Lagoas, MG	CNPMS	Nov. 9	Mar. 22

†Poor germination of initial planting on 10 October prompted need to replant on 31 October.

Post-plant

To ensure the successful completion of growth and development of soybean plants at each field trial, insecticides, fungicides, and herbicides were applied at each location according to local recommendations and as needed to protect the plants from insect, fungal, and weed infestations (Table F-5). Insecticidal chemicals applied included fipronil, teflubenzuron, permethrin, cypermethrin, thiamethoxan, imidaclopride, chlorpyrifos, methamidophos, and avermectin. Chemicals applied for the control of fungi, primarily *Phakopsora pachyrhizi*, included epoxiconazole, pyraclostrobin, flutriafol, and tebuconazole. For treatment T1, weeds were controlled in plots containing CV127 soybean with a single application of imazapyr (70g ai/ha) and controlled with bentazon and acifluorfen (referred to as Volt® herbicide) in all other plots (treatments T2 through T5). The herbicide tepraloxydim was applied for grass weed control in plots of treatments T2-T5 on an as-needed basis. In most locations, more than one application and formulation of insecticide, fungicide, and herbicide was used during the season.

Table F-5. Insecticides, Herbicides, and Fungicides Applied to Plots after Planting, 2006/2007.

Location	Product	Rate		Date of application		Use
		L/ha	kg/ha	2006	2007	
Santo Antônio de Posse	Fastac®	0.12		15-Nov		Insecticide
	Lorsban®	1		15-Nov		Insecticide
	Fastac®	0.12		19-Nov		Insecticide
	Lorsban®	1		19-Nov		Insecticide
	Arsenal®	0.15		22-Nov		Herbicide
	Volt®	1		22-Nov		Herbicide
	Aramo®	0.5		24-Nov		Herbicide
	Opera®	0.5		2-Dec		Fungicide
	Sevin®	2		2-Dec		Insecticide
	Nomolt®	0.08		2-Dec		Insecticide
	Connect®	1		16-Dec		Insecticide
	Nomolt®	0.08		16-Dec		Insecticide
	Opera®	0.6		29-Dec		Fungicide
	Opera®	0.6			11-Jan	Fungicide
	Engeo® Pleno	0.25			11-Jan	Insecticide
	Opera®	0.6			20-Jan	Fungicide
	Impact®	0.6			3-Feb	Fungicide
	Connect®	1			3-Feb	Insecticide
	Folicur®	0.5			19-Feb	Fungicide
	Engeo® Pleno	0.25			19-Feb	Insecticide
Sete Lagoas	Arsenal®		0.07	29-Nov		Herbicide
	Volt®		0.57	29-Nov		Herbicide
	Aramo®		0.1	14-Dec		Herbicide
	Opera®		0.09	21-Dec		Fungicide
	Opera®		0.09		11-Jan	Fungicide
	Opera®		0.09		24-Jan	Fungicide
	Engeo® Pleno		0.066		6-Feb	Insecticide
	Rival™		0.1		16-Feb	Fungicide
Santo Antônio de Goiás	Arsenal®		0.07	28-Nov		Herbicide
	Volt®		0.57	28-Nov		Herbicide
	Talcord®	0.1		6-Dec		Insecticide
	Aramo®		0.1	7-Dec		Herbicide
	Opera®	0.5			4-Jan	Fungicide
	Opera®	0.5			15-Jan	Fungicide
	Folicur®	0.75			20-Jan	Fungicide
	Abametrina	0.3			28-Jan	Insecticide
Uberaba	Arsenal®		0.07	13-Dec		Herbicide
	Volt®		0.57	13-Dec		Herbicide
	Aramo®		0.1	18-Dec		Herbicide
	Opera®		0.09		17-Jan	Fungicide
	Opera®		0.09		31-Jan	Fungicide
	Engeo® Pleno		0.066		31-Jan	Insecticide
	Rival™		0.1		14-Feb	Fungicide

Table F-5 continued.

Location	Product	Rate		Date of application		Use
		L/ha	kg/ha	2006	2007	
Ponta Grossa	Vexter®	1		31-Oct		Insecticide
	Vexter®	1		1-Nov		Insecticide
	Glyphosate	2		3-Nov		Herbicide
	Vexter®	1		3-Nov		Insecticide
	Vexter®	1		21-Nov		Insecticide
	Arsenal®	0.15		28-Nov		Herbicide
	Volt®	1		28-Nov		Herbicide
	Aramo®	0.5		8-Dec		Herbicide
	Opera®	0.5			9-Jan	Fungicide
	Metafos®	0.5			9-Jan	Insecticide
	Fastac®	0.2			9-Jan	Insecticide
	Opera®	0.5			31-Jan	Fungicide
	Engeo® Pleno	0.15			31-Jan	Insecticide
	Folicur®	0.5			23-Feb	Fungicide
	Metafos®	0.5			23-Feb	Insecticide
	Abametrina	0.4			1-Mar	Insecticide
	Engeo® Pleno	0.18			9-Mar	Insecticide
Londrina	Arsenal®	0.15		15-Nov		Herbicide
	Volt®	1		15-Nov		Herbicide
	Tamaron®	0.5		24-Nov		Insecticide
	Klap®	0.15		2-Dec		Insecticide
	Aramo®	0.5		7-Dec		Herbicide
	Opera®	0.5		26-Dec		Fungicide
	Fastac®	0.2		28-Dec		Insecticide
	Nomolt®	0.08		28-Dec		Insecticide
	Opera®	0.5			12-Jan	Fungicide
	Tamaron®	0.5			12-Jan	Insecticide
	Nomolt®	0.08			13-Jan	Insecticide
	Opera®	0.6			30-Jan	Fungicide
	Engeo® Pleno	0.25			30-Jan	Insecticide
	Rival™	0.5			7-Feb	Fungicide
	Engeo® Pleno	0.2			15-Feb	Insecticide
Brasília	Vexter®	1		24-Nov		Insecticide
	Vexter®	1		1-Dec		Insecticide
	Fastac®	0.2		8-Dec		Insecticide
	Arsenal®		0.07	5-Dec		Herbicide
	Volt®		0.57	5-Dec		Herbicide
	Opera®	0.5			2-Jan	Fungicide
	Folicur®	0.75			13-Jan	Fungicide
	Folicur®	0.75			21-Jan	Fungicide
	Engeo® Pleno	0.3			23-Jan	Insecticide
	Folicur®	0.75			3-Feb	Fungicide
	Folicur®	0.75			15-Feb	Fungicide

Harvest

Plants were manually cut below the lowest pod and removed from the middle 6 m of the two center rows of each plot and threshed with a stationary bulk plant thresher. Seed were dried to uniform moisture and weighed. Harvest was initiated on 14 March in Londrina and completed on 29 March in Brasília and Uberaba (Table F-4). A late, but necessary replanting of the trial at Ponta Grossa resulted in uncharacteristic plant growth and late maturity of all CV127, isoline control, and conventional soybean varieties. Thus, all plants at Ponta Grossa were plowed into the soil and destroyed prior to harvest.

Treatments. The five treatments (Table F-6) included in these studies represent a combination of the specific soybean genotypes (test, isoline, and reference) and herbicide formulations (imidazolinone and non-imidazolinone herbicides) required to generate data appropriate for meeting the objectives of each study.

Test (CV127 soybean) and control soybeans.

The imidazolinone-tolerant soybean, CV127, utilized in the study was the F_5 generation of CV127, line 127 (Figure III-1), and the control was the corresponding isoline or null segregant.

Conventional reference varieties.

Two reference soybean varieties were used in these field trials: Monsoy 8001 (M8001) and Coodetec 217 (CD 217). Both of these varieties are common nontransgenic Brazilian commercial varieties with a similar maturity classification as CV127 and the isoline control soybeans.

Two herbicide treatments were used in these evaluations: 1) Imazapyr, an imidazolinone herbicide, sprayed at a rate of 70 g ai/ha, and 2) Volt®, a combination of bentazon (400 g ai/ha) and acifluorfen (170 g ai/ha), sprayed at a rate of 570 g ai/ha (Table F-6).

Table F-6. Experiment Treatments (Entries) Included in Each Soybean Field Trial, 2006/2007.

Treatment	Genotype	Herbicide
Treatment 1 (T1)	CV127	Imazapyr (70 g ai/ha)
Treatment 2 (T2)	CV127	Volt® (570 g ai/ha)
Treatment 3 (T3)	Isoline control	Volt® (570 g ai/ha)
Treatment 4 (T4)	Monsoy 8001	Volt® (570 g ai/ha)
Treatment 5 (T5)	Coodetec 217 (CD 217)	Volt® (570 g ai/ha)

Data Collection and Statistical Analyses. The phenotypic, phenologic, and agronomic similarities of the CV127, isoline control, and conventional soybean varieties were determined by recording various characteristics routinely used to describe the phenotype and behavior of a soybean genotype. The characteristics were recorded for all plots at all locations (unless otherwise noted).

Tukey's test (Steel and Torrie, 1980) was used to compare means of sources of variation determined by ANOVA to have a significant effect on each of the measured traits.

Traits Evaluated

A large number of characteristic agronomic traits were evaluated during the different growth stages of soybean development. The different growth stages of soybean, including vegetative and reproductive stages of soybean plant growth and development are defined below in Table F-7. A description of the evaluation of each measured trait follows.

Table F-7. Description of the Different Soybean Growth Stages

Stage number	Stage name	Stage definition
VE	Emergence	Hypocotyl elongated and hooked; cotyledons at soil surface
VC	Cotyledonary	Hypocotyl straightened; cotyledons unfolded; unifoliates unfolded
V1	First node	First trifoliate leaves unfolded on 50%+ of plants in the plot
V2	Second node	Second trifoliate leaves unfolded on 50%+ of plants in the plot
V3	Third node	Third trifoliate leaves unfolded on 50%+ of plants in the plot
Vn	n^{th} node	N = no. of nodes on 50%+ of plants in the plot with an unfolded trifoliate
R1	Begin flower	50%+ of plants in the plot with a flower on any node
R2	Full flower	50%+ of plants in the plot with a flower on upper two nodes
R3	Begin pod	50%+ of plants in the plot with a 5 mm long pod (upper four nodes)
R4	Full pod	50%+ of plants in the plot with a 2 cm long pod (upper four nodes)
R5	Begin seed	50%+ of plants in the plot with 3 mm long seed (upper four nodes)
R6	Full seed	50%+ of plants in the plot with a full-size seed (upper four nodes)
R7	Begin maturity	50%+ of plants in the plot have a pod with mature (tan/brown) color
R8	Full maturity	All plants in the plot have pods with mature (tan/brown) color

Seed germination: the percentage of emerged plant seedlings at the V2 stage relative to the quantity of seed sown per plot.

Seedling vigor: the overall appearance and rate of plant development of plants in a plot at the V2 stage according to the index in Table F-8.

Table F-8. Scale Used to Evaluate the Average Vigor of Seedlings on a Plot Basis.

Vigor	Description
1	Plant emerged; no trifoliate leaves
2	One trifoliate leaf; unhealthy plant
3	One trifoliate leaf; healthy plant
4	Two trifoliate leaves; unhealthy plant
5	Two trifoliate leaves; healthy plant

Initial and final plant stand: the number of plants per plot at the V2 and R8 stages of plant development.

Green stem: the percentage of plants in a plot that were green at the R8 stage relative to the total number of plants in that plot.

Plant height (cm): the average height of five plants in a plot measured from soil level to the top of the plant at the R8 stage.

Shatter: the percentage of plants in a plot that show premature dehiscence of pods (shatter) resulting in the loss of seed at maturity (R8). Shatter was not identified in any plot at any location and is not considered further here.

Lodging: the average standability (lodging) of plants in a plot at maturity (R8) according to the index in Table F-9.

Table F-9. Scale Used to Evaluate the Average Lodging of Plants on a Plot Basis.

Lodging	Description
1	All plants are standing erect (80-90 degree angle).
2	Most plants have a slight lean (60-70 degree angle).
3	Most plants have a moderate lean (40-50 degree angle).
4	Most plants have a severe lean (20-30 degree angle).
5	All plants are prostrate (0 to 10 degree angle).

Vegetative life cycle (days to flowering): the number of days between planting and full flower (R2).

Total life cycle (days to maturity): the number of days between planting and full plant maturity (R8).

Seed size: the weight (g) of 100 seeds.

Grain yield: the total weight (g) of all grain harvested (adjusted to 12% moisture) converted to kg/ha.

Results and Discussion

Based on analyses of variance (Table F-10):

1) location had a significant effect on all measured traits, including percent germination (G), final plant stand (FS), seedling vigor (V), days to full flower (DF), days to full maturity (DM), percent plants with green stem (GS), plant height (PH), grain yield (Yield), seed size (100SW), and lodging (L),

2) treatment had a significant effect on seedling vigor, days to full maturity, plant height, seed size, and lodging, and

3) the combination of location and treatment had a significant effect on all dependent variables except germination, final plant stand, and seed size.

Table F-10. Agronomic and Phenotypic Evaluations 2006/2007: Statistically Significant and Nonsignificant Sources of Variation in the Model Used to Analyze Data Collected for Each Trait.

Source of variation	Trait[†]									
	G	FS	V	DF	DM	GS	PH	Yield	100SW	L
Location (L)	432.3**	3767.0**	1.8**	142.7**	3857.7**	104.1*	7249.7**	65.32**	38.7**	9.66**
Treatment (T)	58.8	90.3	0.11**	5.1	151.6**	61.1	538.1**	4.78	213.3**	3.98**
L x T	25.9	33.2	0.11**	13.3**	23.1**	35.8**	62.4**	2.64**	1.22	0.87**
Error	17.9	30.3	2.38[a]	0.12	0.05	5.1	13.3	1.11	0.77	0.18
Mean	86.6	92.1	4.89	45.2	124.1	2.0	80.4	3619.8	16.4	1.67

[†]G = germination; FS = final plant stand; V = seedling vigor; DF = days to full flower (vegetative cycle); DM = days to full maturity (total cycle); GS = green stem; PH = plant height; Yield = grain yield; 100SW = seed size (weight of 100 seeds); and L = degree of lodging.
*,** = significant at $P<0.05$ and $P<0.01$, respectively.
[a] the error value for vigor is 2.38×10^{-18}

Due to the naturally different environmental characteristics (temperature, rainfall, humidity, day length, etc.) among trial locations, location had a significant effect on all assessed traits. At Ponta Grossa, the most southerly location (latitude 25^{o} S) and the location for which the Conquista soybean variety is least adapted, plants were taller, green stems were more frequent, lodging was most severe, and number of days to flower and to maturity were higher than at all other locations (Table F-11). As noted previously, yield was not recorded from this site due to a late replanting of the study and subsequent uncharacteristic plant growth and late maturity of the CV127, isoline control, and conventional soybean varieties. Seed size and yield were highest at Londrina (Table F-11). In fact, yield was above the Brazilian average yield at all locations except Brasília. At Brasília, seedling vigor was slightly less than at other locations (rating of 4.2

versus 5.0 for all other locations) and yield appeared to be more adversely affected by a high and late incidence of Asian rust than other locations similarly infected. Due to a high Coefficient of Variation at Brasília for yield, likely due to the Asian rust infection, yield data from Brasília was not included in the across-location analysis for yield.

Table F-11. Agronomic and Phenotypic Evaluations 2006/2007: Location Means and Standard Deviations (Shown in Parentheses) across Treatments for Evaluated Traits.

Location	Trait[†]									
	G	FS	V	PH	GS	L	DF	DM	100SW	Yield
Sto. Antônio de Goiás	89.8 a[‡] (4.9)	106.8 a (5.8)	5.0 a (0)	56.1 d (2.2)	0.0 a (0)	1.0 b (0)	43 b (0.6)	118 c (2.1)	16.8 b (3.1)	3224 c (385.9)
Brasília	80.4 c (4.9)	94.5 b (5.8)	4.2 b (0.4)	57.5 d (1.4)	0.0 a (0)	1.0 b (0)	43 b (1.3)	124 b (3.3)	15.3 c (2.6)	1873 d (623.4)
Sete Lagoas	88.0 ab (3.7)	102.8 a (5.9)	5.0 a (0)	86.6 b (5.5)	1.5 a (2.5)	2.4 a (1.1)	44 b (1.6)	111 d (1.6)	16.0 bc (3.0)	3967 b (221.0)
Londrina	91.5 a (3.2)	89.0 b (3.6)	5.0 a (0)	94.8 a (7.8)	3.3 a (2.3)	1.8 ab (0.8)	45 b (1.2)	128 b (2.2)	19.0 a (2.9)	4459 a (709.0)
Uberaba	91.1 a (4.0)	102.2 a (5.7)	5.0 a (0)	70.0 c (9.2)	0.1 a (0.3)	1.0 b (0)	45 b (1.2)	110 d (1.4)	15.3 c (2.9)	3304 c (217.8)
Sto. Antônio de Posse	83.8 bc (7.1)	81.7 c (7.8)	5.0 a (0)	98.2 a (6.1)	3.3 a (1.8)	1.9 ab (0.7)	44 b (2.7)	126 b (3.2)	16.0 bc (2.8)	3144 c (508.9)
Ponta Grossa	81.5 c (3.8)	68.1 d (4.7)	5.0 a (0)	100.1 a (7.6)	6.0 a (8.5)	2.7 a (0.9)	51 a (1.8)	151 a (5.2)	-	-

[†]G = initial germination (%); FS = final plant stand; V = vigor; PH = plant height (cm); GS = green stem (%); L = degree of lodging; DF = days to full flower (vegetative cycle); DM = days to full maturity (total cycle); 100SW = seed size [weight of 100 seeds (g)]; and Yield = grain yield (kg/ha).
[‡]Means followed by the same letter do not differ statistically significantly by the Tukey test at 5% probability.

As an average across locations, the performance of CV127 treated with imazapyr (T1) or with Volt® (T2) and the performance of the isoline control treated with Volt® (T3) were not significantly different for germination, final stand, green stem, lodging, days to flower, days to maturity, or yield (Table F-12). Only for vigor, plant height and seed size did T1 significantly differ from T3 and only for seed size did T2 significantly differ from T3. Only for seed size, was the difference between both conventional soybean varieties (T4 and T5) significantly different from Treatments 1, 2, and 3.

Table F-12. Agronomic and Phenotypic Evaluations 2006/2007: Treatment Means and Standard Deviations (Shown in Parentheses) Over Location for Each Trait.

Treatment[‡]	Trait[†]									
	G	FS	V	PH	GS	L	DF	DM	100SW	Yield
T1	85.0	90.7	5.0 a[§]	79.0 bc	4.5	1.5 ab	45	126 a	19.7 a	3748
	(7.1)	(13.4)	(0)	(16.8)	(7.6)	(0.6)	(2.2)	(14.8)	(1.5)	(1089.2)
T2	85.8	90.5	4.8 b	83.4 ab	2.0	1.6 ab	45	126 a	18.8 a	3816
	(5.7)	(13.3)	(0.3)	(19.6)	(2.4)	(0.8)	(2.2)	(13.8)	(1.7)	(1165.5)
T3	88.3	94.6	4.8 b	86.0 a	1.1	1.9 a	45	125 ab	16.9 b	3463
	(6.3)	(15.5)	(0.4)	(20.4)	(2.1)	(1.1)	(2.7)	(13.8)	(1.6)	(1047.7)
T4	87.9	93.5	4.8 b	79.0 bc	1.6	1.1 b	45	122 c	13.2 c	3502
	(6.1)	(14.8)	(0.4)	(16.6)	(2.1)	(0.4)	(3.8)	(12.2)	(1.4)	(530.0)
T5	85.9	91.4	4.8 b	74.7 c	0.9	2.1 a	46	122 bc	13.5 c	3570
	(6.0)	(14.8)	(0.4)	(16.6)	(1.7)	(1.2)	(3.6)	(11.4)	(1.8)	(718.7)

[†]G = initial germination (%); FS = final plant stand; V = seedling vigor; PH = plant height (cm); GS = green stem (%); L = degree of lodging; DF = days to full flower (vegetative cycle); DM = days to full maturity (total cycle); 100SW = seed size [weight of 100 seeds (g)]; and Yield = grain yield (kg/ha).
[‡]T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[§]Means followed by the same letter do not differ statistically significantly by the Tukey test at 5% probability.

The Location x Treatment interaction was significant for seedling vigor, days to full flower and maturity, green stem, plant height, yield, and lodging (Table F-10). For all other traits, the mean of the treatments over locations was a reliable indicator of the effect of each treatment on that particular trait. With the exception of plant height, percent green stem, lodging, and days to maturity at Ponta Grossa and seedling vigor at Brasília, the performance of CV127 treated with imazapyr (T1) and CV127 treated with Volt® (T2) was not significantly different (Table F-13). Further, the performance of T2 and T3 were not significantly different for germination, final plant stand, plant height, percent green stem, lodging, seed size or yield at any of the locations. T2 and T3 only differed significantly for days to flowering and days to maturity at Londrina and Santo Antônio de Posse, and for days to flowering at Ponta Grossa (Table F-13). Similarly, the few instances where the performance of T1 and T3 differed significantly included seedling vigor at Brasília, as well as plant height, percent green stem, lodging, days to flowering and maturity at Ponta Grossa, days to flowering and maturity at Londrina and Santo Antônio de Posse, and percent green stem at Sete Lagoas (Table F-13).

Although statistically significant differences did exist between CV127 soybean and the isoline control for specific traits at specific locations, none of the differences are of any biological significance and all trait values are within the range for soybeans that are commercially cultivated in Brazil and elsewhere, including the conventional soybean varieties (T4 and T5) included in these studies.

Table F-13. Agronomic and Phenotypic Evaluations 2006/2007: Means for Each Combination of Treatment and Location for All Traits and Standard Deviations (in Parentheses).

Location	Trt‡	Trait†									
		G	FS	V	PH	GS	L	DF	DM	100SW	Yield
Santo Antônio de Goiás	T1	84.5 (2.4)	101.9 (5.9)	5.0 a§ (0.0)	56.2 ab (1.4)	0.0 a (0.0)	1.0 a (0.0)	43 b (0.0)	119 a (0.0)	20.6 (0.4)	3313 a (332.2)
	T2	86.8 (4.0)	103.1 (4.7)	5.0 a (0.0)	56.9 ab (0.5)	0.0 a (0.0)	1.0 a (0.0)	43 b (0.0)	119 a (0.0)	19.1 (0.5)	3275 a (151.9)
	T3	92.5 (3.7)	109.4 (4.3)	5.0 a (0.0)	57.9 a (1.8)	0.0 a (0.0)	1.0 a (0.0)	43 b (0.0)	119 a (0.0)	17.1 (0.7)	3131 a (85.3)
	T4	94.3 (2.7)	111.1 (3.1)	5.0 a (0.0)	56.5 ab (1.5)	0.0 a (0.0)	1.0 a (0.0)	43 b (0.0)	114 b (0.0)	14.2 (0.9)	3397 a (598.1)
	T5	91.1 (4.8)	108.4 (6.1)	5.0 a (0.0)	53.0 b (2.4)	0.0 a (0.0)	1.0 a (0.0)	44 a (0.5)	119 a (0.0)	13.1 (2.0)	3007 a (562.4)
Brasília	T1	78.6 (4.7)	92.5 (5.6)	5.0 a (0.0)	56.6 a (0.9)	0.0 a (0.0)	1.0 a (0.0)	44 a (0.8)	126 a (0.0)	18.0 (0.5)	1411 b (392.8)
	T2	80.1 (3.7)	94.1 (4.4)	4.0 b (0.0)	57.4 a (0.7)	0.0 a (0.0)	1.0 a (0.0)	44 a (1.3)	126 a (0.0)	18.0 (0.7)	1553 b (386.4)
	T3	81.9 (4.6)	96.3 (5.2)	4.0 b (0.0)	580 a (1.8)	0.0 a (0.0)	1.0 a (0.0)	44 a (0.5)	126 a (0.0)	15.6 (1.4)	1435 b (443.0)
	T4	79.0 (8.8)	92.9 (10.6)	4.0 b (0.0)	58.1 a (1.6)	0.0 a (0.0)	1.0 a (0.0)	42 b (0.6)	118 b (0.0)	12.8 (0.9)	2622 a (321.8)
	T5	82.3 (1.7)	96.9 (1.7)	4.0 b (0.0)	57.3 a (2.0)	0.0 a (0.0)	1.0 a (0.0)	43 ab (1.0)	126 a (0.0)	12.3 (1.1)	2347 ab (367.8)
Sete Lagoas	T1	86.7 (2.6)	102.8 (3.4)	5.0 a (0.0)	88.9 ab (2.2)	5.0 a (3.6)	2.3 ab (0.5)	43 c (0.0)	112 a (0.0)	19.6 (0.7)	4114 a (155.2)
	T2	89.6 (2.2)	99.8 (9.1)	5.0 a (0.0)	87.3 ab (3.8)	2.0 ab (0.8)	2.3 ab (1.0)	43 c (0.0)	112 a (0.0)	18.3 (0.2)	4028 a (66.2)
	T3	90.6 (4.1)	108.0 (4.5)	5.0 a (0.0)	91.9 a (1.3)	0.0 b (0.0)	3.5 a (1.0)	43 c (0.0)	112 a (0.0)	16.8 (0.4)	3829 a (213.1)
	T4	85.5 (4.4)	101.5 (4.8)	5.0 a (0.0)	84.2 ab (3.2)	0.0 b (0.0)	1.0 b (0.0)	45 b (0.0)	112 a (0.0)	12.2 (0.4)	3830 a (217.9)
	T5	87.6 (3.7)	101.9 (5.6)	5.0 a (0.0)	80.7 b (7.7)	0.3 b (0.5)	3.0 a (0.8)	47 a (0.0)	108 b (0.0)	12.9 (0.9)	4033 a (312.7)

Table F-13 is continued on the next page.

Table F-13 continued.

Location	Trt‡	Trait†									
		G	FS	V	PH	GS	L	DF	DM	100SW	Yield
Londrina	T1	94.2 (2.8)	90.3 (4.3)	5.0 a (0.0)	96.9 ab (3.8)	2.8 a (1.5)	2.0 a (0.8)	46 b (0.0)	129 b (0.0)	21.5 (1.6)	4721 ab (572.2)
	T2	91.5 (3.6)	89.0 (4.5)	5.0 a (0.0)	100.3 a (1.6)	3.5 a (2.1)	1.8 a (0.5)	46 b (0.0)	129 b (0.0)	22.1 (0.3)	5171 a (735.0)
	T3	92.1 (3.4)	90.3 (4.1)	5.0 a (0.0)	101.9 a (3.5)	3.0 a (3.5)	1.8 a (1.0)	47 a (0.0)	130 a (0.0)	19.5 (0.6)	4463 ab (676.7)
	T4	89.6 (2.8)	86.4 (2.2)	5.0 a (0.0)	91.3 b (6.8)	4.6 a (1.9)	1.0 a (0.0)	44 c (0.0)	125 c (0.0)	15.0 (1.2)	3799 b (358.3)
	T5	90.1 (2.5)	89.0 (2.9)	5.0 a (0.0)	83.5 c (2.9)	2.8 a (3.0)	2.3 a (1.0)	44 c (0.0)	125 c (0.0)	16.9 (0.5)	4143 ab (498.9)
Uberaba	T1	89.7 (1.5)	100.9 (2.1)	5.0 a (0.0)	70.5 ab (2.0)	0.3 a (0.5)	1.0 a (0.0)	44 b (0.0)	110 a (0.5)	18.9 (1.0)	3432 a (185.0)
	T2	89.9 (3.1)	101.0 (4.2)	5.0 a (0.0)	75.9 a (4.2)	0.0 a (0.0)	1.0 a (0.0)	44 b (0.0)	111 a (0.5)	17.2 (0.4)	3404 a (177.7)
	T3	92.4 (6.3)	105.0 (9.4)	5.0 a (0.0)	79.5 a (6.7)	0.3 a (0.5)	1.0 a (0.0)	44 b (0.0)	111 a (0.6)	16.1 (0.8)	3107 a (252.8)
	T4	92.5 (2.7)	104.3 (3.3)	5.0 a (0.0)	61.6 b (7.3)	0.0 a (0.0)	1.0 a (0.0)	44 b (0.0)	111 a (1.0)	11.9 (0.6)	3303 a (84.4)
	T5	90.9 (5.6)	99.6 (7.4)	5.0 a (0.0)	62.4 b (9.0)	0.0 a (0.0)	1.0 a (0.0)	47 a (0.0)	108 b (1.0)	12.4 (0.3)	3276 a (274.5)
Santo Antônio de Posse	T1	80.8 (11.8)	77.8 (12.1)	5.0 a (0.0)	94.0 a (4.5)	4.0 a (0.8)	1.8 ab (0.5)	47 a (0.0)	130 a (0.0)	19.5 (1.6)	3159 a (305.4)
	T2	82.4 (6.4)	79.5 (7.0)	5.0 a (0.0)	102.3 a (2.1)	4.3 a (2.9)	1.8 ab (0.5)	47 a (0.0)	130 a (0.0)	18.1 (1.2)	3202 a (589.9)
	T3	88.4 (5.2)	88.4 (6.2)	5.0 a (0.0)	105.2 a (1.0)	1.0 a (0.8)	2.3 a (0.5)	45 b (0.0)	126 b (0.0)	16.1 (1.3)	2788 a (799.3)
	T4	87.5 (2.7)	84.9 (4.0)	5.0 a (0.0)	98.2 a (5.0)	4.0 a (0.8)	1.0 b (0.0)	41 c (0.0)	123 c (0.0)	13.0 (1.1)	3182 a (427.7)
	T5	79.8 (4.4)	78.0 (4.4)	5.0 a (0.0)	91.2 a (3.4)	3.0 a (1.2)	2.8 a (0.5)	41 c (0.5)	123 c (0.0)	13.4 (0.7)	3392 a (346.0)

Table F-13 is continued on the next page.

Table F-13 continued.

Location	Trt‡	Trait†									
		G	FS	V	PH	GS	L	DF	DM	100SW	Yield
Ponta Grossa	T1	80.7 (3.6)	69.1 (3.7)	5.0 a (0.0)	90.1 c (4.3)	19.8 a (10.8)	1.8 c (0.5)	49 c (0.0)	157 a (0.0)	-	-
	T2	80.1 (2.9)	67.1 (2.7)	5.0 a (0.0)	104.0 ab (6.3)	4.5 b (2.1)	2.8 b (0.5)	49 c (0.0)	154 b (0.0)	-	-
	T3	80.5 (4.0)	64.6 (3.1)	5.0 a (0.0)	107.8 a (1.7)	3.5 b (2.6)	2.8 b (0.5)	51 b (0.0)	154 b (0.0)	-	-
	T4	86.8 (0.6)	73.6 (2.3)	5.0 a (0.0)	103.5 ab (4.1)	2.3 b (1.3)	2.0 bc (0.0)	53 a (0.0)	148 c (0.0)	-	-
	T5	79.3 (2.2)	66.0 (6.3)	5.0 a (0.0)	94.9 bc (2.8)	0.0 b (0.0)	4.0 a (0.0)	53 a (0.0)	143 d (0.0)	-	-

†G = initial germination (%); FS = final plant stand; V = seedling vigor; PH = plant height (cm); GS = green stem (%); L = degree of lodging; DF = days to full flower (vegetative cycle); DM = days to full maturity (total cycle); 100SW = seed size [weight of 100 seeds (g)]; and Yield = grain yield (kg/ha).
‡Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
§Means followed by the same letter do not differ statistically significantly by the Tukey test at 5% probability.

b) 2007 growing season.

Since the methodology used in the agronomic evaluations was the same in the 2006/2007 and 2007 growing seasons, only important differences from the description of the methodology for the 2006/2007 season are noted below.

Field Locations. The experiments were planted during the short season in 2007 at six experimental stations in Brazil. The stations are located in regions that are representative of areas of commercial soybean production and areas in which the soybean trial entries are adapted. Details of the stations are listed in Table F-14.

Table F-14. Experimental Station Locations with Location Code, 2007.

Research Station	City, State	Location (Experiment Code)
Embrapa / EPAMIG	Uberaba, MG	CTTP (001)
Embrapa Corn and Sorghum	Sete Lagoas, MG	CNPMS (002)
Embrapa Rice and Beans	Santo Antônio de Goiás, GO	CNPAF (003)
Embrapa Horticulture	Brasília, DF	CNPH (005)
Embrapa Soybean in Vilhena	Vilhena, RO	VILH (006)
Embrapa Mid-North	Teresina, PI	EMN (007)

The predominant soil type, the tillage system (conventional disc tillage or no tillage), and the cropping history varied by location (Table F-15). All locations but one (EMN) had been used for crop production within the previous two years.

Table F-15. A Description of the Soybean Field Trial Locations, 2007.

Location	Soil type	Tillage system	Cropping history			
			Short Season 2005	Summer 2005/06	Short Season 2006	Summer 2006/07
CTTP	Sandy	No till	Fallow	Soybean	Fallow	Fallow
CNPMS	Clay	Conventional	Fallow	Fallow	Fallow	Pearl Millet
CNPAF	Clay sand	Conventional	Fallow	Soybean	Fallow	Rice
CNPH	Clay	Conventional	Soybean	Fallow	Potato	Fallow
VILH	Clay	Conventional	Fallow	Soybean	Fallow	Soybean
EMN	Clay	Conventional	Fallow	Fallow	Fallow	Fallow

Rainfall and temperature was recorded at each location for the period from planting to harvest (Table F-16). While irrigation was required to supplement rainfall at each location, the quantity of water and frequency of application was dependent on the quantity and distribution of rainfall. In general, precipitation was greatest during March and April, reaching means of 126 and 106 mm, respectively. Irrigation was necessary during May and June, when the mean rainfall was only 30 and 0.3 mm, respectively. The mean temperature was 23.7°C and, in general,

temperatures were greater in March and April and decreased from May to July (winter season), except at EMN, where temperatures increased in June and July. The EMN location had precipitation and temperatures above the general mean, while the remaining locations experienced precipitation and temperatures near the mean. Excessive rainfall after planting caused the loss of three plots at EMN.

Table F-16. Rainfall Amount and Average Monthly Temperature at Each of the Soybean Field Trial Locations, 2007.

Location	March		April		May		June		July	
	R*	T	R	T	R	T	R	T	R	T
	mm	˚C	mm	˚C	mm	˚C	mm	˚C	mm	˚C
CTTP	104.9	25.9	109.6	24.2	17.3	21.2	0.0	19.8	15.7	19.4
CNPMS	91.8	24.6	119.5	23.5	8.5	20.7	1.9	19.7	0.0	20.0
CNPAF	49.3	24.1	92.6	23.1	6.1	22.1	0.0	21.4	9.3	22.4
CNPH	132.2	25.0	63.7	24.3	6.0	23.1	0.0	22.5	0.0	23.1
VILH	76.0	23.5	90.0	23.6	22.5	21.8	0.0	22.2	19.0	22.2
EMN	300.4	27.2	161.2	27.2	118.6	27.4	0.1	33.2	4.9	34.0

*R=Rainfall (mm) and T=average monthly temperature (˚C).

Field and Plot Design. As described for the 2006/2007 field trial season.

Land and Crop Management. The land and crop management practices at each location, including the management of pests, pathogens, and weeds, were as recommended for each specific region in which the field trials were located.

At-planting

Seeds were planted by hand at a rate of 13-15 seeds/m (depending on local recommendations) or approximately 260,000 seeds per ha between 27 February and 14 March 2007 (Table F-17).

Table F-17. Planting and Harvest Dates for Each Field Trial Location in Brazil, 2007.

Location	Site Code	Planting date (2007)	Harvest date (2007)
Uberaba, MG	CTTP	March 02	July 03
Sete Lagoas, MG	CNPMS	March 06	July 11
Santo Antônio de Goiás, GO	CNPAF	February 27	July 08
Brasília, DF	CNPH	March 01	July 09
Vilhena, RO	VILH	March 12	July 10
Teresina, PI	EMN	March 14	July 04

Post-planting

To ensure the successful completion of each trial, insecticides, fungicides, and herbicides were applied at each location according to local recommendations and as needed to protect the plants from insect, fungal, and weed infestations (Table F-18).

Table F-18. Insecticides, Herbicides, and Fungicides Applied to Plots After Planting, 2007.

Location	Product	Rate l/ha	Active ingredient concentration	Date of application 2007	Use
CNPH	Vexter®	0.8	Chlorpyrifos 480g/l	12 Mar	Insecticide
	Arsenal®	0.15	Imazapyr 480g/l	1 Apr	Herbicide
	Volt®	1	Bentazon 400g/l + Acifluorfen 170g/l	1 Apr	Herbicide
	Nomolt®	0.08	Teflubenzuron 150g/l	15 Apr	Insecticide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	25 Apr	Fungicide
	Folicur®	0.75	Tebuconazole 200g/l	4 May	Fungicide
	Folicur®	0.75	Tebuconazole 200g/l	20 May	Fungicide
	Engeo™ Pleno	0.3	Cypermethrin 220g/l + Thiamethoxam 110g/l	21 May	Insecticide
	Engeo™ Pleno	0.3	Cypermethrin 220g/l + Thiamethoxam 110g/l	2 Jun	Insecticide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	27 May	Fungicide
CTTP	Arsenal®	0.15	Imazapyr 480g/l	1 Apr	Herbicide
	Volt®	1	Bentazon 400g/l + Acifluorfen 170g/l	1 Apr	Herbicide
	Aramo®	0.5	Tepraloxydim 200g/l	7 Apr	Herbicide
	Fastac®	0.2	Alpha-cypermethrin 100g/l	31 Mar	Insecticide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	17 Apr	Fungicide
	Folicur®	0.75	Tebuconazole 200g/l	26 Apr	Fungicide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	1 May	Fungicide
	Folicur®	0.75	Tebuconazole 200g/l	19 May	Fungicide
	Engeo® Pleno	0.3	Cypermethrin 220g/l + Thiamethoxam 110g/l	19 May	Insecticide
CNPMS	Arsenal®	0.15	Imazapyr 480g/l	18 Apr	Herbicide
	Volt®	1	Bentazon 400g/l + Acifluorfen 170g/l	18 Apr	Herbicide
	Aramo®	0.5	Tepraloxydim 200g/l	24 Apr	Herbicide
	Fastac®	0.2	Alpha-cypermethrin 100g/l	24 Apr	Insecticide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	17 May	Fungicide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	25 Apr	Fungicide
	Engeo® Pleno	0.3	Cypermethrin 220g/l + Thiamethoxam 110g/l	17 May	Insecticide
	Folicur®	0.75	Tebuconazole 200g/l	6 Jun	Fungicide
CNPAF	Arsenal®	0.15	Imazapyr 480g/l	27 Mar	Herbicide
	Volt®	1	Bentazon 400g/l + Acifluorfen 170g/l	27 Mar	Herbicide
	Vexter®	0.8	Chlorpyrifos 480g/l	5 Apr	Insecticide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	15 Apr	Fungicide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	25 Apr	Fungicide
	Folicur®	0.75	Tebuconazole 200g/l	4 May	Fungicide
	Engeo® Pleno	0.3	Cypermethrin 220g/l + Thiamethoxam 110g/l	20 May	Insecticide
	Folicur®	0.75	Tebuconazole 200g/l	21 May	Fungicide
	Engeo® Pleno	0.3	Cypermethrin 220g/l + Thiamethoxan 110g/l	3 Jun	Insecticide

Table F-18. continued

Location	Product	Rate l/ha	Active ingredient concentration	Date of application 2007	Use
EMN	Vexter®	0.8	Chlorpyrifos 480g/l	10 Apr	Insecticide
	Arsenal®	0.15	Imazapyr 480g/l	11 Apr	Herbicide
	Volt®	1	Bentazon 400g/l + Acifluorfen 170g/l	11 Apr	Herbicide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	2 May	Fungicide
VIL	Folisuper® 600BR	0.7	Parathion-methyl 600g/l	19 Mar	Insecticide
	Folisuper® 600BR	0.7	Parathion-methyl 600g/l	26 Mar	Insecticide
	Arsenal®	0.15	Imazapyr 480g/l	3 Apr	Herbicide
	Volt®	1	Bentazon 400g/l + Acifluorfen 170g/l	3 Apr	Herbicide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	9 Apr	Fungicide
	Sumithion® 500CE	1.2	Fenitrothion 500g/l	9 Apr	Insecticide
	Opera®	0.5	Epoxiconazole 50g/l + Pyraclostrobin 133g/l	23 Apr	Fungicide
	Sumithion® 500CE	1.2	Fenitrothion 500g/l	23 Apr	Insecticide
	Pirate®	0.7	Chlorfenapyr 240g/l	3 May	Insecticide
	Rival™	0.4	Tebuconazole 200g/l	11 May	Fungicide
	Engeo® Pleno	0.17	Cypermethrin 220g/l + Thiamethoxan 110g/l	11 May	Insecticide
	Vexter®	0.55	Chlorpyrifos 480g/l	11 May	Insecticide
	Engeo® Pleno	0.17	Cypermethrin 220g/l + Thiamethoxan 110g/l	23 May	Insecticide
	Vexter®	0.55	Chlorpyrifos 480g/l	23 May	Insecticide
	Rival™	0.5	Tebuconazole 200g/l	28 May	Fungicide

Treatments. The same five treatments as described for the 2006/2007 field trial season were included in the 2007 field trial season (Table F-6). The only difference was that the F_6 generation of CV127, line 127 (Figure III-1), and the corresponding generation of the isoline control (null segregant) were used in the 2007 studies.

Results and Discussion

Based on analyses of variance (Table F-19):

1) location had a significant effect on all measured traits, including percent germination (G), seedling vigor (V), initial (IS) and final (FS) plant stand, days to full flower (DF), days to full maturity (DM), percent plants with green stem (GS), plant height (PH), grain yield (Yield), seed size (100SW), and lodging (L),

2) no significant effect was observed for treatment for any of the traits,

3) the combination of location and treatment had a significant effect on plant height (PH), percent plants with green stem (GS), seed size (100SW), grain yield (Yield), lodging (L), days to full flower (DF) and days to full maturity (DM).

Table F-19. Agronomic and Phenotypic Evaluations 2007: Statistically Significant and Non-Significant Sources of Variation in the Model Used to Analyze Data for Each Trait.

Source of Variation	Trait[1]										
	G	V	IS	FS	PH	GS	100SW	Yield	L	DF	DM
Location (L)	3678.37**	2.41**	4863.69**	5539.36**	3078.27**	8343.29**	16.86**	3723651.19**	20.83**	2367.07**	2675.40**
Treatment (T)	268.35	0.05	369.06	399.81	664.50	6801.09	85.14	423247.06	0.23	9.72	32.52
L x T	136.96	0.05	190.39	189.34	50.32**	1827.25**	2.61**	306005.05**	0.23**	59.70**	10.80**
Error	103.16	0.17	146.72	152.24	6.26	28.57	0.85	105353.70	0.05	0.15	0.54
Mean	80.04	4.86	93.72	89.59	57.57	24.66	17.83	2324.72	1.42	43.93	114.75
CV%	12.70	8.51	12.92	13.77	4.35	21.68	5.17	13.96	16.00	0.87	0.64

[1]G = initial germination (%); V = seedling vigor; IS = initial plant stand; FS = final plant stand; PH = plant height (cm); GS = green stem (%); 100SW = seed size [weight of 100 seeds (g)]; Yield = grain yield (kg/ha); L = degree of lodging; DF = days to full flower (vegetative cycle) and DM = days to full maturity (total cycle).

*,** = significant at $P<0.05$ and $P<0.01$, respectively.

Due to the naturally different environmental characteristics (temperature, rainfall, humidity, day length, etc.) among trial locations, location had a significant effect on all assessed traits. The plant density was nearly ideal at all locations, except Teresina, where the final stand was the lowest due to low seed germination (Table F-20) caused by excessive rainfall shortly after planting. As expected for short-season growing conditions, plant height was normally low, except at Sete Lagoas, where plants reached a mean stature of 82 cm and the lodging reached the higher index of 3.5. Percentage of green stem was higher at Uberaba, Sete Lagoas and Vilhena. An error in the preparation of the herbicide imazapyr for application of treatment T1 at Uberaba and Sete Lagoas resulted in the application of more than three times the intended application rate (70 g a.i./ha) and caused high phytotoxicity, loss of pods and 100% of plants showing green stem over all the plots with treatment T1. These plots were not harvested and no data was recorded for seed size (100SW), days to full maturity (DM) and yield at these two sites (Table F-22). The higher green stem percentage observed at Vilhena was likely caused by stink bug attack.

The location mean for seed size ranged from 16.4 grams at Uberaba to 19 grams at Vilhena (Table F-20). Flowering occurred earlier at Vilhena and Teresina as a consequence of the lower latitudes of these sites. Similarly, the total cycle was shorter (less than 115 days) in these two sites and also in Uberaba and Sete Lagoas. The yield was relatively high at Brasília and Sete Lagoas, medium at Teresina and Uberaba and low at Santo Antônio de Goiás and Vilhena (Table F-20). The wide-ranging performance obtained for grain yield among the different sites was expected and considered normal for short-season conditions. However, the coefficients of variation for all traits were considered low and acceptable for the purposes of this work (Table F-19).

Table F-20. Agronomic and Phenotypic Evaluations 2007: Location Means Across Treatments and Standard Deviations (in Parentheses) for Evaluated Traits.

Location	Trait[1]										
	G	V	IS	FS	PH	GS	100SW	Yield	L	DF	DM
Uberaba	73.95 c* (7.0)	5.00 a (0.0)	88.70 c (8.5)	79.95 b (9.6)	47.69 b (6.0)	39.20 ab (35.0)	16.44 c (2.4)	2142.00 bc (509.8)	1.00 b (0.0)	45.20 b (0.8)	107.56 d (1.5)
Sete Lagoas	79.75 bc (5.5)	5.00 a (0.0)	95.70 bc (6.4)	90.60 b (8.1)	82.37 a (6.6)	41.35 ab (33.3)	17.37 abc (2.2)	2659.38 ab (368.6)	3.50 a (0.8)	48.90 a (0.8)	113.06 c (1.0)
Santo Antônio de Goiás	92.95 a (2.5)	5.00 a (0.0)	111.43 a (3.0)	110.45 a (3.1)	54.30 b (5.3)	10.00 ab (0.0)	18.00 abc (1.9)	1948.60 c (362.2)	1.00 b (0.0)	44.00 b (0.0)	130.15 a (1.0)
Brasília	89.25 ab (3.3)	5.00 a (0.0)	107.18 ab (3.9)	106.23 a (4.0)	53.20 b (7.1)	3.00 b (2.5)	18.60 ab (1.6)	3054.55 a (356.3)	1.00 b (0.0)	49.00 a (0.0)	126.60 b (1.6)
Vilhena	88.10 ab (5.9)	5.00 a (0.0)	91.85 c (6.2)	84.15 b (7.8)	54.45 b (7.6)	48.40 a (33.7)	19.02 a (2.6)	1975.90 c (427.5)	1.00 b (0.0)	37.30 d (1.4)	99.95 e (3.1)
Teresina	56.25 d (22.7)	4.15 a (1.0)	67.48 d 27.3)	66.15 c (26.8)	53.40 b (3.4)	6.00 ab (5.0)	17.11 bc (2.0)	2175.94 bc (443.4)	1.00 b (0.0)	39.20 c (1.0)	109.40 d (2.0)

[1]G = initial germination (%); V = seedling vigor; IS = initial plant stand; FS = final plant stand; PH = plant height (cm); GS = green stem (%); 100SW = seed size [weight of 100 seeds (g)]; Yield = grain yield (kg/ha); L = degree of lodging; DF = days to full flower (vegetative cycle) and DM = days to full maturity (total cycle).
*Means followed by the same letter do not differ statistically significantly by the Tukey test at 5% probability.

As an average across locations for any given trait, the means of all treatments showed no evidence of a significant difference (Table F-21). However, the Location x Treatment interaction was statistically significant for plant height (PH), green stem (GS), seed size (100SW), yield, lodging (L), days to flowering (DF) and days to maturity (DM) (Table F-19). For all other traits, the means of the treatments over locations were a reliable indicator of the effect of each treatment on that particular trait. With the exception of percent green stem at Uberaba and Sete Lagoas (caused by the application of a higher concentration of imazapyr, as reported previously) and days to flowering at Uberaba the performance of CV127 treated with imazapyr (T1) and CV127 treated with Volt® (T2) were not significantly different (Table F-22). Further, the performance of T2 (CV127 treated with Volt®) and T3 (isoline control treated with Volt®) were not statistically significantly different for germination, seedling vigor, initial and final plant stand, lodging, days to flowering or days to maturity at any of the field trial locations. T2 and T3 only differed statistically significantly for plant height at Sete Lagoas, percent green stem at Uberaba and Vilhena, seed size at Uberaba, Sete Lagoas and Vilhena and yield at Vilhena (Table F-22). Similarly, the instances where the performance of T1 and T3 differed significantly included plant height at Sete Lagoas, green stem at Uberaba, Sete Lagoas and Vilhena, seed size and yield at Vilhena, and days to flowering at Uberaba (Table F-22).

Table F-21. Agronomic and Phenotypic Evaluations 2007: Treatment Means and Standard Deviations (in Parentheses) Over Location for Each Trait.

Trt[2]	Trait[1]										
	G	V	IS	FS	PH	GS	100SW	Yield	L	DF	DM
T1	78.88 (18.2)	4.92 (0.3)	92.42 (21.8)	88.83 (22.5)	59.84 (9.3)	48.75 (43.1)	20.14 (1.8)	2256.27 (787.0)	1.46 (1.1)	43.63 (4.4)	117.63 (12.9)
T2	76.21 (21.6)	4.79 (0.6)	89.15 (25.7)	84.96 (26.2)	60.69 (10.5)	27.92 (22.2)	19.92 (1.3)	2284.04 (614.6)	1.50 (1.1)	44.00 (4.3)	115.42 (11.2)
T3	78.04 (17.2)	4.88 (0.4)	91.42 (19.9)	86.71 (20.8)	62.63 (13.6)	15.83 (9.4)	18.21 (0.9)	2510.04 (466.3)	1.50 (1.2)	44.17 (4.2)	114.96 (11.3)
T4	83.83 (10.6)	4.88 (0.6)	98.02 (18.5)	94.77 (11.2)	55.12 (14.4)	27.50 (32.7)	16.42 (1.3)	2158.75 (508.8)	1.29 (0.7)	43.58 (5.4)	114.04 (9.9)
T5	83.25 (9.1)	4.83 (0.6)	97.60 (10.5)	92.67 (13.3)	49.56 (12.3)	3.29 (4.0)	15.45 (1.3)	2394.83 (538.8)	1.33 (0.8)	44.29 (4.6)	112.67 (11.3)

[1]G = initial germination (%); V = seedling vigor; IS = initial plant stand; FS = final plant stand; PH = plant height (cm); GS = green stem (%); 100SW = seed size [weight of 100 seeds (g)]; Yield = grain yield (kg/ha); L = degree of lodging; DF = days to full flower (vegetative cycle) and DM = days to full maturity (total cycle).
[2]Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

Table F-22. Agronomic and Phenotypic Evaluations 2007: Means and Standard Deviations (in Parentheses) for Each Combination of Treatment and Location for All Traits.

Location	Trt[2]	Trait[1]										
		G	V	IS	FS	PH	GS	100SW	Yield	L	DF	DM
Uberaba	T1	71.25 (9.5)	5.00 (0.0)	85.25 (11.7)	78.50 (12.1)	50.20 ab* (1.5)	100.00 a (0.0)	ND[3]	ND	1.00 a (0.0)	44.00 c (0.0)	ND
	T2	70.25 (10.8)	5.00 (0.0)	84.00 (13.0)	76.50 (11.5)	48.80 ab (1.8)	47.50 b (5.0)	19.53 a (0.7)	1863.50 a (97.5)	1.00 a (0.0)	45.00 b (0.0)	109.00 a (0.0)
	T3	73.75 (2.1)	5.00 (0.0)	88.75 (2.8)	77.50 (11.2)	54.05 a (4.2)	25.00 c (10.0)	17.60 b (0.7)	2593.50 a (811.4)	1.00 a (0.0)	45.00 b (0.0)	109.00 a (0.0)
	T4	78.00 (4.1)	5.00 (0.0)	93.50 (4.7)	88.00 (4.2)	46.90 b (3.1)	22.50 c (5.0)	14.28 c (0.6)	2019.25 a (316.4)	1.00 a (0.0)	46.00 a (0.0)	106.00 b (0.0)
	T5	76.50 (4.5)	5.00 (0.0)	92.00 (5.7)	79.25 (7.0)	38.50 c (4.4)	1.00 d (1.4)	14.35 c (0.3)	2091.75 a (364.4)	1.00 a (0.0)	46.00 a (0.0)	106.25 b (0.5)
Sete Lagoas	T1	87.00 (4.5)	5.00 (0.0)	104.00 (5.4)	100.25 (5.3)	78.35 cd (2.9)	100.00 a (0.0)	ND	ND	3.75 a (0.5)	48.00 b (0.0)	ND
	T2	77.75 (6.1)	5.00 (0.0)	93.25 (7.4)	86.00 (8.1)	81.85 bc (2.5)	35.00 bc (5.8)	20.23 a (0.9)	2446.00 a (102.8)	4.00 a (0.0)	48.50 ab (0.6)	114.00 a (0.0)
	T3	77.25 (3.7)	5.00 (0.0)	93.00 (4.1)	85.75 (8.3)	91.75 a (3.2)	23.75 c (7.5)	18.15 b (0.3)	2742.50 a (349.9)	4.00 a (0.8)	49.00 ab (0.8)	114.00 a (0.0)
	T4	78.00 (4.1)	5.00 (0.0)	93.50 (4.7)	90.75 (5.6)	85.55 ab (2.9)	42.50 b (12.6)	16.10 c (1.0)	2509.50 a (401.2)	2.75 a (0.5)	49.25 ab (0.5)	112.25 b (0.5)
	T5	78.75 (3.6)	5.00 (0.0)	94.75 (4.3)	90.25 (6.4)	74.35 d (2.9)	5.50 d (3.3)	14.95 c (0.4)	2939.50 a (425.8)	3.00 a (0.8)	49.75 a (0.5)	112.00 b (0.0)

Table F-22 is continued on the next page.

Table F-22 continued.

Location	Trt[2]	Trait[1]										
		G	V	IS	FS	PH	GS	100SW	Yield	L	DF	DM
Santo Antônio de Goiás	T1	91.50 (4.3)	5.00 (0.0)	110.00 (5.2)	109.38 (5.4)	59.00 a (1.8)	10.00 a (0.0)	20.13 a (1.0)	2017.75 a (238.0)	1.00 a (0.0)	44.00 a (0.0)	131.00 a (0.0)
	T2	94.50 (1.8)	5.00 (0.0)	113.25 (2.2)	113.13 (2.0)	58.00 a (1.4)	10.00 a (0.0)	19.18 a (1.3)	1928.75 a (326.9)	1.00 a (0.0)	44.00 a (0.0)	131.00 a (0.0)
	T3	93.25 (2.0)	5.00 (0.0)	111.63 (2.4)	110.38 (2.2)	58.00 a (2.0)	10.00 a (0.0)	18.53 a (1.0)	2179.50 a (347.5)	1.00 a (0.0)	44.00 a (0.0)	130.50 a (1.0)
	T4	92.50 (2.1)	5.00 (0.0)	110.88 (2.6)	109.50 (2.7)	48.50 b (1.0)	10.00 a (0.0)	16.28 b (0.5)	1985.50 a (287.9)	1.00 a (0.0)	44.00a (0.0)	129.25 b (0.5)
	T5	93.00 (1.7)	5.00 (0.0)	111.38 (2.1)	109.88 (1.7)	48.00 b (1.6)	10.00 a (0.0)	15.90 b (0.3)	1631.50 a (499.5)	1.00 a (0.0)	44.00 a (0.0)	129.00 b (0.0)
Brasília	T1	89.75 (2.2)	5.00 (0.0)	107.75 (2.6)	107.3 (2.3)	57.50 a (4.5)	5.00 a (0.0)	20.20 a (1.2)	3343.25 ab (332.8)	1.00 a (0.0)	49.00 a (0.0)	128.00 a (0.0)
	T2	90.00 (4.8)	5.00 (0.0)	108.00 (5.8)	107.63 (5.8)	59.00 a (2.2)	5.00 a (0.0)	19.70 ab (0.9)	3385.50 a (241.4)	1.00 a (0.0)	49.00 a (0.0)	128.00 a (0.0)
	T3	87.25 (1.6)	5.00 (0.0)	104.88 (1.9)	103.50 (2.1)	58.75 a (1.5)	5.00 a (0.0)	18.88 ab (0.6)	2898.25 ab (260.4)	1.00 a (0.0)	49.00 a (0.0)	127.25 a (1.5)
	T4	87.75 (4.3)	5.00 (0.0)	105.50 (5.1)	104.25 (5.0)	45.75 b (1.5)	0.00 b (0.0)	16.60 c (0.9)	2874.00 ab (305.5)	1.00 a (0.0)	49.00 a (0.0)	125.00 b (0.0)
	T5	91.50 (1.9)	5.00 (0.00	109.75 (2.3)	108.38 (2.5)	45.00 b (4.1)	0.00 b (0.0)	17.63 bc (1.4)	2771.75 b (185.4)	1.00 a (0.0)	49.00 a (0.0)	124.75 b (0.5)

Table F-22 is continued on the next page.

Table F-22 continued.

Location	Trt[2]	Trait[1]										
		G	V	IS	FS	PH	GS	100SW	Yield	L	DF	DM
Vilhena	T1	83.50 (5.0)	5.00 (0.0)	87.00 (5.2)	77.00 (4.0)	58.50 a (2.6)	67.50 b (15.0)	21.50 a (1.9)	1701.5 b (206.6)	1.00 a (0.0)	36.75 bc (0.5)	100.50 b (1.9)
	T2	83.25 (1.4)	5.00 (0.0)	86.50 (1.5)	77.00 (4.1)	60.00 a (2.3)	60.00 b (14.1)	21.58 a (1.4)	1800.50 b (187.8)	1.00 a (0.0)	37.50 ab (1.3)	99.50 bc (1.0)
	T3	88.00 (5.4)	5.00 (0.0)	92.00 (5.6)	86.25 (5.3)	58.25 a (1.9)	21.25 c (6.3)	18.58 b (0.9)	2371.25 a (128.6)	1.00 a (0.0)	38.00 ab (0.0)	98.00 bc (1.2)
	T4	96.50 (2.4)	5.00 (0.0)	100.50 (2.5)	93.25 (1.3)	54.25 a (4.9)	90.00 a (7.1)	18.20 b (0.3)	1518.75 b (128.3)	1.00 a (0.0)	35.25 c (0.5)	104.75 a (2.5)
	T5	89.25 (1.2)	5.00 (0.0)	93.25 (1.2)	87.25 (6.9)	41.25 b (2.4)	3.25 c (2.4)	15.25 c (0.7)	2487.75 a (261.1)	1.00 a (0.0)	39.00 a (0.0)	97.00 c (0.0)
Teresina	T1	50.25 (26.9)	4.50 (0.6)	60.50 (32.3)	60.50 (32.3)	55.50a (2.5)	10.00 a (0.0)	18.27 a (2.3)	1865.00 a (837.0)	1.00 a (0.0)	40.00 a (0.0)	111.00 a (0.0)
	T2	41.50 (31.3)	3.75 (1.0)	49.88 (37.6)	49.50 (37.6)	56.50 a (1.7)	10.00 a (0.0)	19.10 a (1.7)	2278.67 a (520.5)	1.00 a (0.0)	40.00 a (0.0)	111.00 a (0.0)
	T3	48.75 (22.0)	4.25 (1.0)	58.25 (26.4)	56.88 (26.0)	55.00 a (2.2)	10.00 a (0.0)	17.33 ab (1.1)	2197.00 a (292.2)	1.00 a (0.0	40.00 a (0.0)	111.00 a (0.0)
	T4	70.25 (11.6)	4.25 (1.5)	84.25 (13.9)	82.88 (14.0)	49.75 b (1.7)	0.00 b (0.0)	17.05 ab (0.5)	2045.50 a (236.6)	1.00 a (0.0)	38.00 b (0.0)	107.00 b (0.0)
	T5	70.50 (4.1)	4.00 (1.2)	84.50 (4.9)	81.00 (4.4)	50.25 b (1.3)	0.00 b (0.0)	14.63 b (1.0)	2446.75 a (245.3)	1.00 a (0.0)	38.00 b (0.0)	107.00 b (0.0)

[1]G = initial germination (%); V = seedling vigor; IS = initial plant stand; FS = final plant stand; PH = plant height (cm); GS = green stem (%); 100SW = seed size [weight of 100 seeds (g)]; Yield = grain yield (kg/ha); L = degree of lodging; DF = days to full flower (vegetative cycle) and DM = days to full maturity (total cycle).

[2]Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

[3]ND = Not determined

[*]Means followed by the same letter do not differ statistically significantly by the Tukey test at 5% probability.

Conclusions

The above studies compared the agronomic, phenotypic, and phenologic characteristics of CV127 soybean relative to the isoline control and to other conventional soybean varieties. Although statistically significant differences did exist between CV127 and the isoline control for specific traits at specific locations, the only trait for which there was a trend toward a consistent difference between CV127 and the isoline control was seed size. For all other traits, including seedling vigor, yield, germination, plant height, green stem, lodging, and days to flowering and maturity, there were no consistent significant differences between CV127 and the isoline control. The lack of any biologically significant differences between CV127 and the isoline control for any of the evaluated traits demonstrates that CV127 is agronomically, phenotypically, and phenologically equivalent to the isoline control as well as conventional soybean varieties under standard soybean production practices.

2.0. Disease susceptibility in the a) 2006/2007 and b) 2007 growing seasons

Introduction

Plant diseases can cause serious losses to soybean yield in commercial production in Brazil. Since 2001, Asian soybean rust, caused by the fungus *Phakopsora pachyrhizi*, has become one of the most important diseases in Brazilian soybean agriculture due to yield losses and the expense of control measures. In addition to rust, downy mildew (*Peronospora manshurica*) can cause yield losses in soybean, and other leaf diseases known as end-of-cycle diseases (DC) caused mainly by *Septoria glycines* (brown spot or Septoriose) and *Cercospora kikuchii* (soybean leaf spot), can reduce the yield by more than 20%. According to Sinclair and Hartman (1999), powdery mildew can cause a 10 to 35% reduction in the yield of susceptible soybean cultivars and can occur at any phase of the plant's growth. These pathogens are currently disseminated throughout the soybean-producing regions in Brazil, and the use of resistant cultivars and fungicide treatment are the main control methods.

The purpose of the current study was to evaluate the disease susceptibility characteristics of CV127 soybean relative to the isoline control and to two conventional soybean varieties. Field studies were conducted during the 2006/2007 and 2007 growing seasons at the thirteen field locations in Brazil described above in section 1.0 of Appendix F. Disease susceptibility of CV127 soybean relative to the isoline control and conventional soybean varieties was determined at different stages of plant growth and development throughout the growing season.

Materials and Methods

Field design, management and treatments. The field experimental design and management practices at each field trial location, and treatments in each study have been described above in section 1.0 of Appendix F ("Agronomic and Phenotypic Evaluations").

Disease evaluations. The incidence of all diseases present at the time of assessment was recorded for each plot as the percent of affected leaf area. The dominant diseases causing infection were identified at each assessment. Assessments were made at or near vegetative stage

V4 and reproductive stages R1, R5, and R7. Exact stages in which assessments were made are indicated in the data tables. Due to the potential in Brazil for fungal diseases to devastate the soybean crop and thereby compromise the goals of this study, all plots were uniformly treated with fungicides in order to maintain plant health. Evaluations of disease incidence in each plot were typically made prior to disease control treatments.

Statistical Analyses. Data were analyzed by ANOVA on a per location, per disease, and per time-of-assessment basis. In those cases where differences among treatments were significant, Tukey's procedure (Steel and Torrie, 1980) was used to make pair-wise comparisons of treatment means.

Results and Discussion

a) 2006/2007 growing season

Despite multiple applications of fungicide, Asian rust (*Phakopsora pachyrhizi*) was present at all locations. In addition, powdery mildew (*Erysiphe diffusa*), downy mildew (*Peronospora manshurica*), and late-season diseases, brown spot (*Septoria glycines*) and leaf spot (*Cercospora kikuchii*), were present at most locations (Tables F-23 to F-29). In the 2006/2007 field trials, no diseases were evident prior to the V4 stage of development. In the data presented in Tables F-23 to F-29, where any of the four diseases listed above were not observed on plants grown at a particular field location and stage of plant development, then no data is presented.

No significant differences in disease incidence were observed among CV127 treated with imazapyr (T1), CV127 treated with Volt® (T2), and the isoline control (T3), (Tables F-23 to F-29). Significant differences in affected leaf area due to Asian rust between the treatments containing the Conquista genetic background (T1, T2 and T3) and the conventional reference varieties, Monsoy 8001 (T4) and CD 217 (T5), were detected at one or more of the assessment stages (R1, R5, or R7) in Ponta Grossa (Table F-23), Londrina (Table F-24), Santo Antônio de Posse (Table F-25), and Brasília (Table F-28). These differences in disease susceptibility were attributed to genetic differences between the Conquista germplasm and the germplasm of the conventional reference soybean varieties.

Other than a high incidence of Asian rust at several locations, the incidence of other diseases was generally low. Although a few isolated differences in disease susceptibility were noted among the treatments, these were not consistent across field trial locations and in general there were no differences in susceptibility between CV127 and the isoline control. Where susceptibility differences were observed, they were generally between varieties with the Conquista genetic background (CV127 and the isoline control) and the two conventional soybean varieties with different genetic backgrounds.

Table F-23. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Ponta Grossa Field Site in 2006/2007.

Treatment[†]	Growth Stage at Assessment						
	V4	R1		R5		R7	
	Diseases[‡]						
		PM	DM	DC	AR	DC	AR
T1	ND[§]	6 a[¶]	2	5	8.4 a	5	48 a
T2	ND	6 a	2	5	6.0 ab	5	43 ab
T3	ND	7 a	2	5	6.0 ab	5	40 ab
T4	ND	16 b	2	5	5.3 b	5	30 b
T5	ND	8 a	2	5	4.8 b	5	36 ab

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡]PM = powdery mildew (*Erysiphe diffusa*); DM = downy mildew (*Peronospora manshurica*); DC = end-of-cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.
[¶]Means followed by the same letter do not differ statistically significantly by the Tukey's test at 5% probability.

Table F-24. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Londrina Field Site in 2006/2007.

Treatment[†]	Growth Stage at Assessment						
	V4	R1		R5		R7	
	Diseases[‡]						
		DM	AR	PM	AR	DC	AR
T1	ND[§]	1.8	2.0 c[¶]	2	50 a	5	60 a
T2	ND	1.5	2.0 c	2	51 a	5	60 a
T3	ND	1.8	2.0 c	2	51 a	5	60 a
T4	ND	1.8	2.8 b	2	44 b	5	51 b
T5	ND	1.8	4.0 a	2	30 c	5	40 c

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡]PM = powdery mildew (*Erysiphe diffusa*); DM = downy mildew (*Peronospora manshurica*); DC = end-of-cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.
[¶]Means followed by the same letter do not differ statistically significantly by the Tukey's test at 5% probability.

Table F-25. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Santo Antônio de Posse Field Site in 2006/2007.

Treatment[†]	Growth Stage at Assessment					
	V4	R1	R5		R7	
	Diseases[‡]					
		AR	DM	AR	DC	AR
T1	ND[§]	7.3	2.3	48 a[¶]	5	58 a
T2	ND	6.8	2.0	45 a	5	58 a
T3	ND	7.3	1.5	50 a	5	60 a
T4	ND	7.5	1.8	30 b	5	40 b
T5	ND	6.9	2.8	43 a	5	52 a

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡]DM = downy mildew (*Peronospora manshurica*); DC = end-of-cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.
[¶]Means followed by the same letter do not differ statistically significantly by the Tukey's test at 5% probability.

Table F-26. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Uberaba Field Site in 2006/2007.

Treatment[†]	Growth Stage at Assessment				
	V4	R1	R5		R7
	Diseases[‡]				
			PM	AR	AR
T1	ND[§]	ND	1.5	2.0	2.5
T2	ND	ND	1.5	2.0	2.5
T3	ND	ND	1.5	2.0	2.5
T4	ND	ND	1.5	2.0	2.5
T5	ND	ND	1.5	2.2	2.5

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡]PM = powdery mildew (*Erysiphe diffusa*); AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.

Table F-27. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Sete Lagoas Field Site in 2006/2007.

Treatment[†]	Growth Stage at Assessment			
	V4	R1	R5	R7
	Diseases[‡]			
		AR	AR	AR
T1	ND[§]	1.5	2.5	2.5
T2	ND	1.5	2.5	2.5
T3	ND	1.5	2.5	2.5
T4	ND	1.5	2.5	2.5
T5	ND	1.5	2.5	2.5

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡]AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.

Table F-28. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Brasília Field Site in 2006/2007.

Treatment[†]	Growth Stage at Assessment					
	V4	R1	R5		R7	
	Diseases					
		AR	DC	AR	DC	AR
T1	ND[§]	1.6	0.4 ab[¶]	39 a	4.4	45 a
T2	ND	2.0	0.6 ab	42 a	4.1	55 a
T3	ND	1.7	0.4 ab	40 a	4.4	45 a
T4	ND	1.9	1.1 a	19 b	5.0	23 b
T5	ND	1.8	0.0 b	19 b	4.7	23 b

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡]DC = end-of-cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.
[¶]Means followed by the same letter do not differ statistically significantly by the Tukey's test at 5% probability.

Table F-29. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Santo Antônio de Goiás Field Site in 2006/2007.

Treatment[†]	Growth Stage at Assessment					
	V6	R1	R5		R7	
	Diseases[‡]					
	DC	AR	DC	AR	DC	AR
T1	ND[§]	0.8	0.9	18	2.8	24
T2	ND	1.0	0.5	17	2.9	22
T3	ND	0.9	0.1	19	2.4	22
T4	ND	0.6	0.3	18	3.6	21
T5	ND	0.8	0.6	18	3.1	22

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡]DC = end-of-cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.

b) 2007 growing season

The short-season in Brazil typically has a low disease incidence of Asian rust (*Phakopsora pachyrhizi*) and this was reflected in the data collected. Asian rust was present in five of the six field site locations in 2007, but with very low severity. In addition, powdery mildew (*Erysiphe diffusa*) and late-season diseases, brown spot (*Septoria glycines*) and leaf spot (*Cercospora kikuchii*), were also present at some locations (Tables F-30 to F-34) but with low severity. No diseases were evident prior to the V4 stage of development, with the exception of powdery mildew in Santo Antônio de Goiás (Table F-32). In Tables F-30 to F-35, in cases where any of the four diseases listed above were not present at a specific field location or stage of plant development, then no data is presented.

No statistically significant differences in disease incidence occurred between the isoline control and CV127 treatments in any of the locations in the 2007 field trials, except in Uberaba where CV127 treated with imazapyr had a higher level of end-of-cycle diseases than the isoline at the R7 stage of development (Table F-30).

Conclusions

Although a few isolated differences in disease susceptibility were noted among the treatments across both the 2006/2007 and 2007 growing seasons, these differences were not consistent across field trial locations and in general there were no differences in susceptibility between CV127 and the isoline control. Where susceptibility differences were observed, they were generally between treatments with the Conquista genetic background (CV127 and the isoline

control) and the two conventional soybean varieties with different genetic backgrounds. Therefore disease susceptibility of CV127 was found to be no different from that of the isoline control plants. This study demonstrates that the insertion of the *csr1-2* gene into the genome of CV127 did not affect the disease susceptibility of CV127.

Table F-30. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Uberaba Field Site in 2007.

Treatment[†]	Growth Stage at Assessment					
	V4	R1	R5.1		R7	
	Diseases[‡]					
		AR	DC	AR	DC	AR
T1	ND[§]	1.5	0.7 b[¶]	0.8	1.5 a	0.6
T2	ND	1.5	1.1 ab	0.7	1.4 ab	0.6
T3	ND	1.5	0.9 ab	0.6	1.3 b	0.5
T4	ND	1.5	1.2 a	1.0	1.5 a	0.6
T5	ND	1.5	0.8 ab	0.8	1.5 a	0.5

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡] DC = end-of-cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.
[¶]Means followed by the same letter do not differ statistically significantly by the Tukey's test at 1% probability.

Table F-31. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Sete Lagoas Field Site in 2007.

Treatment[†]	Growth Stage at Assessment				
	V4	R1	R5.1		R7
	Diseases[‡]				
		AR	AR	PM	AR
T1	ND[§]	0.5	0.7	1.0 b[¶]	1.0
T2	ND	0.6	0.8	0.8 b	1.1
T3	ND	0.6	1.0	0.7 b	1.0
T4	ND	0.6	0.7	1.7 a	1.1
T5	ND	0.5	0.6	0.6 b	1.0

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡]AR = Asian rust (*Phakopsora pachyrhizi*); PM = powdery mildew (*Erysiphe diffusa*).
[§]ND = no diseases were observed.
[¶]Means followed by the same letter do not differ statistically significantly by the Tukey's test at 1% probability.

Table F-32. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Santo Antônio de Goiás Field Site in 2007.

Treatment[†]	Growth Stage at Assessment			
	V4	R1	R5.1	R7
	Diseases[‡]			
	PM	PM	AR	AR
T1	5	6.1	3.4	1.3
T2	5	7.1	2.2	1.9
T3	5	6.6	2.5	0.6
T4	5	6.3	2.1	1.3
T5	5	6.4	3.1	0.9

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡] PM = powdery mildew (*Erysiphe diffusa*); AR = Asian rust (*Phakopsora pachyrhizi*).

Table F-33. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Brasília Field Site in 2007.

Treatment[†]	Growth Stage at Assessment			
	V4	R1	R5.1	R7
	Diseases[‡]			
		PM	AR	AR
T1	ND[§]	1.4	1.4	1.6
T2	ND	1.6	0.9	1.6
T3	ND	1.3	0.7	1.6
T4	ND	1.6	0.9	1.3
T5	ND	1.6	1.4	1.6

[†] T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
[‡] PM = powdery mildew (*Erysiphe diffusa*); AR = Asian rust (*Phakopsora pachyrhizi*).
[§]ND = no diseases were observed.

Table F-34. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Vilhena Field Site in 2007.

Treatment†	Growth Stage at Assessment				
	V4	R1	R5.1	R7	
	Diseases‡				
		AR	AR	DC	AR
T1	ND§	3.7	8.4	5.0	22.0
T2	ND	3.7	8.6	5.0	20.6
T3	ND	3.6	7.6	4.4	20.2
T4	ND	3.6	8.0	5.0	19.8
T5	ND	2.1	5.2	5.0	10.5

† T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
‡ DC = end-of-cycle diseases (*Septoria glycines, Cercospora kikuchii*); AR = Asian rust (*Phakopsora pachyrhizi*).
§ND = no diseases were observed.

Table F-35. Disease Assessment Presented as Mean Percent Affected Leaf Area at the Teresina Field Site in 2007.

Treatment†	Growth Stage at Assessment			
	V4	R1	R5.1	R7
	Diseases			
T1	ND§	ND	ND	ND
T2	ND	ND	ND	ND
T3	ND	ND	ND	ND
T4	ND	ND	ND	ND
T5	ND	ND	ND	ND

† T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = CD 217 treated with Volt®.
§ND = no diseases were observed.

3.0. Insect interactions in the a) 2006/2007 and b) 2007 growing seasons

Introduction

Populations of several different insect orders are common in commercial soybean production fields in Brazil and include the Coleopterans (*Diabrotica* and *Aracanthus* spp.), Lepidopterans (*Anticarsia*, *Agrotis*, *Pseudoplusia*, and *Spodoptera* spp.), and Hemipterans (*Euschistus*, *Piezodorus*, and *Nezara* spp.). Insect pests in these orders can cause serious losses to soybean yield in commercial production in Brazil. The purpose of the current study was to evaluate resistance to insect damage and the effect on in-field insect populations of imidazolinone-tolerant CV127 soybean relative to the isoline control and the two conventional soybean varieties. Field studies were conducted during the 2006/2007 and 2007 growing season at the thirteen field locations in Brazil described above in section 1.0 of Appendix F. Resistance to insect damage and effects on in-field insect populations of CV127 soybean relative to the isoline control and conventional soybean varieties were determined at different stages of plant growth and development throughout each growing season.

Materials and Methods

Field design, management and treatments. The field experimental design and management practices at each field trial location, and treatments in each study have been described above in section 1.0 of Appendix F ("Agronomic and Phenotypic Evaluations").

Insect interaction evaluations. Insects were collected by placing a shake cloth (a 1.0 m x 0.8 m tarp supported on either side by a wooden rod) on the ground between the center two rows of each plot. The plants in the two rows adjacent to the shake cloth were bent over the cloth and shaken vigorously to remove all insects within the canopy. All insects that fell on the cloth were counted and identified by order. This method was initially conducted at or near the V4 stage and repeated at or near the R1, R5, and R7 stages. Leaf, stem, and/or pod damage caused by feeding of each insect type was also evaluated at each sampling date using a damage rating scale (Table F-36). However, insect feeding damage across all treatments and across locations was considered minimal and no differences were distinguished among the treatments. Therefore these data are not presented.

Table F-36. Rating Scale Used to Evaluate Each Plot for Damage Caused by Insect Feeding.

Insect damage rating	Percent of leaves with insect damage
0	0
1	1 – 10
2	11 – 20
3	21 – 30
4	31 – 40
5	41 – 50
6	51 – 60
7	61 – 70
8	71 – 80
9	81 – 90
10	91+

Statistical Analysis. Data were analyzed by ANOVA on a per location, per insect order, and per time-of-assessment basis. In those cases where differences among treatments were significant, Tukey's procedure (Steel and Torrie, 1980) was used to make pair-wise comparisons of treatment means.

Results and Discussion

a) 2006/2007 growing season

Insect orders that were common across most or all locations included the Coleopterans (*Diabrotica* and *Aracanthus* spp.), Lepidopterans (*Anticarsia*, *Agrotis*, *Pseudoplusia*, and *Spodoptera* spp.), and Hemipterans (*Euschistus, Piezodorus*, and *Nezara* spp.). The Coleopterans were the most common order at Ponta Grossa, Londrina, Santo Antônio de Goiás, and Brasília, while the Lepidopterans were most common at Santo Antônio de Posse, Uberaba, and Sete Lagoas (Table F-37). The location with the largest insect populations was Ponta Grossa, followed by Santo Antônio de Posse and Londrina. Relatively few insects were found at Sete Lagoas, Santo Antônio de Goiás, Uberaba, and Brasília. The largest populations of insects were detected at the R5 and R7 sampling dates.

A statistical analysis of the data detected no evidence of a significant difference among treatments for the number of individual insect species detected at any location in the 2006/2007 season.

Regardless of the size of each insect population at each sampling date, at no time were populations high enough to compromise plant health. In fact, leaf, stem, or pod damage from insect feeding was below 2% for all treatments and was therefore not statistically analyzed nor is it presented herein.

Table F-37. The Mean Number of Coleopteran, Lepidopteran, and Hemipteran Insects at Each Field Site in 2006/2007.

Location	Trt†	Growth Stage											
		V1			R1			R5			R7		
		Order											
		Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera
Ponta Grossa	T1	0.25	0.25	0	2.5	1.5	0	6.75	3.25	2.5	2.5	0	1
	T2	0.25	0.25	0	2	2	0	5.75	3	2	2.25	0	0.75
	T3	0.75	0.25	0	2.25	1.25	0	5.5	2.75	2.5	2.75	0	0.75
	T4	0.25	0.25	0	3.75	2.25	0	4.75	3.25	2.75	2	0	1.25
	T5	0.5	0.25	0	3.25	2.25	0	6	3	2.25	2.25	0	1
	Mean	0.4	0.25	0	2.75	1.85	0	5.75	3.05	2.4	2.35	0	0.95
Londrina	T1	0.5	0	0.5	1.75	2.5	0	1.5	0.5	0.75	0.25	0	1.75
	T2	1	0	0.25	2.25	2.25	0	1	2.25	1.25	0	0.25	2.25
	T3	1	0	1	1.75	1.75	0	1.25	1.25	0.25	0.75	0.25	1.5
	T4	1	0	0.25	2.25	2.25	0	0.75	2.25	1.25	0.25	0.75	1.25
	T5	1.25	0	0.5	1.75	1.75	0	1.25	1	2.25	0.25	0.25	2.75
	Mean	0.95	0	0.5	1.95	2.1	0	1.15	1.45	1.15	0.3	0.3	1.9
Santo Antônio de Posse	T1	0.5	0.5	0	0	0	0	2	4	4	0	3.75	2.75
	T2	0.25	0	0.25	0	0	0	2.25	3.75	4	0	4.25	3
	T3	0.5	0.5	0.25	0	0	0.5	1.75	4	3.75	0	3.25	2.75
	T4	0.5	0.5	0.25	0	0	0.5	2.25	4	3.75	0	4.25	3.25
	T5	0.5	0.5	0.25	0	0	0.25	2.75	4.5	4.25	0	4.25	3.25
	Mean	0.45	0.4	0.2	0	0	0.25	2.2	4.05	3.95	0	3.95	3
Uberaba	T1	0.5	0	0	0	0.25	0	0	0.5	0	0	0.5	0.25
	T2	0.25	0	0	0	0	0	0	0.5	0	0	0	0.25
	T3	0	0	0	0	0	0	0	0.25	0	0	0.25	0.5
	T4	0.75	0	0	0	0.25	0	0	0	0.25	0	0	0
	T5	0.5	0	0	0	0.25	0.25	0	0.75	0.25	0	0.75	0.25
	Mean	0.4	0	0	0	0.15	0.05	0	0.4	0.1	0	0.3	0.25

† Treatment: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

Table F-37 continued.

Location	Trt†	Growth Stage											
		V1			R1			R5			R7		
		Order											
		Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera
Sete Lagoas	T1	0	0	0	0	0	0	0.25	0	0	1	0.5	0
	T2	0.25	0	0	0	0.25	0.25	0	0.25	0	0.5	1	0
	T3	0.5	0	0	0	0.25	0	0	0.25	0	0	1.25	0.5
	T4	0.5	0	0	0	0.5	0	0.25	0	0	0.25	0.75	0.25
	T5	0.75	0	0	0	0.5	0	0.75	0.25	0	0.75	0.5	0
	Mean	0.4	0	0	0	0.3	0.05	0.25	0.15	0	0.5	0.8	0.15
Santo Antônio de Goiás	T1	1.5	0	0	0.5	0.25	0	0	0	0	0	0	0.25
	T2	0.25	0	0	0	0	0	0	0	0.5	0	0	0.5
	T3	0.5	0	0	1	0	0	0	0	0.25	0	0	0.25
	T4	1.25	0	0	0.75	1.25	0	0	0	0	0	0	0
	T5	0.25	0	0	0	0.5	0	0	0	0.25	0	0	0.25
	Mean	0.75	0	0	0.45	0.4	0	0	0	0.2	0	0	0.25
Brasília	T1	0.5	0.25	0	0	0	0	0	0	0.25	0	0	0
	T2	0	0.25	0	0.5	0	0	0	0	0	0	0	0
	T3	0.25	0	0	0.25	0.25	0	0	0.25	0.5	0	0	0
	T4	0.25	0.25	0	0	1	0	0	0	0.5	0	0	0.25
	T5	0.5	0	0	0.25	0	0	0	0	0	0	0	0.25
	Mean	0.3	0.15	0	0.2	0.25	0	0	0.05	0.25	0	0	0.1

† Treatment: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

b) 2007 growing season

Insect orders that were common across most or all locations in the trials conducted in the 2007 short season included the Coleopterans (*Diabrotica* and *Aracanthus* spp.), Lepidopterans (*Anticarsia*, *Agrotis*, *Pseudoplusia*, and *Spodoptera* spp.), and Hemipterans (*Euschistus*, *Piezodorus*, and *Nezara* spp.). The Coleopterans and Hemipterans were the most common orders in Vilhena, while Lepidopterans were common at Teresina (Table F-38). The location with the largest insect populations was Vilhena, followed by Sete Lagoas and Uberaba. Relatively few insects were found at Santo Antônio de Goiás and Brasília. The largest populations of insects of the orders Coleoptera and Lepidoptera were detected at the V4 growth stage, while insects of the order Hemiptera were greatest at the R5 and R7 growth stages.

A statistical analysis of the data did not detect any statistically significant differences among treatments for the number of individual insect species detected at any location, except for one instance at the Teresina field site in 2007 for Coleopterans at the R5 growth stage, where levels on the conventional soybean variety Monsoy 8001 were statistically significantly higher compared to the other treatments.

Regardless of the size of each insect population at each sampling date, at no time were populations high enough to compromise plant health. In fact, leaf, stem, or pod damage from insect feeding was below 2% of the total leaf area for all treatments and was therefore not statistically analyzed nor is it presented herein.

Table F-38. The Mean Number of Coleopteran, Lepidopteran, and Hemipteran Insects at Each Field Trial Site in 2007.

Location	Trt[1]	Growth Stage											
		V4			R1			R5			R7		
		Order											
		Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera
Uberaba	T1	1.39	0.71	0.71	1.06	1.10	0.71	0.71	1.10	0.71	0.71	0.71	0.84
	T2	1.56	0.71	0.71	0.97	0.84	0.71	0.71	0.93	0.84	0.71	0.84	0.71
	T3	1.48	0.71	0.71	1.06	0.84	0.71	0.71	1.06	0.84	0.71	0.84	0.71
	T4	1.56	0.71	0.71	0.84	0.71	0.71	0.71	0.84	0.84	0.71	0.71	0.71
	T5	1.40	0.71	0.71	0.97	0.97	0.71	0.71	0.97	0.71	0.71	0.71	0.71
	Mean	1.48	0.71	0.71	0.98	0.89	0.71	0.71	0.98	0.78	0.71	0.76	0.73
Sete Lagoas	T1	1.22	0.71	0.71	0.84	0.84	0.84	0.71	1.40	0.84	0.71	0.71	0.84
	T2	1.06	0.71	0.71	0.84	1.06	0.71	0.71	1.31	0.84	0.71	0.84	0.97
	T3	1.18	0.71	0.71	1.06	1.06	0.71	0.71	1.10	0.71	0.71	0.84	0.71
	T4	1.22	0.71	0.71	0.97	1.18	0.71	0.71	0.84	0.97	0.71	0.97	0.71
	T5	1.10	0.71	0.71	0.97	0.97	0.71	0.71	1.06	0.97	0.71	1.06	0.71
	Mean	1.16	0.71	0.71	0.93	1.02	0.73	0.71	1.14	0.86	0.71	0.88	0.78
Santo Antônio de Goiás	T1	1.36	0.71	0.71	0.84	0.84	0.84	0.71	0.71	0.84	0.71	0.71	0.84
	T2	1.06	0.84	0.71	0.84	0.71	0.71	0.71	0.71	0.84	0.71	0.71	0.71
	T3	1.06	0.71	0.71	0.84	0.84	0.71	0.71	0.71	0.84	0.71	0.71	0.84
	T4	0.93	0.84	0.71	0.84	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
	T5	0.93	0.71	0.71	0.71	0.84	0.71	0.71	0.71	0.84	0.71	0.71	0.71
	Mean	1.06	0.76	0.71	0.81	0.78	0.73	0.71	0.71	0.81	0.71	0.71	0.76
Brasília	T1	0.71	0.71	0.71	0.71	0.84	0.71	0.71	0.84	0.84	0.71	0.71	0.71
	T2	0.71	0.84	0.71	0.71	0.84	0.71	0.71	0.71	0.71	0.71	0.71	0.84
	T3	0.84	0.71	0.71	0.84	0.71	0.71	0.71	0.84	0.97	0.71	0.71	0.84
	T4	0.84	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
	T5	0.97	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
	Mean	0.81	0.73	0.71	0.73	0.76	0.71	0.71	0.76	0.78	0.71	0.71	0.76

[†] Treatment: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

Table F-38 continued.

Location	Trt[1]	Growth Stage											
		V4			R1			R5			R7		
		Order											
		Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera	Coleoptera	Lepidoptera	Hemiptera
Vilhena	T1	0.97	0.71	0.71	1.49	0.71	0.71	1.40	1.10	1.18	0.71	0.71	1.10
	T2	0.97	0.71	0.71	1.49	0.71	0.71	1.61	0.84	1.27	0.71	0.71	1.22
	T3	1.10	0.71	0.71	1.65	0.71	0.71	1.31	1.10	1.40	0.71	0.71	1.10
	T4	1.06	0.71	0.71	1.49	0.71	0.71	1.79	1.13	1.44	0.71	0.71	1.18
	T5	0.97	0.71	0.71	1.40	0.71	0.71	1.59	0.84	1.49	0.71	0.71	1.06
	Mean	1.01	0.71	0.71	1.51	0.71	0.71	1.54	1.00	1.36	0.71	0.71	1.13
Teresina	T1	0.71	1.78	0.71	0.71	0.71	0.71	0.71 b*	0.71	0.84	0.71	0.71	0.97
	T2	0.71	2.04	0.71	0.71	0.84	0.71	0.71 b	0.71	0.84	0.71	0.71	0.71
	T3	0.71	1.90	0.71	0.71	0.97	0.71	0.71 b	0.71	0.71	0.71	0.71	0.71
	T4	0.71	1.83	0.71	0.71	0.71	0.71	1.79 a	1.13	0.71	0.71	0.71	0.84
	T5	0.71	1.63	0.71	0.71	0.71	0.71	0.71 b	0.71	0.71	0.71	0.71	0.71
	Mean	0.71	1.84	0.71	0.71	0.78	0.71	0.92	0.79	0.76	0.71	0.71	0.78

[†] Treatment: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

*Means followed by the same letter, in the same column, are not statistically different at the 5% level.

Conclusions

The numbers of Coleopteran, Lepidopteran, and Hemipteran insects detected on CV127 soybean plants were not statistically significantly different from numbers detected on plants of the isoline control at any of four sampling dates during both the 2006/2007 and 2007 growing seasons. Furthermore, insect feeding damage in both growing seasons across all treatments and across locations was considered minimal and no biologically significant differences were distinguished among the treatments. Therefore, resistance to insect damage and effect on in-field insect populations of CV127 soybean was found to be no different from that of the isoline control plants or conventional soybean varieties. These results further support the conclusion that the cultivation of CV127 soybeans poses no different environmental impact than the cultivation of conventional soybean varieties that have a long history of safe cultivation and food and feed use.

4.0. Nitrogen fixation characteristics in the a) 2006/2007 and b) 2007 growing seasons

Introduction

An important agronomic characteristic of soybeans is the ability to support a nitrogen-fixing symbiosis with the soil bacterium *Bradyrhizobium japonicum*. *B. japonicum* cells in the soil stimulate the development of root nodules on the soybean. The *B. japonicum* cells populate the interior of the nodule and the plant creates an environment within the nodule that is conducive to nitrogen fixation. Through this symbiosis, soybeans are able to convert atmospheric nitrogen to fixed forms of nitrogen and reduce their need for chemical fertilizer. It is a common practice in Argentina and Brazil for farmers to inoculate soybean seed with *B. japonicum* at planting to ensure the development of plentiful root nodulation and strong nitrogen fixation activity. Studies have demonstrated that the rotation of soybean with other crops generally leads to increased yields for the soybean and subsequent crops (Johnson, 1987; Wesley, 1999). Other benefits for the farmer from rotation with soybeans include reduced nitrogen input in the following crop, increased residue cover, and the reduction of pest and weed cycles.

The purpose of the current study was to confirm that CV127 soybean is equivalent to the isoline control soybean and comparable to conventional soybean varieties with respect to nitrogen fixation and plant N accumulation. Characteristics of nitrogen fixation in CV127 soybean were evaluated and compared with the characteristics of the isoline control and conventional soybean varieties, including measurements of soil populations of *B. japonicum*, plant growth and nodulation and total nitrogen and ureide nitrogen accumulation in the plants. These determinations were made on plants grown in field studies during the 2006/2007 growing season and the 2007 short growing season at thirteen field locations in Brazil described above in section 1.0 of Appendix F; these locations are representative of areas of commercial soybean production and the soybean varieties included in the study are adapted to these areas.

Materials and Methods

Field design, management and treatments. The field experimental design and management practices at each field trial location, and treatments in each study have been described above in section 1.0 of Appendix F ("Agronomic and Phenotypic Evaluations").

Evaluation of biological nitrogen fixation. Evaluation of biological nitrogen fixation was performed following the protocols of RELARE (Rede de Laboratórios para Recomendação de Estirpes de Rizóbio e Inoculantes Microbianos), a network of Brazilian microbiologists officially recognized by the Ministry of Agriculture.

Population of soybean Bradyrhizobium japonicum. The *Bradyrhizobium japonicum* population was estimated with soil samples collected at the pre-sowing stage, using the most probable number (MPN) technique (Vincent, 1970) and the statistical tables of Andrade and Hamakawa (1994). Evaluation of the nodulation capacity of the *Bradyrhizobium japonicum* population was performed using soybean plants of cultivar Conquista, grown under greenhouse conditions, that were inoculated with soil dilutions. Each replicate was analyzed in triplicate.

Plant growth, nodulation, total N and N-ureides accumulated in plant tissues. At the R2 stage, ten plants were randomly collected per replicate (avoiding areas established for harvesting grains) for evaluation of nodulation and plant growth. In the laboratory, shoots were separated from roots and the latter were carefully washed and placed in a forced-air dryer at 65ºC until constant weight was obtained (approximately 72 h). Nodules were removed from the roots and dried again. Nodulation (nodule number and dry weight), shoot dry weight and shoot total N (Kjeldahl digestion and determination of N concentration using a Tecator automatic N analyzer) were determined (Hungria *et al*., 2006). In soybeans, nitrogen is fixed in the nodules and converted and exported to the plant principally in the form of N-ureides. Therefore, biological nitrogen fixation by nodules associated with the soybean plant was estimated using the N-ureide technique in dry tissues of plants harvested at the R2 stage, as described by Hungria *et al*. (2006). Analysis was performed with stems including the petioles according to Herridge and Peoples (1990). Dry stems (generated by incubation at 65ºC until a constant weight) of ten plants per replicate were ground (20 mesh), extracted with 0.1 M phosphate buffer (pH 7.0) and 2.5 mL of ethanol (2:1, v/v, buffer:ethanol), and heated at 80°C for 5 min. Samples were filtered in cheesecloth, centrifuged at 10,000 g for 5 min. and stored at -5°C. Concentrations of ureides (allantoin and allantoic acid) were determined based on the method of Vogels and Van der Drift (1970), as described by Hungria (1994). Nitrate was evaluated in the same extracts by the salicylic acid technique (Cataldo *et al*., 1975) that was adapted for smaller sample sizes as described by Hungria (1994). Therefore N-ureides were expressed as a percent of total N (N-ureides plus nitrate) in the stem and petiole tissues. N-ureides are representative of the major forms of nitrogen translocated in the soybean plant and provide an estimate of the contribution of nitrogen fixation to total N accumulated by the plant. The N-ureide concentration in the stem and

petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot (TNU) for treatment comparison purposes.

Statistical Analyses. Statistical analyses were conducted by analysis of variance (ANOVA) on a within- and across-location basis. Mean comparisons across treatments at each site (within-location) were conducted by the Least Significant Difference (LSD) statistical procedure and across sites (location across treatment and treatment across location) by the Tukey's test (Steel and Torrie, 1980).

Results and Discussion

a) 2006/2007 growing season

Population of Bradyrhizobium japonicum in the soils. The soil collected from all of the experimental areas prior to planting in the 2006/2007 growing season had high populations of *Bradyrhizobium japonicum* capable of nodulating soybean (Table F-39). The values are in agreement with those usually obtained in studies of nodulation in soils cropped with soybeans at the Embrapa Soja field trial site that have previously received rhizobial inoculants.

Table F-39. Population of Bradyrhizobium Measured in Soils Prior to Planting the CV127 Soybean Field Trials at the Seven Trial Locations Conducted in the 2006/2007 Growing Season. The values are expressed in number of *B. japonicum* cells g^{-1} dry soil and were obtained using the most probable number (MPN) method (Vincent, 1970). These data are not specific to any one treatment.

Locations	Number of Cells[1]
Santo Antônio de Posse	$2.75x10^3$
Ponta Grossa	$3.00x10^4$
Londrina	$4.83x10^5$
Uberaba	$3.98x10^3$
Sete Lagoas	$1.60x10^3$
Santo Antônio de Goiás	$1.39x10^4$
Brasília	$0.95x10^3$

[1]Means of four replicates performed in triplicate.

Soybean nodulation. Nodulation was evaluated in each of seven trials at the R2 growth stage in ten plants per replicate. At all trial sites the coefficients of variance (CV) for the nodule number values were acceptable and were in the range of the mean values (between 12.03 and 37.73%) proposed by Souza *et al*. (2008), (Table F-40).

In the within-location analysis, good nodulation was observed in all field trials and no statistical differences were observed for nodule number among the five treatments in the field trials performed at Ponta Grossa, Sete Lagoas, Santo Antônio de Goiás and Brasília (Table F-40). At Santo Antônio de Posse higher nodulation was observed in CV127

soybean and the isoline control compared to the other treatments. However, at Santo Antônio de Posse, Londrina, and Uberaba, there was no evidence of statistically significant differences among the CV127 soybean and isoline control treatments in terms of nodule number. At these three sites, nodulation of soybean cultivars Monsoy 8001 and CD 217 was lower than the nodulation exhibited by the CV127 and isoline control soybean plants (Table F-40). These results demonstrate that the capacity for nodulation in CV127 soybean is equivalent to that of the isoline control.

In the analysis of nodule number across treatments for each field trial location, higher nodule number at the R2 stage occurred in Santo Antônio de Goiás, Uberaba, Ponta Grossa, and Londrina (Table F-47). In the analysis of nodule number for each treatment across field trial locations (the treatment effect), highest nodulation was demonstrated for CV127 and the isoline control treated with Volt® herbicide (treatments T2 and T3, respectively) compared to CV127 treated with imazapyr (T1) and the conventional varieties Monsoy 8001 (T4) and CD 217 (T5) (Table F-48). However, there were no statistically significant differences between nodule number for CV127 treated with either imazapyr or Volt® herbicide compared to the isoline control (Table F-48). Furthermore, there were no statistically significant differences in nodule number between CV127 treated with either imazapyr herbicide or with Volt® herbicide or the isoline control within any field trial location (Table F-40). These results confirm that there is no difference in root nodule formation capacity between CV127 and the isoline control and show that CV127 is equivalent to other conventional soybean varieties. Where nodulation differences were observed among treatments, these were generally between CV127 together with the isoline control compared to the conventional soybean varieties, and this was most likely associated with differences in genetic backgrounds.

Table F-40. Nodule Number (number plant^{-1}) in Soybeans at the R2 Stage, in the Field Trials Performed in Brazil During the 2006/2007 Growing Season[1].

Treatment[2]	Locations						
	Santo Antônio de Posse	Ponta Grossa	Londrina	Uberaba	Sete Lagoas	Santo Antônio de Goiás	Brasília
T1	28.9 ab*	32.8	44.4 ab	50.7 ab	27.2	30.8	28.5
T2	42.3 a	54.3	58.4 a	56.0 ab	43.2	70.1	43.8
T3	42.9 a	45.4	43.5 ab	72.3 a	29.8	56.0	33.3
T4	22.0 b	57.8	25.6 b	35.1 b	33.5	48.3	34.8
T5	19.2 b	41.8	32.8 b	37.6 ab	32.0	51.9	21.3
CV(%)	27.5	24.9	21.3	32.4	36.0	36.9	31.4
Pr>F	0.004	0.063	0.002	0.044	0.425	0.126	0.084
LSD	19.2	n.s.[3]	19.6	36.7	n.s.	n.s.	n.s.

[1] The data represent the mean of four replicates, with ten plants per replicate.

[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®

[3] n.s. = no significant differences

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

For the within-location analysis of nodule dry weights, the CV values were within the medium variability range (between 8.84 and 32.60%), as proposed by Souza *et al*. (2008), in four sites (Ponta Grossa, Londrina, Uberaba, and Sete Lagoas), but higher values were observed at Santo Antônio de Posse (41.1%), Santo Antônio de Goiás (49.1%) and Brasília (36.7%), (Table F-41). Significant statistical differences in nodule dry weight were detected among the treatments at three out of the seven sites, Ponta Grossa, Londrina, and Brasília (Table F-41). Lower nodule dry weights at those sites were observed in the conventional soybean varieties Monsoy 8001 (T4) and CD 217 (T5), except for Monsoy 8001 in Ponta Grossa. There was no evidence of a statistical difference in the nodule dry weights among CV127 soybean and the isoline control at any of the locations (Table F-41).

In the analysis of nodule dry weight across treatments for each field trial location, the results confirm normal levels of nodulation at all field trial locations (Table F-47). There were no statistically significant differences in nodule dry weights between CV127 soybean and the isoline control or the conventional soybean varieties when treatments were analyzed across locations (Table F-48). These results confirm that there is no difference in root nodule formation capacity between CV127 soybean and the isoline control or other conventional soybean varieties.

Table F-41. Nodule Dry Weight (mg plant^{-1}) of Soybean at the R2 Stage, in the Field Trials Performed in Brazil During the 2006/2007 Growing Season[1].

Trt[2]	Location						
	Santo Antônio de Posse	Ponta Grossa	Londrina	Uberaba	Sete Lagoas	Santo Antônio de Goiás	Brasília
T1	138.1	94.9 b*	104.0 ab	167.8	117.4	79.9	187.0 ab
T2	199.8	143.6 ab	123.2 a	160.8	189.5	154.2	207.8 a
T3	184.1	138.0 ab	111.0 ab	236.3	149.7	162.9	163.2 ab
T4	106.5	194.4 a	75.0 b	171.3	238.3	204.1	122.8 ab
T5	89.7	106.6 b	70.1 b	176.9	193.7	158.4	74.1 b
CV(%)	41.1	26.6	19.4	25.4	32.4	49.1	36.7
Pr>F	0.089	0.017	0.007	0.207	0.093	0.276	0.035
LSD	n.s.[3]	81.2	42.3	n.s.	n.s.	n.s.	124.9

[1] The data represent the mean of four replicates, with ten plants per replicate.

[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

[3] n.s. = no significant differences

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Shoot biomass production. The analysis of shoot dry weight values across treatments for each field trial location indicated that a generally higher plant dry weight production was obtained at Santo Antônio de Posse, Ponta Grossa and Brasília (Table F-47). In the analysis of shoot dry weight for each treatment at each field location, no statistical

differences in plant dry weight were detected among treatments in five of the seven locations, including Santo Antônio de Posse, Ponta Grossa, Londrina, Sete Lagoas, and Uberaba, while at two sites, Santo Antônio de Goiás and Brasília, there was no evidence of statistically significant differences in shoot dry weight values between CV127 (T1 and T2 treatments) and the isoline control (T3), but lower values were determined for Monsoy 8001 (T4) and CD 217 (T5), (Table F-42), which most likely reflects varietal differences in growth characteristics.

In the across-location analysis of treatments, there were no statistically significant differences in shoot dry weights between CV127 treated with either imazapyr or Volt® herbicide compared to the isoline control but values for the conventional soybean varieties were significantly lower compared to the other treatments (Table F-48). These results demonstrate that there is no evidence of a significant difference in the production of shoot biomass, as indicated by shoot dry weights, between CV127, treated with either imazapyr herbicide or with Volt® herbicide, and the isoline control.

Table F-42. Shoot Dry Weight (g plant^{-1}) of Soybean at the R2 Stage, in the Field Trials Performed in Brazil During the 2006/2007 Growing Season[1].

Treatment[2]	Location						
	Santo Antônio de Posse	Ponta Grossa	Londrina	Uberaba	Sete Lagoas	Santo Antônio de Goiás	Brasília
T1	23.25	35.07	15.43	11.25	15.70	19.30 a*	27.48 ab
T2	24.05	37.40	19.94	10.25	13.40	15.28 ab	25.08 ab
T3	26.69	35.90	20.23	10.70	14.33	14.54 abc	30.45 a
T4	19.49	27.33	17.28	7.88	12.03	12.66 bc	21.98 b
T5	20.44	33.40	16.17	7.24	12.86	9.43 c	20.57 b
CV(%)	14.8	20.8	15.4	31.0	24.8	18.1	14.5
Pr>F	0.066	0.348	0.096	0.266	0.610	0.002	0.014
LSD	n.s.[3]	n.s.	n.s.	n.s.	n.s.	5.80	8.20

[1] The data represent the mean of four replicates, with ten plants per replicate.
[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[3] n.s. = no significant differences
* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Total N and N as ureides in the soybean shoot. The total N concentration in soybean shoot tissues at the R2 stage was higher in the trials at Santo Antônio de Posse, Ponta Grossa, and Brasília (Table F-47) based on the analysis across treatments for each field trial location and most likely reflects differences in nodulation and soil fertility between field trial locations. However, the within-location analyses demonstrated no statistical differences among the three treatments including CV127 and the isoline control (treatments T1, T2, and T3). Some statistical differences were observed between these treatments and the conventional soybean varieties T4 and T5 (Table F-43). Total N accumulated in the shoot (column "TNS" of Table F-48) was statistically significantly

lower in the conventional soybean varieties (T4 and T5 treatments) compared to the CV127 and isoline control treatments (T1-T3), but total N concentration in the soybean shoot tissue (column "CNS" of Table F-48) was similar across all treatments with the exception of treatment T1 (CV127 treated with imazapyr) and treatment T4 (Monsoy 8001) which were statistically different due to the higher concentration of N in the shoots of Monsoy 8001. Therefore, the differences observed for total N accumulated in the shoot (column "TNS" of Table F-48) between treatments T1-T3 and the conventional soybean varieties were attributed to the statistically significantly lower shoot dry weight values of treatments T4 and T5 compared to treatments T1-T3 (column "SDW" of Table F-48).

Table F-43. Total N Concentration in Shoot (g N kg^{-1} $plant^{-1}$) of Soybean at the R2 Stage, in the Field Trials Performed in Brazil During the 2006/2007 Growing Season[1].

Treatment[2]	Location						
	Santo Antônio de Posse	Ponta Grossa	Londrina	Uberaba	Sete Lagoas	Santo Antônio de Goiás	Brasília
T1	56.65 ab*	55.70	47.05	36.20	41.18	44.62 ab	53.55 ab
T2	54.90 ab	51.82	48.00	42.35	44.70	42.65 ab	52.50 b
T3	56.95 ab	57.18	54.50	42.65	40.95	47.98 a	54.45 ab
T4	59.55 a	59.90	54.18	43.48	52.82	43.42 ab	56.42 a
T5	50.78 b	58.18	51.28	40.95	49.35	36.30 b	54.65 ab
CV(%)	5.6	7.2	9.5	8.0	11.7	8.7	2.84
Pr>F	0.020	0.129	0.156	0.059	0.053	0.012	0.039
LSD	6.97	n.s.[3]	n.s.	n.s.	n.s.	8.42	3.84

[1] The data represent the mean of four replicates, with ten plants per replicate.
[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[3] n.s. = no significant differences
* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

These data were also expressed as total N in the shoot per plant (Tables F-44, F-47 and F-48). In the analysis across treatments for each field location, considerable differences were detected among locations for total N accumulated in shoots per plant (Table F-47) as expected, since this value is a combination of shoot dry weight and N accumulation. Higher total N values were found at Santo Antônio de Posse, Ponta Grossa, and Brasília, and the lowest value was observed at Uberaba (Table F-47). In the analysis of total shoot N for each treatment at each field location, at five of the sites there was no evidence of statistical differences between CV127 treated with either imazapyr herbicide or with Volt® herbicide and the isoline control treatment (Table F-44). At the Londrina field site, shoot N accumulation in CV127 treated with imazapyr herbicide (T1) was statistically significantly lower than CV127 treated with Volt® herbicide (T2) and the isoline control (T3). In contrast, at the Brasília field site, total shoot N in treatment T2 was statistically significantly lower than treatment T3. Therefore, there was no consistent trend of any

differences between these treatments with respect to shoot N content. This was further confirmed in the across-location analysis for each treatment, where there were no statistical differences in the total N content in the shoot per plant between the treatments with CV127 (T1 and T2) and the isoline control (expressed as total N in the shoots per plant in Table F-48). Overall, these results demonstrate that there is no significant difference in either N concentration or total N in the shoot between CV127, treated with either imazapyr herbicide or with Volt® herbicide, and the isoline control.

Table F-44. Total N in Shoot (mg N plant^{-1}) of Soybean at the R2 Stage, in the Field Trials Performed in Brazil During the 2006/2007 Growing Season[1, 4].

Treatment[2]	Location						
	Santo Antônio de Posse	Ponta Grossa	Londrina	Uberaba	Sete Lagoas	Santo Antônio de Goiás	Brasília
T1	1317.1ab*	1953.4 ab	725.5 c	407.3	646.5	861.2	1471.5 ab
T2	1320.3 ab	1938.1 ab	957.1 ab	434.1	599.0	651.7	1316.7 bc
T3	1520.0 a	2052.7 a	1102.5 a	456.4	586.8	697.6	1658.0 a
T4	1160.6 b	1637.1 b	936.2 ab	342.6	635.4	549.7	1240.1 bc
T5	1037.9 b	1943.2 a	829.2 bc	296.5	634.6	342.3	1124.2 c
CV(%)	22.3	19.2	21.1	27.7	33.6	32.1	25.2
Pr>F	0.005	0.003	0.004	0.368	0.414	0.556	0.003
LSD	295.0	350.1	200.2	n.s.[3]	n.s.	n.s	340.5

[1] The data represent the mean of four replicates, with ten plants per replicate.
[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[3] n.s. = no significant differences
[4] Total shoot N was calculated from the shoot N concentration and the total shoot dry weight.
* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

In the analysis across treatments for each field location of the contribution of biological nitrogen fixation to the total N accumulated by the soybean crop, determined as percent N-ureides of total translocated nitrogen (N-ureides plus nitrate) in the stem and petiole tissues, the % N-ureide values measured ranged from 67.36% in the trial at Uberaba to 76.28% in the trial at Sete Lagoas, with no evidence of statistical differences among the seven sites (Table F-47). When % N-ureide values were presented for the different treatments at each field site (within-location analysis), there was no evidence of statistical differences among the three treatments including CV127 and the isoline control (treatments T1, T2, and T3), and no statistical differences between these treatments and the conventional soybean varieties T4 and T5 at four of the seven field sites (Table F-45). However, in three out of the seven sites, Santo Antônio de Posse, Santo Antônio de Goiás and Brasília, there was a statistically lower contribution of biological nitrogen fixation in the conventional soybean varieties, Monsoy 8001 and CD 217, compared to CV127 and the isoline control (Table F-45), and this difference in shoot ureide concentration is to be expected between different soybean varieties grown at different locations.

When all data were analyzed for treatments across locations (across-location analysis), there were no differences in N-ureide concentration between CV127, treated with either imazapyr herbicide or with Volt® herbicide, and the isoline control, but lower values were present in the conventional soybean varieties Monsoy 8001 and CD 217 (Table F-48).

Table F-45. N-ureides as Percent of Total N (N-ureides plus nitrate)[§] in the Stem and Petiole Tissues of Soybean at the R2 Stage, in the Field Trials Performed in Brazil During the 2006/2007 Growing Season[1].

Trt[2]	Location						
	Santo Antônio de Posse	Ponta Grossa	Londrina	Uberaba	Sete Lagoas	Santo Antônio de Goiás	Brasília
T1	72.1 a*	76.8	67.9	68.9	78.2	80.2 a	74.2 a
T2	73.3 a	75.0	73.1	73.3	77.1	77.3 a	76.2 a
T3	76.1 a	79.9	74.4	72.8	76.7	76.9 a	80.1 a
T4	63.2 b	69.1	68.8	61.1	75.0	70.2 b	65.0 b
T5	60.0 b	73.9	67.2	60.7	74.4	65.5 b	62.1 b
CV(%)	8.2	7.7	8.1	6.6	9.2	9.0	8.2
Pr>F	0.003	0.111	0.092	0.066	0.071	0.004	0.004
LSD	8.2	n.s.[3]	n.s.	n.s.	n.s.	6.5	7.2

[1] The data represent the mean of four replicates, with ten plants per replicate.

[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

[3] n.s. = no significant differences

[§] N-ureides were measured in stem and petiole tissues only and expressed as a percent of total N (N-ureides plus nitrate) in these tissues. N-ureides are an estimate of the contribution of nitrogen fixation to total N accumulated by the plant.

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

The final parameter used to evaluate biological nitrogen fixation was the total N-ureide content on a per-plant basis. The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot for treatment comparison purposes. Higher levels of total N-ureide shoot content per plant were observed in the trials performed in Ponta Grossa, Brasília and Santo Antônio de Posse, with lower values in Londrina, Uberaba, Santo Antônio de Goiás and Sete Lagoas according to the analysis across treatments for each field location (Table F-47).

In the within-location analysis of each treatment, there was statistically lower N-ureide content in the conventional soybean varieties Monsoy 8001 and CD 217 at two field trial locations (Santo Antônio de Posse and Santo Antônio de Goiás) compared to CV127 and the isoline control (Table F-46). Among the CV127 and isoline control treatments (T1, T2, T3), there was no evidence of a significant difference in N-ureide content at five of the seven sites, including Santo Antônio de Posse, Ponta Grossa, Uberaba, Sete Lagoas, and Brasília (Table F-46). At the Santo Antônio de Goiás site, the CV127 treated with

imazapyr (T1) had a statistically significantly higher N-ureide content compared to the CV127 treated with Volt® herbicide and the isoline control, while at the Londrina site, the reverse was noted (Table F-46).

In the across-location analysis for each treatment for total N-ureide shoot content per plant, there was no evidence of a statistically significant difference between the CV127 soybean treatments or the isoline control (Table F-48). These results demonstrate that there is no significant difference in either N-ureide concentration or total N-ureide in the shoot between CV127, treated with either imazapyr herbicide or with Volt® herbicide, and the isoline control. These results are consistent with previous results showing no differences in nodule number or dry weight associated with CV127 compared to the isoline control.

Table F-46. Total N-Ureide Content (mg N-ureide plant^{-1})[§] in Soybean at the R2 Stage, in the Field Trials Performed in Brazil During the 2006/2007 Growing Season[1].

Treatment[2]	Location						
	Santo Antônio de Posse	Ponta Grossa	Londrina	Uberaba	Sete Lagoas	Santo Antônio de Goiás	Brasília
T1	949.6 a*	1500.2 a	492.6 d	280.6	505.6	690.7 a	1091.9 ab
T2	967.8 a	1453.6 a	699.6 ab	318.2	461.8	503.8 b	1003.3 abc
T3	1156.7 a	1640.2 a	820.3 a	332.2	450.1	536.5 b	1328.1 a
T4	733.5 b	1131.2 b	644.1 bc	209.3	476.6	385.9 c	806.1 bc
T5	622.7 b	1436.0 a	557.2 cd	180.0	472.2	224.2 d	698.1 c
CV(%)	20.2	23.1	17.7	32.3	27.7	11.9	16.6
Pr>F	0.005	0.005	0.005	0.411	0.334	0.005	0.004
LSD	212.7	223.0	128.6	n.s.[3]	n.s.	126.4	236.5

[1] The data represent the mean of four replicates, with ten plants per replicate.
[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[3] n.s. = no significant differences
[§] The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot for treatment comparison purposes.
* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

The across-location analysis for each treatment of shoot N and N-ureides has shown that there was no evidence of statistical differences between CV127 soybean and the isoline control in the total N or N-ureide contents (Table F-48). In general, the values of total N and N-ureide observed in CV127 soybean and the isoline control were higher than those obtained with conventional soybean varieties Monsoy 8001 and CD 217 (Table F-48).

Table F-47. Effect of Location on Nitrogen Fixation Parameters in the 2006/2007 Growing Season.

Location	Parameter[1]						
	NN	NDW	SDW	CNS	TNS	%NU	TNU
Santo Antônio de Posse	31.0 c*	143.7 ab	22.78 b	55.77 a	1271.2 b	68.94	886.1 b
Ponta Grossa	46.4 abc	135.5 ab	33.82 a	56.56 a	1904.9 a	74.94	1432.2 a
Londrina	41.0 abc	96.7 b	17.81 c	51.00 ab	910.1 c	70.28	642.8 c
Uberaba	50.3 ab	182.6 a	9.46 d	41.12 c	387.4 e	67.36	264.1 d
Sete Legoas	33.1 bc	177.7 a	13.66 c	45.80 bc	620.5 d	76.28	473.2 c
Santo Antônio de Goiás	51.4 a	151.9 ab	14.25 c	43.00 c	620.5 d	74.02	468.2 cd
Brasília	32.3 c	151.0 ab	25.11 b	54.32 a	1362.1 b	71.52	985.5 b

[1] Nodule number (NN, number plant^{-1}) and dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), concentration of N in shoots (CNS, g N kg^{-1} plant^{-1}), total N accumulated in shoots (TNS, mg N plant^{-1}), %N as N-ureide (%NU)[2] and total N as N-ureide (TNU, mg N-ureide plant^{-1})[3] in soybean harvested at the R2 stage, in the seven trials performed in Brazil during the 2006/2007 growing season.

[2] N-ureides were measured in stem and petiole tissues only and expressed as a percentage (%NU) of total N (N-ureides plus nitrate) in these tissues. N-ureides are an estimate of the contribution of nitrogen fixation to total N accumulated by the plant.

[3] The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot (TNU) for treatment comparison purposes.

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Table F-48. Effect of the Different Treatments on Nitrogen Fixation Parameters in the 2006/2007 Growing Season.

Treatment[2]	Parameter[1]						
	NN	NDW	SDW	CNS	TNS	%NU	TNU
1	34.8 b*	127.0	21.07 a	47.85 b	1054.6 a	74.04 a	787.3 a
2	52.6 a	168.4	20.78 a	48.13 ab	1031.0 a	75.04 a	772.6 a
3	46.2 ab	163.6	21.84 a	50.66 ab	1153.4 a	76.70 a	894.9 a
4	36.7 b	158.9	16.95 b	52.82 a	928.8 b	67.49 b	626.7 b
5	33.8 b	124.2	17.16 b	48.78 ab	886.8 b	66.26 b	598.6 b

[1] Nodule number (NN, number plant^{-1}) and dry weight (NDW, mg plant^{-1}), shoot dry weight (SDW, g plant^{-1}), concentration of N in shoots (CNS, g N kg^{-1} plant^{-1}), total N accumulated in shoots (TNS, mg N plant^{-1}), %N as N-ureide (%NU)[3] and total N as N-ureide (TNU, mg N-ureide plant^{-1})[4] in soybeans harvested at the R2 stage, in the seven trials performed in Brazil during the 2006/2007 growing season.

[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

[3] N-ureides were measured in stem and petiole tissues only and expressed as a percentage (%NU) of total N (N-ureides plus nitrate) in these tissues. N-ureides are an estimate of the contribution of nitrogen fixation to total N accumulated by the plant.

[4] The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot (TNU) for treatment comparison purposes.

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

b) 2007 growing season

Population of soybean Bradyrhizobium japonicum in the soils. The soil collected from all of the experimental field areas had high populations of *B. japonicum* capable of nodulating soybean (Table F-49). The values are in agreement with those usually obtained at the Embrapa Soja field trial site in studies evaluating nodulation in soils cropped with soybean that have previously received rhizobial inoculants.

Table F-49. Population of *Bradyrhizobium japonicum* in Soils Cropped with Soybean in the Six Trials Performed in the 2007 Growing Season.
The values are expressed in number of *Bradyrhizobium japonicum* cells g^{-1} dry soil and were obtained using the most probable number (MPN) method (Vincent, 1970).

Locations	No. cells g^{-1} soil*
Teresina, PI, EMN	$1.89x10^3$
Vilhena, RO, ER	$2.41x10^3$
Uberaba, MG, CTTP	$1.58x10^4$
Sete Lagoas, MG, CNPMS	$2.60x10^4$
Santo Antônio de Goiás, GO, CNPAF	$2.05x10^4$
Brasília, DF, CNPH	$1.48x10^3$

*Means of four replicates with analyses performed in triplicate.

Soybean nodulation. In the trials conducted during the 2007 short season, nodulation at the R2 stage was evaluated at six locations on ten plants per replicate. Field nodulation by legumes, especially nodule number (NN) per plant, can present high variability, and the maximum acceptable value of coefficient of variation (CV) established by Souza *et al.* (2008) was 37.7% (medium). The observed variability for nodule number was very high at all locations, as evident by the within-location analysis, and medium CV was observed only in Vilhena (Table F-50). In this case, as recommended by Souza *et al*. (2008), values were transformed into $\log_{10}$, promoting a significant reduction in the coefficients of variation to values between 8.2% and 12.8% in Santo Antônio de Goiás and Sete Lagoas, respectively (data not shown). Despite the high coefficients of variation, good nodulation was observed in Teresina, Brasília, Vilhena, and Santo Antônio de Goiás. Due to adverse climatic conditions, nodulation was lower in Uberaba and Sete Lagoas (Table F-50).

The within-location analysis demonstrated that significant differences among treatments for nodule number were observed in Vilhena and Uberaba, with the highest values observed in treatment T3, the isoline control treated with conventional herbicide, and the lowest in T4, conventional soybean variety Monsoy 8001 (Table F-50). The analysis across treatments for each field location showed that a lower number of nodules were present on plants at the Uberaba and Sete Lagoas sites (Table F-57). However, no significant differences were observed among the five treatments in the across-location analysis (Table F-58).

Table F-50. Number of Nodules (number plant^{-1}) in Soybeans at the R2 Stage in the Field Trials Conducted in Brazil During the 2007 Growing Season[1].

Treatment[2]	Location					
	Teresina	Brasília	Vilhena	Uberaba	Sete Lagoas	Santo Antônio de Goiás
T1	30.8	45.0	48.4 ab*	12.8 b	23.0	32.0
T2	27.0	39.6	53.6 a	15.7 b	17.2	23.2
T3	25.6	36.1	62.9 a	26.1 a	20.0	36.7
T4	33.4	32.2	25.2 c	5.7 b	11.5	20.3
T5	26.8	30.7	30.2 bc	8.6 b	8.0	26.6
CV(%)	45.6	38.6	27.8	48.8	64.3	47.9
Pr>F	0.891	0.0016	0.009	0.032	0.513	0.657
LSD	n.s. [3]	n.s.			n.s.	n.s.

[1] Data are means of four replicates with analyses performed in duplicate.
[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[3] n.s. = no significant differences
* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

In the observations for nodule dry weight, CV values of up to 32.6% were within the range of medium variability proposed by Souza *et al*. (2008) at two locations, Vilhena and Santo Antônio de Goiás (Table F-51). Based on the within-location analysis, significant differences for nodule dry matter were observed among treatments at three locations, Brasília, Vilhena and Uberaba. At Uberaba the isoline control soybean (T3) had higher nodule mass compared to the CV127 treatments T1 and T2 (Table F-51). No statistically significant differences in nodulation parameters were observed between CV127 treated with either imazapyr or Volt® herbicide in the 2007 season (Tables F-50 and F-51).

The data analysis across treatments for each field location showed differences among locations with respect to nodulation parameters, with poor nodulation at Uberaba, Sete Lagoas, Santo Antônio de Goiás and Teresina (Table F-57). The excellent nodulation potential of CV127 soybean and the isoline control soybean, which were superior to the conventional soybean varieties Monsoy 8001 and CD217, was also observed when the treatments were compared across all field trial locations (across-location analysis) with respect to nodulation parameters (Table F-58). These results confirmed that there is no evidence of a statistically significant difference in root nodule formation capacity between CV127 compared to the isoline control. Even though CV127 soybean and the isoline control had slightly higher nodule numbers and nodule dry weights compared to the conventional soybean varieties, the only statistically significant difference was between CD217 and the other treatments with respect to nodule dry weight (Table F-58).

Table F-51. Nodule Dry Weight (mg plant^{-1}) of Soybeans at the R2 Stage in the Field Trials Conducted in Brazil During the 2007 Growing Season[1].

Treatment[2]	Location					
	Teresina	Brasília	Vilhena	Uberaba	Sete Lagoas	Santo Antônio de Goiás
T1	121.3	319.1 a*	154.0 ab	51.8 b	147.8	125.1
T2	94.3	313.9 a	196.7 ab	58.0 b	99.6	137.7
T3	99.2	253.2 ab	209.0 a	106.8 a	131.1	193.3
T4	155.6	187.7 b	147.6 ab	21.6 b	82.2	126.5
T5	117.6	180.9 b	131.4 b	31.4 b	66.0	149.9
CV(%)	61.0	38.4	25.6	49.2	59.0	29.2
Pr>F	0.912	0.0072	0.065	0.018	0.621	0.303
LSD	n.s. [3]	n.s.			n.s.	n.s.

[1] Data are means of four replicates with analyses performed in duplicate.
[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[3] n.s. = no significant differences
* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Shoot biomass production. Analysis of shoot dry weight values across treatments for each field location showed a high level of variability among locations, with the highest values observed at Santo Antônio de Goiás and Brasília and the lowest values at Uberaba (Table F-57). The variation in shoot dry weight values most likely reflects environmental differences between field locations with, for example, higher rainfall at the Uberaba site compared to Santo Antônio de Goiás. The within-location analysis for each treatment at each location showed there were no significant differences in shoot dry weights between the CV127 and isoline control soybeans except at Uberaba (Table F-52). Furthermore, there were no significant differences in this parameter between CV127 soybean and the two conventional soybean varieties at any location except for a statistically significant difference observed with CD217 (T5) at Santo Antônio de Goiás (Table F-52). It is concluded that shoot dry matter accumulation in CV127 soybean is not different from that of the isoline control or other conventional soybean varieties. Comparison of shoot dry weight accumulation for each treatment across locations confirmed that CV127 soybeans were not statistically significantly different for shoot dry matter accumulation compared to the isoline control or the conventional soybean varieties (Table F-58).

Table F-52. Shoot Dry Weight (g plant^{-1}) of Soybean at the R2 Stage in Field Trials Conducted in Brazil During the 2007 Growing Season[1].

Treatment[2]	Location					
	Teresina	Brasília	Vilhena	Uberaba	Sete Lagoas	Santo Antônio de Goiás
T1	9.82	14.27	6.86	2.63 b*	8.23	14.96 a
T2	9.24	14.92	7.36	1.67 b	7.21	14.01 a
T3	11.05	14.83	7.44	4.46 a	8.55	13.99 a
T4	10.79	14.53	7.00	3.22 ab	7.25	12.39 ab
T5	9.46	12.32	7.51	3.26 ab	5.84	9.71 b
CV(%)	29.5	24.5	16.0	45.5	26.4	13.95
Pr>F	0.891	0.444	0.924	0.103	0.406	0.023
LSD	n.s.[3]	n.s.	n.s.		n.s.	

[1] Data are means of four replicates with analyses performed in duplicate.
[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[3] n.s. = no significant differences
* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Total N and N as ureides in the soybean shoot. The total N concentration in soybean shoot tissues at the R2 stage across treatments for each field location was comparable among all locations (Table F-57), and small differences observed most likely reflect differences in nodulation and soil fertility between field locations. For each treatment at each field location, there was no evidence of statistically significant differences among treatments for total shoot N concentration at any of the field trial locations (Table F-53), and this was confirmed in data analysis comparing treatments across field trial locations (Table F-58).

Table F-53. Shoot N Concentration (g N kg^{-1} $plant^{-1}$) of Soybean at the R2 Stage in Field Trials Conducted in Brazil During the 2007 Growing Season[1].

Treatment[2]	Location					
	Teresina	Brasília	Vilhena	Uberaba	Sete Lagoas	Santo Antônio de Goiás
T1	30.5	49.9	44.0	39.4	42.9	47.0
T2	37.6	42.9	42.7	40.5	42.4	47.8
T3	28.0	44.2	41.1	43.1	45.8	46.6
T4	33.1	46.6	41.7	39.8	41.4	41.1
T5	32.5	43.0	42.0	39.8	40.0	37.3
CV(%)	11.77	19.89	8.36	10.88	15.73	17.21
Pr>F	0.131	0.916	0.370	0.943	0.824	0.246
LSD	n.s.[3]	n.s.	n.s.	n.s.	n.s.	n.s.

[1] Data are means of four replicates with analyses performed in duplicate.

[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

[3] n.s. = no significant differences

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

The values of total N accumulated in the shoots of the soybean plants were also determined (Table F-54). The data analysis across treatments for each location detected statistically significant differences among locations for total N accumulated in shoots per plant (Table F-57), as expected, since this value is a combination of shoot dry weight and N accumulation. In the analysis of total shoot N within a location for each treatment, at four of the locations there was no evidence of statistically significant differences for total shoot N among any of the treatments. Only at the Uberaba site was a statistically significant difference observed between CV127 treated with Volt® herbicide and the isoline control, and at the Santo Antônio de Goiás field site the total shoot N content of the conventional soybean variety CD 217 was statistically significantly less than the CV127 and isoline control treatments (Table F-54). Across locations, there was no evidence of statistically significant differences in total shoot N accumulation among the treatments (Table F-58). Overall, these results demonstrate that there is no evidence of statistically significant differences in either N concentration or total N in the shoot between CV127, treated with either imazapyr herbicide or with Volt® herbicide, and the isoline control and conventional soybean varieties (Table F-58).

Table F-54. Total N in the Shoot (mg N plant^{-1}) of Soybean at the R2 Stage in Field Trials Conducted in Brazil During the 2007 Growing Season[1, 4].

Treatment[2]	Location					
	Teresina	Brasília	Vilhena	Uberaba	Sete Lagoas	Santo Antônio de Goiás
T1	302	720	304	103 ab*	354	699 a
T2	351	660	314	69 b	306	666 a
T3	306	648	304	191 a	395	652 a
T4	382	718	292	123 ab	300	517 ab
T5	308	529	314	132 ab	230	363 b
CV(%)	29.5	38.83	14.47	42.56	28.56	19.10
Pr>F	0.940	0.822	0.948	0.068	0.1779	0.0056
LSD	n.s. [3]	n.s.	n.s.		n.s.	

[1] Data are means of four replicates with analyses performed in duplicate.
[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.
[3] n.s. = no significant differences
[4] Total shoot N was calculated from the shoot N concentration and the total shoot dry weight.
* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

In the analysis across treatments for each field location of the contribution of biological nitrogen fixation to the total N accumulated by the soybean crop determined as percent N-ureides of total translocated nitrogen (N-ureides plus nitrate) in the stem and petiole tissues, the % N-ureide values measured ranged from 58.0% in the trial at Teresina to 82.2% in the trial at Sete Lagoas, with statistically significant differences in % N-ureide among the six locations (Table F-57). These differences among locations were most likely related to soil fertility and plant nodulation levels between sites. When % N-ureide values were presented for the different treatments within a location, there was no evidence of a statistically significant difference in this parameter among the five different treatments at five of the field trial sites (Table F-55). The only statistically significant difference for % N-ureide content was a lower value for the conventional soybean variety CD 217 compared to the isoline control treatment at Sete Lagoas. Across locations, there were no differences in % N-ureide values between CV127, treated with either imazapyr herbicide or with Volt® herbicide, and the isoline control as well as the Monsoy 8001 conventional soybean variety. There was a statistically significantly lower value in the conventional soybean variety CD 217 compared to the isoline control and Monsoy 8001 (Table F-58).

Finally, total N-ureide content on a per-plant basis was measured to evaluate biological nitrogen fixation contribution to overall nitrogen accumulation in the plant. The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot for treatment comparison purposes. An analysis across treatments for each field location showed that total N-ureide varied between field sites, as expected, since this parameter is the product of both

plant growth as well as N-ureide accumulation (Table F-57). When total N-ureide content per plant was presented for the different treatments within each field site, there was no evidence of statistically significant differences between any of the five treatments at four of the six field trial locations (Table F-56). At the Uberaba field site, total N-ureide content was statistically significantly higher for the isoline control soybean (T3) compared to CV127 soybean treated with Volt® herbicide (T2). Also, at the Santo Antônio de Goiás field site, levels of total N-ureide content were statistically significantly higher in CV127 soybean treated with imazapyr herbicide (T1) compared to the conventional soybean variety CD 217. Levels of total N-ureide per plant were equivalent between all other treatments at these two field trial locations.

When total N-ureide content per plant was analyzed for each treatment across all locations, there was no evidence of statistically significant difference between the CV127 soybean treatments and the isoline control as well as the conventional soybean variety Monsoy 8001 (Table F-58). However, levels of total N-ureide content per plant were statistically significantly lower in conventional soybean variety CD 217 (T5) compared to the other four treatments in the study. These results demonstrate that there is no evidence of a significant difference in either N-ureide concentration or total N-ureide in the shoot between CV127, treated with either imazapyr herbicide or with Volt® herbicide, and the isoline control, aside from the above-mentioned difference at Uberaba. These results are consistent with results reported for the same studies conducted in the 2006/2007 growing season.

The analysis of shoot N and N-ureides across all field trial locations has shown that there was no evidence of statistical differences between CV127 soybean and the isoline control in the total N or N-ureide content (Table F-58). In general, the values of total N and N-ureide observed in CV127 soybean and the isoline control were higher than, or comparable to, those obtained with conventional soybean varieties Monsoy 8001 and CD 217 (Table F-58).

Table F-55. Percent N-Ureides (%NU) of Total N (N-ureides Plus Nitrate)[§] in the Stem and Petiole Tissues of Soybean at the R2 Stage in Field Trials Conducted in Brazil During the 2007 Growing Season[1].

Treatment[2]	Location					
	Teresina	Brasília	Vilhena	Uberaba	Sete Lagoas	Santo Antônio de Goiás
T1	56.3	76.2	60.8	68.5	85.2 ab*	86.2
T2	58.3	73.8	60.2	60.0	81.2 ab	78.2
T3	57.0	74.8	59.0	77.2	89.0 a	84.5
T4	60.0	73.2	59.0	68.8	81.0 ab	81.0
T5	58.3	63.5	59.5	72.5	74.8 b	71.0
CV(%)	11.75	20.06	6.77	9.17	5.84	12.05
Pr>F	0.981	0.745	0.960	0.173	0.0141	0.2516
LSD	n.s. [3]	n.s.	n.s.	n.s.		n.s.

[1] Data are means of four replicates with analyses performed in duplicate.

[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

[3] n.s. = no significant differences

[§] N-ureides were measured in stem and petiole tissues only and expressed as a percent of total N (N-ureides plus nitrate) in these tissues. N-ureides are an estimate of the contribution of nitrogen fixation to total N accumulated by the plant.

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Table F-56. Total N-Ureide (mg N-ureide plant^{-1})[§] in Soybean at the R2 Stage in Field Trials Conducted in Brazil During the 2007 Growing Season[1].

Treatment[2]	Location					
	Teresina	Brasília	Vilhena	Uberaba	Sete Lagoas	Santo Antônio de Goiás
T1	174	567	186	71 ab*	303	605 a
T2	210	516	190	43 b	251	526 ab
T3	173	496	179	150 a	354	552 ab
T4	245	582	173	90 ab	245	431 ab
T5	180	345	187	100 ab	176	276 b
CV(%)	50.58	55.85	19.47	52.19	31.72	27.5
Pr>F	0.8388	0.768	0.954	0.073	0.096	0.0302
LSD	n.s.[3]	n.s.	n.s.		n.s.	

[1] Data are means of four replicates with analyses performed in duplicate.

[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

[3] n.s. = no significant differences

[§] The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot for treatment comparison purposes.

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Table F-57. Effect of Location on Nitrogen Fixation Parameters in the 2007 Growing Season.

Location	Parameter[1]						
	NN	NDW	SDW	CNS	TNS	%NU	TNU
Teresina	28.7 ab	117.6 bc	10.07 b	32.4 b	330 b	58.0 d	196 bc
Brasilia	36.7 a	251.0 a	14.18 a	45.3 a	655 a	72.3 bc	501 a
Vilhena	44.1 a	167.7 ab	7.23 c	42.3 a	305 b	59.7 d	184 bc
Uberaba	13.8 b	53.9 c	3.05 d	40.5 a	124 c	69.4 c	91 c
Sete Lagoas	15.9 b	105.3 bc	7.42 c	42.5 a	317 b	82.2 a	266 b
Santo Antônio de Goiás	27.8 ab	146.5 bc	14.18 a	44.0 a	580 a	80.2 ab	487 a

[1] Number (NN, number plant^{-1}) and dry weight (NDW, mg plant^{-1}) of nodules, shoot dry weight (SDW, g plant^{-1}), concentration of N in shoots (CNS, g N kg^{-1} plant^{-1}), total N accumulated in the shoots (TNS, mg N plant^{-1}), %N as N-ureide (%NU)[2] and total N as ureides (TNU, mg ureide-N plant^{-1})[3] in soybean at the R2 stage in field trials conducted at six locations in Brazil during the 2007 growing season.

[2] N-ureides were measured in stem and petiole tissues only and expressed as a percent (%NU) of total N (N-ureides plus nitrate) in these tissues. N-ureides are an estimate of the contribution of nitrogen fixation to total N accumulated by the plant.

[3] The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot (TNU) for treatment comparison purposes.

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Table F-58. Effect of the Different Treatments on Nitrogen Fixation Parameters in the 2007 Growing Season.

Treatment[2]	Parameters[1]						
	NN	NDW	SDW	CNS	TNS	%NU	TNU
T1	32.0	153.2 a*	9.45 ab	42.8	418	72.9 ab	324 a
T2	29.4	150.0 a	9.06 ab	42.5	396	69.1 ab	293 ab
T3	34.6	165.4 a	10.01 a	42.0	421	74.3 a	324 a
T4	21.4	120.2 ab	9.12 ab	40.9	388	71.0 a	297 ab
T5	21.8	112.9 b	7.96 b	39.4	313	67.0 b	212 b

[1] Number (NN, number plant^{-1}) and dry weight (NDW, mg plant^{-1}) of nodules, shoot dry weight (SDW, g plant^{-1}), concentration of N in shoots (CNS, g N kg^{-1} plant^{-1}), total N accumulated in the shoots (TNS, mg N plant^{-1}), %N as N-ureide (%NU)[3] and total N as ureides (TNU, mg ureide-N plant^{-1})[4] in soybean at the R2 stage in field trials conducted in Brazil at six locations in the 2007 growing season.

[2] Treatments: T1 = CV127 treated with imazapyr, T2 = CV127 treated with Volt®, T3 = Isoline control treated with Volt®, T4 = Monsoy 8001 treated with Volt®, T5 = Coodetec 217 treated with Volt®.

[3] N-ureides were measured in stem and petiole tissues only and expressed as a percentage (%NU) of total N (N-ureides plus nitrate) in these tissues. N-ureides are an estimate of the contribution of nitrogen fixation to total N accumulated by the plant.

[4] The N-ureide concentration in the stem and petiole plant tissues was transformed to total N-ureide content in the above-ground portion of the plant or shoot (TNU) for treatment comparison purposes.

* Means followed by no letter or by the same letter, in the same column, are not statistically different at the 5% level.

Conclusions

Studies on nitrogen fixation characteristics of CV127 soybean compared to the isoline control and two conventional soybean varieties were conducted in both the 2006/2007 and the 2007 growing seasons in Brazil. The results across both growing seasons were consistent and showed that CV127 soybean, irrespective of herbicide treatment, is equivalent to the isoline control soybean with respect to biological nitrogen fixation, including nodule number and dry weight, shoot dry weight, total N content and the contribution of nitrogen fixation to the total N accumulated in tissues as evaluated by the N-ureide content of the shoot. Comparison of CV127 and the isoline control as a group to the conventional soybean varieties showed some differences in nitrogen fixation parameters, which were attributed to basic genetic differences between soybean varieties in biological nitrogen fixation capacities. Therefore, these results support the conclusion that CV127 soybean is no different in its interactions with the symbiotic nitrogen-fixing *Bradyrhizobium japonicum* or in nitrogen fixation capacity than conventional soybean varieties.

Appendix G

Best Management Practice (BMP) Program for the Imidazolinone-tolerant CV127 Soybean Production System

The Imidazolinone-tolerant Soybean Production System

Introduction

The Production System for imidazolinone-tolerant soybean is an innovative cropping system that offers broad-spectrum weed control. It creates a number of new opportunities for soybean producers:

- Superior one-pass, broad-spectrum control of grass and annual broadleaf weeds with imidazolinone herbicides.
- Superior in-crop weed control of a range of difficult-to-control weeds, including glyphosate-resistant weeds. It is expected that growers adopting this technology will be able to reduce the number of herbicide applications and benefit from an overall reduction in weed control costs.
- An additional weed management tool for weed control and resistance management.
- Allows the maintenance of glyphosate as an effective pre-seed tool.

Soybean (*Glycine max* L.) plants have been developed by BASF Plant Science, L.P and EMBRAPA (Empresa Brasileira de Pesquisa Agropecuaria, Brasil) that are tolerant to the imidazolinone class of agricultural herbicides. The herbicide-tolerant CV127 soybean plants (also referred to as CV127) are derived from a single transformation event and were produced by introduction of the imidazolinone-tolerance conferring acetohydroxyacid synthase large subunit (*AHAS*) gene *csr1-2* with its native promoter from *Arabidopsis thaliana* into the soybean plant genome via biolistics transformation technology.

BASF Plant Science is developing imidazolinone-tolerant CV127 soybeans for cultivation primarily in Brazil and Argentina. The herbicide **OnDuty®** (525 grams ai/L imazapic + 175 grams ai/L imazapyr) is the commercial product being developed for this production system. These imidazolinone-tolerant CV127 soybeans are distinct from the imidazolinone-tolerant crops sold under the **Clearfield®** brand name; **Clearfield®**-brand crops are not derived from recombinant DNA technology.

Regulatory approval will be sought in the United States and Canada, as well as other countries, for importation of soybean grain from CV127 for food, feed, and processing uses. [

[CBI] deleted

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The introduction of CV127 soybeans in Argentina and Brazil is expected to provide enhanced soybean yield potential and offer growers excellent weed control options in addition to the environmental benefits that result from the cultivation of herbicide-tolerant soybeans. The imidazolinone herbicides possess several environmentally beneficial characteristics compared to other herbicide classes (Tan *et al*., 2005). Imidazolinone herbicides control a wide spectrum of grass and broadleaf weeds. The growing use of glyphosate with glyphosate-tolerant soybeans in Argentina and Brazil has led to a shift in the species of prevalent weeds, with those that are more tolerant to glyphosate predominating. The most common weeds in this category include Benghal dayflower (*Commelina benghalensis* L.), morning glory (*Ipomoea* spp.), Brazil pusley (*Richardia brasiliensis*), and winged false buttonweed (*Spermacoce alata*). These weeds are sensitive to imidazolinone herbicides. Imidazolinone herbicides are effective at low application rates (Shaner and Singh, 1998), possess residual activity in the soil and are absorbed by roots and translocated throughout the plant, resulting in fewer herbicide applications. For example, in Brazil, the recommended application rate and frequency that are proposed for imidazolinones used with CV127 soybean is one application per season at a rate of approximately 70 g ai/hectare.

Imidazolinone herbicides have a very favorable toxicology profile. Due to the fact that imidazolinones are rapidly excreted before they can accumulate in blood or tissues (Gagne *et al*., 1991) and since animals do not possess the AHAS enzyme that is the target for imidazolinones, imidazolinone herbicides have very low mammalian toxicity. Based on the known safety profile of imidazolinone herbicides, it is anticipated that the Imidazolinone-tolerant Soybean Production System will provide a safe and environmentally beneficial system of weed control for soybean growers.

Key Sustainability Issues

The users, developers and marketers of herbicide-tolerant cropping systems are responsible for sustaining the production system and must address the following key issues:

- Herbicide resistance management using an integrated approach.
- Control of herbicide-tolerant crop volunteers.
- Managing out-crossing to other crops and weedy relatives.

These key sustainability issues form the basis for our stewardship plans for crops grown using the Imidazolinone-tolerant Soybean Production System. The Imidazolinone-tolerant Soybean Stewardship Plan can be summarized by the following guiding principles:

Imidazolinone-tolerant Soybean Production System – GUIDING PRINCIPLES

There are a number of GUIDELINES that must be understood and followed by Agronomists and Growers when using the Imidazolinone-tolerant Soybean Production System.

- DO NOT exceed a maximum of 2 exclusive Group 2 herbicides on any one field, in any 4-year period.
- ALWAYS follow an Integrated Weed Management (IWM) program that includes a wide range of herbicides, cultural practices and crop rotations in order to manage weed populations and minimize weed seed development.
- ALWAYS control volunteers in the season following an imidazolinone-tolerant soybean crop.
- USE practices which minimize the likelihood of out-crossing to similar crops or related weeds.
- Scout fields for weeds or volunteer crops that are uncontrolled by herbicides.
- FOLLOW the Best Management Practices outlined in the Imidazolinone-tolerant Soybean Stewardship Guide.

Resistance Management in the Imidazolinone-tolerant Soybean Production System

Herbicides have been grouped based on their mode of action. Herbicides that are in Group 2 are those classified as ALS (acetolactate synthase)/AHAS (acetohydroxyacid synthase) inhibitors. BASF markets herbicides that are in the imidazolinone chemical family and they are members of Group 2. BASF is therefore a key stakeholder in resistance management of ALS/AHAS-resistant weeds.

BASF is committed to maintaining the efficacy of all of its herbicides in order to provide growers with effective, high-performance, environmentally sound products for many years. The key to the performance of the Imidazolinone-tolerant Soybean Production System is effective weed resistance management. BASF is committed to delivering sustainable cropping systems that incorporate best practice principles. The Imidazolinone-tolerant Soybean Production System provides alternative options for growers within a well-managed crop rotation system.

Herbicide Resistance Management Using an Integrated Weed Management (IWM) Approach

The objectives of herbicide resistance management are to achieve weed control while preserving the value of each herbicide and each herbicide group for the longer term. *An integrated approach to weed control is the Best Management Practice to delay the onset of weed resistance to herbicides.* Integrated weed management involves the use of a range of methods available to the grower in order to provide effective weed control in-crop. The use of herbicides is one of a number of useful tools available to growers.

Development of Resistance:

The Herbicide Resistance Action Committee (HRAC) is an industry initiative which fosters co-operation between plant protection manufacturers, government researchers, advisors and farmers. The objective of the working group is to facilitate the effective management of herbicide resistance. HRAC has identified a number of factors to consider when evaluating herbicide resistance risk. The most important factors influencing a plant's potential to develop resistance are the following:

- *Biology and genetic make up of the weed species in question:* Points of consideration include the following. Weeds that are extremely susceptible to an herbicide or that are prolific seed producers and have a large amount of genetic variation within the species may have a greater potential to become resistant to an herbicide. The initial frequency of naturally-occurring resistant biotypes in a weed population influences a weed population's potential to develop resistance. Also, the relative fitness or vigor of resistant weed biotypes affects resistance development. Generally speaking, for any particular weed species, the greater the initial frequency of resistance and the greater the fitness of the resistant biotype, the greater the potential for herbicide resistance to develop.
- *History of herbicide use and mode of action*: Continuous use of the same mode-of-action herbicide for several consecutive years, without tank mixing or sequentially applying herbicides with other modes-of-action, may increase the risk for resistant populations to develop. The greater the selection pressure exerted by an herbicide, the greater the potential for resistance. Although a higher rate of application or sequential applications results in a high level of weed control, it also represents an increased potential for the development of resistance. Likewise, lower herbicide rates, which provide less effective weed control, exert less selection pressure. Label herbicide rates are a reflection of efficacy trials that indicated the best control and crop yield responses. Some herbicides, including Group 2 herbicides, have a greater ability to select for resistant weeds than others because of the frequency of resistance alleles in weedy populations.
- *Crop management practices*: Weed control that relies solely on herbicide use and does not combine tillage, or other cultural practices, with herbicide applications may increase the potential for resistant populations to develop. Crop rotation practices

that allow for non-chemical control options in addition to chemical group rotations are sound management procedures.

- *Environmental conditions*: Environmental conditions that are not conducive to herbicide breakdown in the soil may increase the potential for resistant populations to develop. Continuous dry weather can slow the breakdown of many herbicides (e.g. imidazolinones). Also, high soil pH inhibits the breakdown of some herbicides, such as some of the sulfonylurea herbicides. The longer a herbicide persists, the longer it exerts selection pressure on a weed population, particularly if there are multiple weed flushes in one growing season.
- *Weed seed bank/Seed soil dormancy*: A high soil seed bank within an individual field increases the selection pressure, which in turn increases the likelihood of resistance developing. Seed soil dormancy will also have an impact on weed resistance development. Plants with longer soil dormancy will tend to exhibit slower resistance development since selection pressure is reduced. Seeds that can survive for years in the soil may slow the onset of resistance. Weeds with a long seed life may create a large seed bank in the soil. This seed bank serves as a buffer against genetic changes in the weed population, since the seeds do not normally all germinate within one year. Conversely, weed seed with a short seed life germinate within one or two years. This rapidly depletes the quantity of susceptible weed seed and gives any resistant seed a competitive advantage when a selection pressure is applied.

Many exceptions to these generalizations exist, and this makes it difficult to predict which species will develop a resistant population. The time required for a weed population to develop resistance will vary, and depends on many factors, including the following:

- Selection pressure exerted by the herbicide;
- Herbicide rotation patterns;
- Seed germination dynamics;
- Use of herbicide combinations with different modes of action;
- Initial frequency of naturally occurring resistant individuals in the weed population; and
- The relative vigor of resistant biotypes of weeds.

Based on these factors, models have been developed to predict the development of resistance in a weed population. Current models provide an indication of the development of resistance and these indications are an essential input to the development of resistance management strategies and practices.

Identifying Weed Resistance:

It is important to avoid confusing herbicide failure caused by weed resistance with herbicide failure caused by other factors. All other possible reasons for poor herbicide performance should be ruled out before considering the possibility of resistance. These include application error and poor environmental conditions at the time of herbicide application. Shifts in weed populations from susceptible species to species that are less

sensitive can also cause weed control problems. Herbicide resistance should be suspected under the following conditions:

- A weed species that is normally controlled by the herbicide now escapes treatment, while other weeds on the label are controlled.

- Other factors such as application error or adverse weather conditions are ruled out.

- Irregular-shaped patches of a weed develop in the field and are not controlled with the particular herbicide or herbicide group.

- Weed control records for the field indicate repeated use of a particular herbicide, or herbicides from the same herbicide group.

General Recommendations to Minimize Development of Weed Resistance:

The following is a discussion and proposed strategies for managing ALS/AHAS herbicide resistance in weed populations under the Imidazolinone-tolerant Soybean Production System. The guidelines for managing the development of weed resistance presented here are consistent with recommendations put forward in crop protection guides and the Weed Resistance Education and Action Program (WREAP).

Development of herbicide-resistant weeds can be avoided or delayed through good management practices. The recommendations listed below take into consideration many of the points discussed so far and are designed to provide an integrated approach to weed management in order to prevent or delay the onset of weed resistance. Three key areas of integrated weed management are chemical control, cultural practices and crop management.

<u>Chemical Control</u>

- Know your herbicide groups.

  Reliance on one product or on products within the same group may eventually lead to weed resistance. Understanding herbicide classification by product group is necessary to develop an effective weed management strategy.

- Keep records of herbicide application.

  Herbicide application records and field mapping are necessary to effectively rotate herbicide groups.

- Always read and follow herbicide label recommendations.

The recommended rate of herbicide provides the most effective control over a wide range of environmental conditions. This will help to ensure weed seed is not added to the seed bank, while minimizing selection pressure.

- Use tank mixes or sequential applications of herbicides that have different modes of action.

  Herbicide tank mixes allow weed control in more than one way by combining two or more modes of herbicide action on the same weed. In order to be effective, both active ingredients need to provide control of the target weed. This minimizes selection pressure and delays the development of weed resistance.

- Rotate among herbicide groups.

  Rotate among herbicide groups for both grass and broadleaf weed control. Alternating products used according to mode-of-action is one of the most effective means of delaying development of weed resistance. Use the minimum number of applications of any one herbicide or herbicide group per season.

- Utilize non-selective herbicides.

  Non-selective herbicides applied pre-emergence are an effective means of controlling early flushes of weeds and/or weed escapes. They should be used in conjunction with an in-crop herbicide that has an alternative mode of action.

Cultural Control/Crop Management

Cultural (non-chemical) weed control practices do not exert any chemical selection pressure and can help to reduce the level of weeds in the soil seed bank. These practices are important components of an integrated weed control strategy.

- Use crop rotations, notably rotations from broadleaf crops to grass crops, winter-seeded and spring-seeded crops, and perennial and annual crops.

  Different crops can help to alter the weed spectrum. It also makes it easier to rotate between herbicide groups.

- Plant competitive crops.

  Crops vary in their ability to compete with weeds. Crops such as barley that establish a heavy stand are much more competitive with weeds than crops such as lentils or flax. In most instances, crop competition will increase with higher seeding rates and by planting varieties with a tall stature. A competitive crop can reduce weed pressure.

- Seed early.

  Early seeding generally leads to better crop establishment and increased crop competitive ability. Delayed seeding of most crops results in a potential yield loss and is not recommended.

- Plant quality seed/Increase the seeding rate.

  Certified seed offers a number of benefits and helps to ensure a uniform stand that will compete more effectively with weeds. A more dense plant canopy helps to reduce weed competition.

- Combine tillage and/or timely cultivation with herbicide treatments, if practical.

  Direct seeding has been shown to reduce the number of annual weeds over time. However, if the crop production system includes spring cultivation, seed as soon as possible after working the ground to give the crop a head start over the weeds.

An effective weed management strategy is comprised of multiple weed control options. Herbicide-tolerant cropping systems provide yet another mechanism for effective weed control and should be considered as one of the tools for managing the development of weed resistance.

Integrated Weed Management (IWM) in the Imidazolinone-tolerant Soybean Production System

Imidazolinone-tolerant soybean is tolerant to the imidazolinones, which are Group 2 herbicides. Group 2 herbicides work by inhibiting ALS/AHAS, an enzyme that is required for the production of the amino acids leucine, isoleucine and valine in plants. Group 2 herbicides are known as 'ALS inhibitors'.

Continuous use of Group 2 herbicides may result in the selection of weed biotypes with a resistance to this Group of herbicides. Preservation of the effectiveness of this Group of herbicides is vital for efficient and cost-effective agricultural production in North America. Therefore effective management strategies for weed control delay or avoid the potential for the development of resistant weeds and are an important focus of the Imidazolinone-tolerant Soybean Production System.

In addition to the general chemical and cultural control recommendations outlined in the previous section, a number of specific management strategies outlined below, should be followed when using the Imidazolinone-tolerant Soybean Production System. Growers and agronomists should give consideration to each of the following points, in order to develop an integrated weed management system when using imidazolinone-tolerant soybeans as part of their production system.

- Make only one application of a Group 2 herbicide per season.

- Apply no more than two (2) exclusive Group 2 herbicides in any four (4) year period on the same field. The use of no more than two applications in four years will slow the development of resistance. Consideration should be given to less frequent use to delay the onset of resistance development. This includes the use of Group 2 herbicides both within the Imidazolinone-tolerant Soybean Production System and in conventional crops.

- If a Group 2 herbicide has been applied as a pre-emergence application, DO NOT apply further Group 2 herbicides to that crop. Make any further post-emergence applications with herbicides from a different mode-of-action group.

- Where it is possible, care should be taken to avoid applications of Group 2 herbicides in consecutive years unless in at least the two previous years, effective weed control has been achieved with methods other than the use of Group 2 herbicides.

- Farm practices, including herbicide and crop rotations, should be developed which allow for the use of alternative mode-of-action herbicides.

- Where Group 2 resistance is suspected within a weed population, testing of the relevant weeds should be carried out prior to the use of crops in the Imidazolinone-tolerant Soybean Production System.

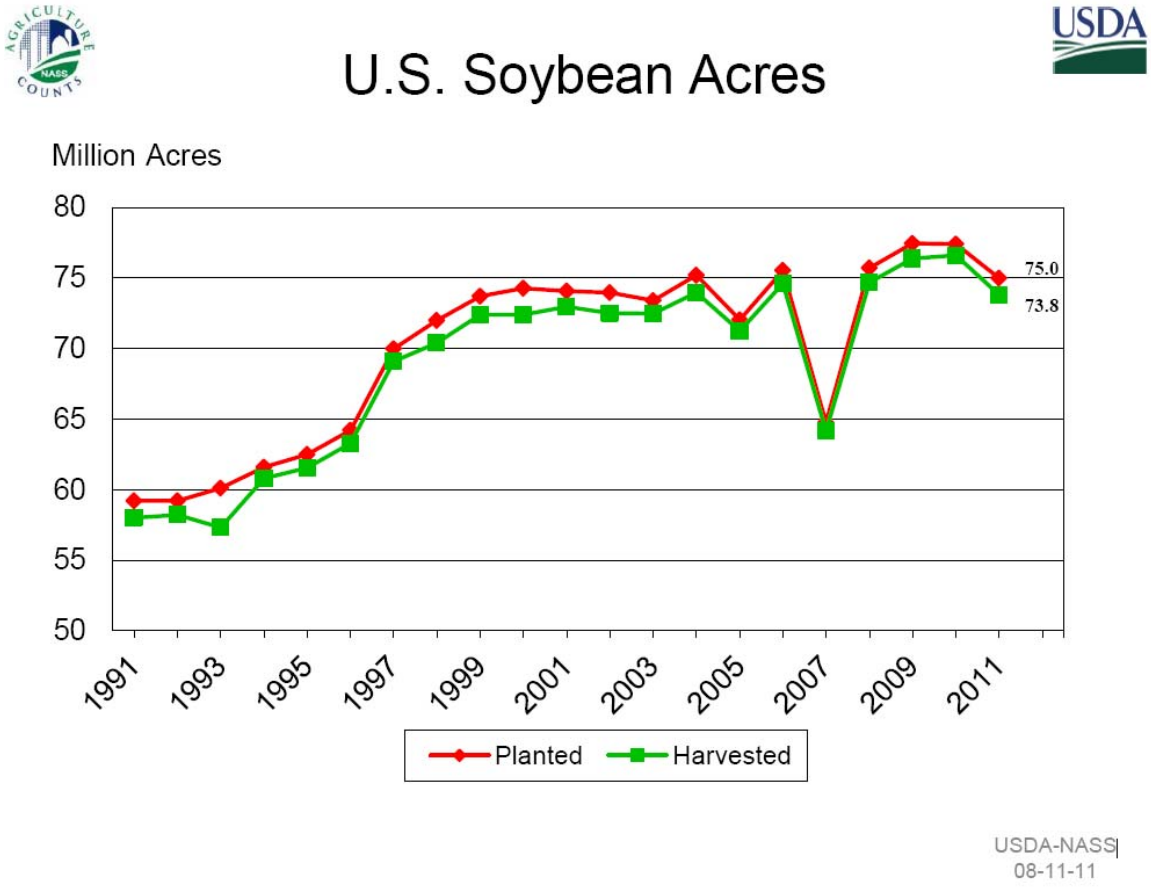
- Integrated Weed Management should be undertaken on a field-by-field basis. Specific field planning should take into consideration the history of the field well as the future use options.

- Resistance management guidelines for other herbicide Groups, especially Group 1, should be taken into account when developing and planning rotations.

Crop Rotation and Imidazolinone-tolerant Soybeans

The most common rotational crops for soybeans in the United States are corn, other row crops, and small grains. In 2002, approximately 80% of the soybeans were grown in rotation with corn (USDA-ERS, 2006b). The area planted to soybean in the U.S. over the past 20 years is presented in Figure G-1. For the years 2006-2011, the area planted to soybeans in the United States has ranged between 65 and 77 million acres (USDA-NASS, 2011; USDA-ERS, 2011b).

Figure G-1. Area Planted to Soybeans in the United States.



Controlling Volunteers from Imidazolinone-tolerant Soybeans

Objective: control of all volunteers from Imidazolinone-tolerant Soybeans before flowering.

Summary

- Best Management Practice is to control volunteer plants in the year following the cultivation of imidazolinone-tolerant soybeans.
- Do NOT rely on Group 2 herbicides to control imidazolinone-tolerant soybean volunteers. Group 4 + 5 herbicides are the most commonly used management tool for control of imidazolinone-tolerant soybean volunteers.
- Volunteers from imidazolinone-tolerant soybeans will be controlled by all herbicides currently registered for control of volunteer conventional soybeans, except for imidazolinone herbicides.
- Clean-up of farm equipment during all stages of sowing, harvesting, storage and transport are important in the effective control of volunteers.
- Best Management Practice is to make volunteer control part of weed, pest and disease management strategies for the farm.

Important reasons for control of volunteers

- Volunteer plants act as competitive weeds in subsequent crops or pastures.
- Volunteers may be important in the build-up and spread of major diseases.
- Volunteers increase the risk of herbicide tolerance spreading from cross-pollination of volunteer plants and conventional plants of the same crop species in neighboring fields.

Cultural and chemical options for volunteer management

A number of cultural and chemical control options are available to control volunteers including the following:

- Minimize seed losses at harvest by the following means:
    - Pay close attention to timeliness of harvesting.
    - Make correct adjustments in the header.
    - Seal all holes and cracks in harvesting equipment which allow spillage, even of small quantities of seed (especially in the table, front elevator and grain tank).
    - Clean out harvest equipment before switching fields.
- Practice good hygiene at harvest and during transport of grain. Secure loads to prevent losses during transport.
- Practice good stubble management after harvest in light of crop rotation decisions. Leaving soybean seed on the surface will increase seed mortality and help to reduce the seed bank population in the soil.
- Use pre-seed glyphosate application to control weeds and volunteers that emerge prior to seeding.
- Control volunteers along field edges and borders.
- Control ALL volunteers in subsequent crops with the proper selection of in-crop herbicides or other agronomic practices such as tillage where appropriate.
- Keep good field records of herbicides used in previous crops and herbicide-tolerant varieties in neighboring fields to develop effective plans for controlling volunteers.
- Soybeans should not follow an imidazolinone-tolerant soybean crop, as controlling volunteers within the same crop species is difficult. Generally speaking, this would also not be a good agronomic practice for disease and weed management.

Management of volunteer Imidazolinone-tolerant Soybean

Cultivated soybean seed rarely displays any dormancy characteristics, and only under certain environmental conditions will it grow as a volunteer (OECD, 2000). If soybean plants do grow as a volunteer, they do not compete well with the succeeding crop.

Volunteer soybean management is normally not a significant management concern in North America because of environmental conditions, crop rotation and the numerous herbicide control options available. When soybean seeds are left on the soil surface they are typically not viable after winter. When a rotational crop such as winter wheat is

planted after soybean harvest, it is possible for soybean seed to be incorporated into the soil and to germinate that fall. These volunteer plants will be controlled by freezing temperatures in the fall and do not become a competitive consideration. Normally volunteer soybean is not a target for weed control measures in winter wheat.

When corn is the rotational crop, the first option available to control volunteer soybean the following spring is pre-plant tillage or the use of a pre-plant burn-off herbicide such as glyphosate. Table G-1 lists the herbicide active ingredients applied to corn in the United States in 2010. Herbicides from Group 4 (auxinic herbicides), Group 5 (triazines), Group 10 (phosphorylated amino acids) and Group 27 or Group 28 (4-hydroxyphenyl pyruvate dioxygenase or HPPD inhibitors) all will control volunteer soybeans in corn. Seventeen of the 58 active ingredient reported as used on corn (29% of the total) in 2010 are members of one of these herbicide groups. Therefore, there are numerous tools available to control volunteer soybeans in corn.

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In Roundup Ready® (RR) corn, volunteer conventional or imidazolinone-tolerant soybeans will be controlled by any of the glyphosate products (Group 9) applied. In some cases the glyphosate is tank-mixed or used in sequence with another broadleaf herbicide. Any of the broadleaf herbicide products from Groups 4, 5 or 27 will also control volunteer soybeans. Therefore, virtually all the herbicide options available in Roundup Ready® corn will control volunteer soybeans. The two most commonly-used herbicide active ingredients for use on corn, glyphosate and atrazine, [ ] will both control volunteer soybeans.

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In the United States, volunteer soybean management is normally not a concern in common rotational crops such as corn and wheat. In addition to cultural control measures such as tillage, there are numerous herbicide options available in these crops to

control volunteer soybeans. In corn, post-emergence application of products such as AAtrex® (atrazine), **Clarity®** (dicamba), **Distinct®** (diflufenzopyr + dicamba), Hornet® (flumetsulam + clopyralid) and Widematch® (clopyralid + fluroxypyr) will control volunteer conventional, volunteer Roundup Ready® or imidazolinone-tolerant soybean (Zollinger, 2011). Similarly, in wheat there are also a number of products available for volunteer soybean management. Products such as Bronate® Advanced™ (bromoxynil), **Clarity®** (dicamba) and Widematch® (clopyralid + fluroxypyr) will control all types of volunteer soybean (Zollinger, 2011).

Managing Out-Crossing to Imidazolinone-sensitive Crops and Weeds

What is out-crossing and gene flow?

Gene flow is the movement of gametes, zygotes (seeds), individuals, or groups of individuals from one place to another and their subsequent incorporation in the gene pool of the new locality (Slatkin, 1987). Gene flow is a natural biological process and in plants it primarily occurs via pollen or seed dispersal (Levin and Kerster, 1974). The relative importance of gene flow to a population's genetic structure depends on the distance between donor and recipient populations, population size, how long the process has been in effect, and whether the new gene confers any fitness advantage to the recipient population (Waines and Hegde, 2003).

Out-crossing (or cross-pollination) is a type of mating in plants in which a male gamete of one individual fertilizes a female gamete of another individual (Waines and Hegde, 2003). The term out-crossing generally refers to mating within a species and the term has been used synonymously with gene flow (Gleaves, 1973; Handel, 1983). However, as defined earlier, gene flow can occur through means other then cross-pollination. This discussion is restriced to pollen-mediated gene flow (out-crossing).

A wide range of factors influence the ability of a given plant to out-cross with others, including synchrony of flowering, stigma receptivity, pollen production, pollen dispersal, pollen viability and environmental factors. The likelihood of out-crossing varies greatly from species to species and variety to variety. Successful out-crossing may result in the offspring displaying characteristics of both parent plants.

The risk of out-crossing can vary among crop and weed species. The focus for management must be on the control of volunteers and managing herbicide-tolerant crops and related species.

Description of the Imidazolinone-tolerant Soybean technology

Cultivated soybean, *Glycine max* (L.) Merr., is a diploidized tetraploid (2n=40), in the family Fabaceae. Three types of growth habit can be found amongst soybean cultivars: determinate, semi-determinate and indeterminate (Bernard and Weiss, 1973). Generally determinate types are grown in the southern United States and indeterminate types are grown in central and northern regions of North America. In Canada the majority of the soybean cultivars have an indeterminate growth habit.

The herbicide-tolerant CV127 soybean plants are derived from a single transformation event and were produced by introduction of the imidazolinone-tolerance conferring acetohydroxyacid synthase large subunit gene *csr1-2* with its native promoter from *Arabidopsis thaliana* into the soybean plant genome via biolistics transformation technology.

Potential for out-crossing of Imidazolinone-tolerant Soybean

Soybean is considered to be a self-pollinating species. Based on the reproductive morphology of soybean, the anthers mature in the bud and directly pollinate the stigma of the same flower (OECD, 2000). Natural out-crossing levels range from less than 0.5% to about 1.0% (Carlson and Lersten, 1987). Therefore the potential for gene transfer from an Imidazolinone-tolerant Soybean variety to another commercial variety is very low.

Potential for out-crossing to species related to Imidazolinone-tolerant Soybean

Soybeans, *Glycine max*, belong to the subgenus *Soja*. This subgenus also includes *G. soja* and *G. gracilis* which are wild and semi-wild soybean relatives from Asia. Interspecific, fertile hybrids between *G. max* and *G. soja* or between *G. max* and *G. gracilis* have been easily obtained (as reviewed in OECD, 2000). However, the potential for gene flow to wild soybean relatives is limited geographically. Wild soybean species are limited to Korea, Taiwan, Japan, China and areas around the border of the former USSR. These wild relatives do not exist naturally in North America.

The genus *Glycine* also contains the subgenus *Glycine*. This subgenus consists of 22 wild perennial species that are indigenous to Australia, the South Pacific Islands, China, Papua New Guinea, the Philippines and Taiwan. Intersubgeneric hybrids between the subgenus *Soja* and the subgenus *Glycine* have been created artificially with great difficulty, and the progeny of such hybrids were sterile (as reviewed in OECD, 2000).

The risk for introgression into other relatives is extremely low. Soybeans can only cross with other members of *Glycine* subgenus *Soja*. Therefore, the potential for outcrossing to other members of the subgenus *Soja* is limited by geographic isolation and the wild soybean species of the subgenus *Soja* are not naturalized in North America (OECD, 2000).

Conclusion

In summary, the accidental presence of Imidazolinone-tolerant Soybean in the North American environment would have no impact on current soybean control practices, since all of the conventional control methods (including all herbicides currently registered for control of volunteer conventional soybeans, except for imidazolinone herbicides) would continue to control this plant. There are no significant differences between the growth and productivity of Imidazolinone-tolerant Soybean and the corresponding conventional varieties of the equivalent maturity group. Therefore, there would be no change to agronomic practice due to the occurrence of Imidazolinone-tolerant Soybean.

Always read and follow label directions.

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Appendix H

Digestive Fate of the AtAHAS Produced in CV127 Soybean and in a Recombinant *E. coli* Expression System

INTRODUCTION

The purpose of this study was to determine the susceptibility of 1) a test substance (Lot number AtAHAS-0107) containing AtAHAS protein encoded by the imidazolinone-tolerant acetohydroxyacid synthase large subunit gene isolated from *Arabidopsis thaliana (*AtAHAS, containing R272K and S653N mutations) and 2) AHAS protein present in extracts of leaf and grain from CV127 plants, to digestion in both simulated mammalian gastric fluid (SGF) and simulated mammalian intestinal fluid (SIF).

The introduction of an imidazolinone-tolerant acetohydroxyacid synthase large subunit gene from *Arabidopsis thaliana* into the plant genome allows growth and, hence, selection of the transformed cells in the presence of imidazolinone herbicides. The *Arabidopsis thaliana* AHAS large subunit (AtAHAS) protein is a member of the class of AHAS large subunit proteins found ubiquitously in plants. The mutation in the *Arabidopsis* acetohydroxyacid synthase large subunit gene responsible for imidazolinone herbicide tolerance is a single nucleotide change of guanine to adenine, which results in a codon change from AGT to AAT and a single amino acid substitution of serine to asparagine at position 653 of the AtAHAS protein. In addition, a second mutation was identified after this gene was introduced into the soybean genome, resulting in the transformation event designated as CV127. The second mutation, in which arginine at position 272 was replaced by lysine (R272K), does not impact the enzymatic function of the AHAS enzyme (Stevenson-Paulik, 2011).

The AHAS enzyme catalyzes the first step in the biosynthesis of branched-chain amino acids in plants. In conventional plants, inhibition of the AHAS enzyme by imidazolinone herbicides leads to a deficiency in branched-chain amino acids and other compounds derived from this pathway that are needed for plant survival. The acetohydroxyacid synthase large subunit gene from *Arabidopsis* confers tolerance to imidazolinone herbicides by encoding an AtAHAS enzyme (large subunit) with altered herbicide binding properties. However, the enzyme has normal biosynthetic function in the transgenic plant.

The lot of test substance, AtAHAS-0107, was prepared from a recombinant *Escherichia coli* over-expression system and was intended for use in an acute oral mouse toxicity study, as well as other studies to confirm the food, feed and environmental safety of the protein. The AtAHAS protein preparation was characterized to determine identity, purity, functionality, concentration, and solubility (Privalle, 2007a) and has been found to be substantially equivalent to that produced by CV127 plants (Privalle, 2007b). The purpose of Phase A of this study was to confirm that the microbially-produced AtAHAS protein is rapidly digested, in a similar manner as typical dietary proteins. The purpose of also examining the digestion of the AtAHAS protein as expressed in leaf and grain of CV127 (Phase B of this study) was to determine whether the plant matrix has an effect on the digestibility and to confirm that the plant-produced AtAHAS protein had a similar digestive fate to the *E. coli*-produced AtAHAS protein as well as the endogenous soybean AHAS protein. After incubation for various times in the appropriate simulated digestive

fluids, the remaining protein was subjected to electrophoresis and visualized by either Coomassie blue staining or western blot analysis.

MATERIALS AND METHODS

PHASE A.

Preparation of test substance. The *Arabidopsis thaliana* acetohydroxyacid synthase large subunit gene (*ahas R272K, S653N*) was cloned into the inducible, over-expression vector pTrcHis A® (Invitrogen, Carlsbad, CA) in *E. coli* strain BL21(DE3)pLysS. The AtAHAS protein encoded by this vector lacks the predicted 85-amino acid N-terminal leader sequence that targets the protein *in planta* to the chloroplast. This leader sequence has been replaced in this vector with 38 amino acids which include a 6x His tag to allow for ease in purification, a gene 10 leader sequence to enhance solubility, and an Xpress™ tag for detection. The remainder of the protein is identical in amino acid sequence to that produced by the native gene from *Arabidopsis thaliana* (Mazur *et al*., 1987) except for the two aforementioned point mutations. The replacement of serine with asparagine at amino acid residue 653 of the AtAHAS protein results in decreased binding of imidazolinone herbicide to AtAHAS, and imidazolinone tolerance. The point mutation that results in replacement of arginine with lysine at amino acid residue 272 has no apparent impact on AHAS functionality.

AtAHAS protein was produced and purified by Invitrogen, Inc. (Madison, WI) and transferred as an ammonium sulfate pellet to the Regulatory Science Laboratory at BASF Plant Science, Research Triangle Park, NC where it was received on February 27, 2007. The AtAHAS protein was purified from 2,400 g of cell paste after lysis in 20 L buffer (50 mM sodium phosphate, 300 mM NaCl, 10 mM β-mercaptoethanol, 30 mM imidazole) using an Emulsiflex cell disruptor (Avestin, Ontario, Canada). Insoluble material was removed by centrifugation. The His-tagged AtAHAS present in the supernatant was purified by chromatography using Talon® cobalt resin (Clontech, Inc., Mountain View, CA). Once eluted, the fractions containing AtAHAS were pooled and immediately precipitated by ammonium sulfate at 40% saturation. The ammonium sulfate pellet was resuspended in 100 mM Tris-HCl, pH 7.0 and dialyzed against 20 mM ammonium bicarbonate buffer, pH 7.9, containing 20 µM FAD. The dialyzed material was then lyophilized and designated AtAHAS-0107. This material has been characterized (Privalle, 2007a). The test substance in this study is also the test system. The test solution used in the digestion reactions below was prepared by resuspending 2.2 mg of AtAHAS-0107 in 1.1 ml of 8 mM sodium phosphate, 2 mM potassium phosphate, 0.14 M sodium chloride, 10 mM potassium chloride, pH 7.4, and used on the day of preparation.

Control substances. An extract of nontransgenic soybean leaves was prepared by homogenizing 0.67 g of leaf material with two ml extraction buffer (8 mM sodium phosphate, 2 mM potassium phosphate, 0.14 M sodium chloride, 10 mM potassium chloride, pH 7.4). An extract of nontransgenic soybean grain was prepared similarly except that two grams of material was extracted with nine ml of buffer. Extracts were

centrifuged 15 min at approx. 13,000 x g. The protein concentration was determined in the supernatants after centrifugation and the supernatants were used directly in the digestive reactions. Digestion reactions were conducted the same day that the extracts were prepared.

Protein quantification. Total protein in the leaf and grain extracts was quantified by the BCA™ procedure (bicinchoninic acid procedure; Pierce Biotechnology, Inc., Rockford, IL) using bovine serum albumin as the standard. Dilutions of the extract (final volume 100 µl) were prepared such that the expected concentration of protein would be within the standard curve. Samples were reacted with two ml of a 1:50 (B:A) mixture of BCA reagent, incubated at 37ºC for 30 min and allowed to cool at room temperature for 10 min. The absorbance at 562 nm was measured using a UV1600 Spectrophotometer (Shimadzu, Columbia, MD). The results were analyzed by the instrument's software using the linear regression curve fit.

PHASE B.

Preparation of test substances. Extracts of CV127 leaves were prepared by homogenizing two grams of leaf material with six ml extraction buffer (8 mM sodium phosphate, 2 mM potassium phosphate, 0.14 M sodium chloride, 10 mM potassium chloride, pH 7.4). Extracts were centrifuged 15 min at approx. 13,000 x g. An extract of CV127 grain was prepared similarly except that two grams of material was extracted with nine ml of buffer. The protein concentration was determined in the supernatants after centrifugation and the supernatants were used directly in the digestive reactions. Digestion reactions were conducted the same day extracts were prepared.

Protein quantification. Total protein in the leaf and grain extracts was quantified by the BCA™ procedure (bicinchoninic acid procedure; Pierce Biotechnology, Inc., Rockford, IL) using bovine serum albumin as the standard. Dilutions of the extract were prepared such that the expected concentration of protein would be within the standard curve. Samples (25 µl) were loaded into a multiwell plate in triplicate, reacted with 200 µl of a 1:50 (B:A) mixture of BCA reagent, incubated at 37ºC for 30 min and allowed to cool at room temperature for 10 min. The absorbance at 550 nm was measured using a Multiskan Ascent V1.24 multiwell plate reader (Therma Labsystems, Helsinki, Finland) and concentrations were determined using a standard curve analyzed by linear regression.

PHASE A and B.

Simulated mammalian gastric fluid reactions. Simulated mammalian gastric fluid [2X SGF; 0.016 N HCl, 0.75 mM NaCl, pH 1.2, and 3500 Units of pepsin (Sigma)] was prepared as described by Thomas *et al.* (2004) and proteolytic activity was confirmed using azoalbumin as a substrate (SOP BPS 510.01). A single tube containing sufficient reaction mix for all assay time points was prepared by mixing 350 µl of SGF (2X) with 350 µl of AtAHAS-0107 solution or CV127 leaf extract. For CV127 grain extract, a single tube containing sufficient reaction mix for all assay time points was prepared by mixing 665 µl 1X SGF with 35 µl extract. The reaction mixtures were immediately placed in a 37°C water bath and 100 µl samples were removed at 0.5, 2, 5, 10, 30 and 60 min after initiation of the experiment. Each sample was quenched by the addition of 35

µl of 200 mM $NaHCO_3$, pH 11, and 35 µl 5X Laemmli buffer (40% glycerol, 5% β-mercaptoethanol, 10% SDS, 0.33 M Tris, 0.05% bromophenol blue, pH 6.8). Quenched samples were then heated to >75°C for 10 min and stored at *ca*. –20°C until subjected to electrophoresis as described below. The "zero" time point for all reactions was prepared by first quenching SGF and then adding the test protein. In addition, reactions that served as controls for pepsin auto-digestion and test protein stability were prepared containing SGF without test protein and SGF with test protein but without pepsin ("G-con"), respectively. These control reactions were treated exactly as described above except samples were only taken at 0 and 60 minutes after the start of the 37°C incubation.

Simulated mammalian intestinal fluid reactions. Simulated mammalian intestinal fluid (SIF) containing phosphate (8.2 mg KH_2PO_4/ml), tap water and pancreatin (12 mg/ml), pH 7.5, was prepared as described in the United States Pharmacopoeia (2000) and was checked for proteolytic activity using azoalbumin as a substrate (SOP BPS 510.01). A single tube containing enough reaction mix for all time points was prepared by mixing 280 µl of SIF with 70 µl of AtAHAS-0107 solution or CV127 leaf or grain extract. The reaction mixtures were immediately placed in a 37°C water bath and 50 µl samples were removed at 0.5, 2, 5, 10, 30 and 60 min after initiation of the experiment. Each sample was quenched by addition of 50 µl of 2X Laemmli buffer (20% glycerol, 2% β-mercaptoethanol, 4% SDS, 0.13 M Tris, 0.02% bromophenol blue, pH 6.8). Samples were then heated to >75°C for 10 min and stored at *ca*. –20°C until subjected to electrophoresis as described below. The "zero" time point was prepared by first heating SIF for 10 min at > 75°C and then adding the test protein and 50 µl 2X Laemmli buffer and reheating for 10 min at > 75°C. In addition, reactions that served as controls for pancreatin auto-digestion and test protein stability were prepared containing SIF without test protein and SIF with test protein but without pancreatin ("I-con"), respectively. These control reactions were treated exactly as described above except samples were only taken at 0 and 60 minutes after the start of the 37°C incubation.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. To monitor the integrity of AtAHAS (*ca*. 64,000 mol. wt.) in sample AtAHAS-0107 after incubation in the various digestion mixtures, aliquots of the quenched and heated mixes were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, SOP BPS 510.02), using a 4 - 20% polyacrylamide gradient Tris-glycine gel (Invitrogen). Aliquots of the samples that contained *ca.* 2.5 µg of protein based on determinations made prior to digestion were loaded onto the gel. Mark 12™ molecular weight markers (Invitrogen) were used to establish the approximate molecular weight of proteins. The protein bands were stained with 0.1% Coomassie Brilliant Blue R (Sigma Chemical, Saint Louis, MO).

Western blot analysis. To monitor the integrity (intactness) of the AtAHAS protein in the leaf and grain extracts as well as in sample AtAHAS-0107 after incubation in the various digestion mixtures, western blot analysis was performed. Aliquots of the quenched and heated mixes were subjected to SDS-PAGE on a 4 - 20% polyacrylamide gradient gel followed by electroblotting onto PVDF membrane (Invitrogen; SOP BPS 510.03). The samples were loaded onto the gel to achieve maximum total protein based on determinations made prior to digestion. For the SGF samples, the amounts of protein

loaded were 24 μg for leaves and grain, and 8 and 30 μg for CV127 and conventional soybean leaves, respectively. For the SIF samples, the amounts loaded onto the gel were 84 and 67 μg for CV127 and conventional soybean grain, respectively. For western blot analysis of the AtAHAS-0107 SIF reactions, 0.2 μg of protein was loaded per lane. After electroblotting, the membranes were probed with rabbit anti-AHAS peptide 2 polyclonal antibody. Donkey anti-rabbit IgG linked to horseradish peroxidase (Pierce), diluted 1:3000 in blocking buffer (3% nonfat dry milk in 0.1% Tween-20, 10 mM Tris-HCl, 150 mM NaCl, pH 7.5) was used to bind to the primary antibody and was visualized by development with the chemiluminescent ECL kit from Amersham (Buckinghamshire, UK) according to the manufacturer's instructions.

RESULTS

PHASE A.

Sensitivity of *E. coli*-produced AtAHAS (Lot number AtAHAS-0107) to degradation in SGF. The *E. coli*-produced AtAHAS protein in test material lot number AtAHAS-0107 was rapidly degraded in SGF (Figure H-1 panel A). AtAHAS (molecular weight *ca*. 64,000) is readily detected in the time 0 sample and in the "G-con" samples. However, no full-length protein is visible in the sample removed at 0.5 min after the initiation of the reaction. Some low molecular weight, Coomassie blue-staining bands (<6000) were observed in the 0.5 min sample, but these were degraded after 2 min in the reaction. A low molecular weight band of approx. 6000 MW is apparent that is also present in the "SGF only" lane and hence must be derived from the pepsin enzyme preparation.

Sensitivity of *E. coli*-produced AtAHAS (Lot number AtAHAS-0107) to degradation in SIF. The *E. coli*-produced AtAHAS protein in test material lot number AtAHAS-0107 was also rapidly degraded in SIF (Figure H-2 panel A). Similar to the SGF results, AtAHAS is detected in the time 0 and "I-con" samples. However, no full-length protein is visible in the sample after 0.5 min of incubation. Some degradation of the AtAHAS is observed in the 60 min "I-con" sample indicating protein sensitivity to incubation at 60 min in simulated intestinal fluid in the absence of pancreatin. Pancreatin is comprised of multiple proteases and lipases and this accounts for the multiple staining bands in the SIF lanes. All of the lower molecular weight bands visible in the digestion time course correspond to those present in the "SIF only" sample (no AtAHAS-0107). The results of the AtAHAS digestion in SIF using western blot analysis (Figure H-3 panel A) confirm that no immunoreactive degradation products of AtAHAS were obscured by bands associated with the pancreatin, and confirm that the AtAHAS protein is rapidly degraded in SIF within a 0.5 min incubation time.

PHASE B.

Sensitivity of CV127 soybean leaf and grain AtAHAS to degradation in SGF. The AHAS protein produced in CV127 soybean leaves and grain is a mixture of the endogenously encoded soybean AHAS proteins and the AtAHAS protein encoded by the introduced imidazolinone-tolerant *ahas R272K, S653N* gene. Due to the high amino acid

sequence similarity of the endogenous AHAS and AtAHAS proteins, they are immunologically indistinguishable. Both soybean AHAS and AtAHAS proteins are rapidly digested in SGF and, although the AHAS protein band is visible at time 0, no AHAS protein band is detectable within two minutes of incubation (Figure H-1 panel B). An additional immunoreactive band at approximately 50,000 molecular weight is observed in leaf extracts (Figure H-1 panel B). It is stable in the absence of pepsin ("G-con" lanes) but is not visible during the digestion time course. A different immunoreactive band at approximately 36,000 molecular weight is observed in the grain extracts, and it is also rapidly digested by pepsin within 0.5 min (Figure H-1 panel C). These bands may represent proteins that cross-react with the AHAS-specific antibody or smaller fragments of the AHAS protein, but in either case, they are rapidly degraded.

Sensitivity of CV127 soybean leaf and grain AtAHAS to degradation in SIF. The full-length soybean leaf AHAS protein is rapidly digested and is no longer visible within 0.5 min in SIF (Figure H-2 panel B). Similar to the results observed with the *E. coli*-produced AtAHAS protein, the leaf AHAS protein is not stable in SIF without pancreatin ("I-con"). Also, a lower molecular weight protein of approximately 50,000 molecular weight appears to increase in intensity with time of digestion. Furthermore, an immunoreactive band of approximately 16,000 molecular weight appears to be associated with the pancreatin since the band was not visible in the absence of SIF ("I-con" lanes) or at the zero time point where the SIF was inactivated by heating prior to addition of the leaf extract. A smaller immunoreactive band of >6000 molecular weight is also observed and since it is also present in the "SIF only" incubations, it appears to be associated with pancreatin. This band may be a degradation product of pancreatin. Full-length AHAS protein is just barely detected by western blot analysis in the leaf extract of conventional soybean and the lower molecular weight protein at approximately 50,000 molecular weight (observed in the leaf extract of CV127 soybean) is apparently below the detection limit for this method as the 50,000 molecular weight band was not observed in the conventional soybean leaf extract subjected to digestion in SIF (Figure H-3 panel B). This result was not unexpected since the AHAS protein expression levels, as reported in da Silva (2007) were approximately 600 ng AHAS/g dry weight in young CV127 leaves as compared to approx. 75 ng AHAS/g dry wt in young leaves of conventional soybean. The lower molecular weight bands at approximately 16,000 and >6000 molecular weights, previously observed in SIF digestion of leaf extracts of CV127, were again observed in SIF digestion of the leaf extract of conventional soybean (Figure H-3 panel B), supporting the conclusion that these bands are associated with pancreatin and not with the AtAHAS transgenic protein encoded by the introduced imidazolinone-tolerance-conferring *ahas R272K, S653N* gene.

In the grain extracts of CV127, full-length AHAS is rapidly degraded by pancreatin and is not observed at 0.5 min (Figure H-2 panel C). Full-length AHAS protein was not detected in the SIF digest of the conventional soybean grain sample (Figure H-3 panel C). This result was not unexpected since the expression levels, as reported in da Silva (2007) were below the level of quantification in the conventional soybean grain, and approximately 28 ng/g dry weight in CV127 grain. As observed with digestion of the leaf extract of CV127, where a lower molecular weight (approx. 50,000) protein band was

observed, a lower molecular weight immuno-reactive band at approximately 36,000 molecular weight appears to accumulate with digestion time in SIF digestions of both CV127 and the conventional soybean grain (Figures H-2 panel C and H-3 panel C respectively). Also, the same immunoreactive bands associated with pancreatin (at approximately 16,000 and >6000 molecular weights), which appeared in the digest of the leaf extracts, are also observed in digestion of the grain extracts (Figures H-2 panel C and H-3 panel C). The grain AtAHAS appears more stable in the SIF without pancreatin ("I-con") and perhaps this is due to the presence of other components in the grain extract that stabilize AHAS (Figure H-2 panel C).

In leaf and grain extracts, protein bands corresponding to approximately 50,000 and 36,000 molecular weights, respectively, appeared to increase in intensity with increasing time of SIF digestion. Because no lower molecular weight protein bands at approximately 50,000 or 36,000 molecular weights were observed to accumulate in SIF digestion of AtAHAS-0107 (Figure H-3 panel A), it is unlikely that these protein bands are a degradation product of the full-length AHAS protein produced in leaf or grain tissue, and are more likely immunoreactive peptides generated from digestion of a different protein present in the leaf or grain extracts.

CONCLUSIONS

E. coli-produced AtAHAS, and both forms of AHAS produced in CV127 leaves and grain (endogenous soybean AHAS and AtAHAS), are rapidly degraded in simulated mammalian gastric fluid. Full-length AHAS, regardless of source, is also rapidly degraded in simulated mammalian intestinal fluid. Degradation of the AHAS and AtAHAS proteins in plant extracts was slightly slower compared to the purified *E. coli*-produced protein in both SGF and SIF, showing that the plant matrix has only a slight effect on protein digestion. Therefore, AtAHAS expressed in CV127 soybean is digested the same as conventional dietary protein, and the same as endogenous soybean AHAS has been digested in conventional plants to date.

Although large quantities of a range of proteins are consumed in human diets each day, rarely do any of these tens of thousands of proteins elicit an allergenic response (Taylor, 1992). There are no predictive bioassays available to assess the allergenic potential of proteins in humans; however, physicochemical and human exposure profiles of the protein provide a basis for assessing potential allergenicity relative to known protein allergens. Results of the current study show that the AtAHAS protein expressed in CV127 soybean is highly digestible under the simulated digestion conditions, which is typical of most proteins exposed to the proteases of the mammalian digestive tract. Furthermore, the transgenic AtAHAS protein expressed in CV127 soybean was immunologically indistinguishable from the endogenous soybean AHAS protein in the current studies, and results suggest that the digestive fate of both proteins in SGF and SIF is identical. Therefore, the AtAHAS protein shares the same digestive fate properties with the endogenous soybean AHAS protein, which has a history of safe consumption in food and feed products.

GLP COMPLIANCE AND PROTOCOL CHANGES: This study was conducted in compliance with the relevant provisions of Good Laboratory Practice Standards (40 CFR 160, *Federal Register*, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act and subsequent revisions with the exception of Phase B, the digestibility of AtAHAS produced in CV127 soybean. A protocol change was made to add digestion reactions with conventional soybean grain and leaf preparations. This was judged to have no adverse impact on the study and allowed better interpretation of background protein bands.

Standard Operating Procedures

SOP BPS 510.01 Simulated Mammalian *In vitro* Digestibility Method

SOP BPS 510.02 SDS-Polyacrylamide Gel Electrophoresis

SOP BPS 510.03 Western Blot Analysis

Figure H-1. Susceptibility of AtAHAS to Digestion in Simulated Mammalian Gastric Fluid (SGF).

Incubation time at 37ºC is indicated in minutes. "SGF" is simulated mammalian gastric fluid containing pepsin; "G-con" is SGF without pepsin. "SGF only" indicates no AtAHAS sample was included. **Panel A**. Coomassie blue stained 4-20% polyacrylamide gel containing digestion reactions carried out with test substance AtAHAS-0107. **Panel B**. Western blot analysis of digestion reactions carried out with leaf extract from CV127 soybean. **Panel C**. Western blot analysis of digestion reactions carried out with grain extract from CV127 soybean. AtAHAS is *ca.* 64,000 mol wt.; pepsin is ca. 40,000 mol. wt. Molecular weight (x 10^{-3}) markers are indicated.

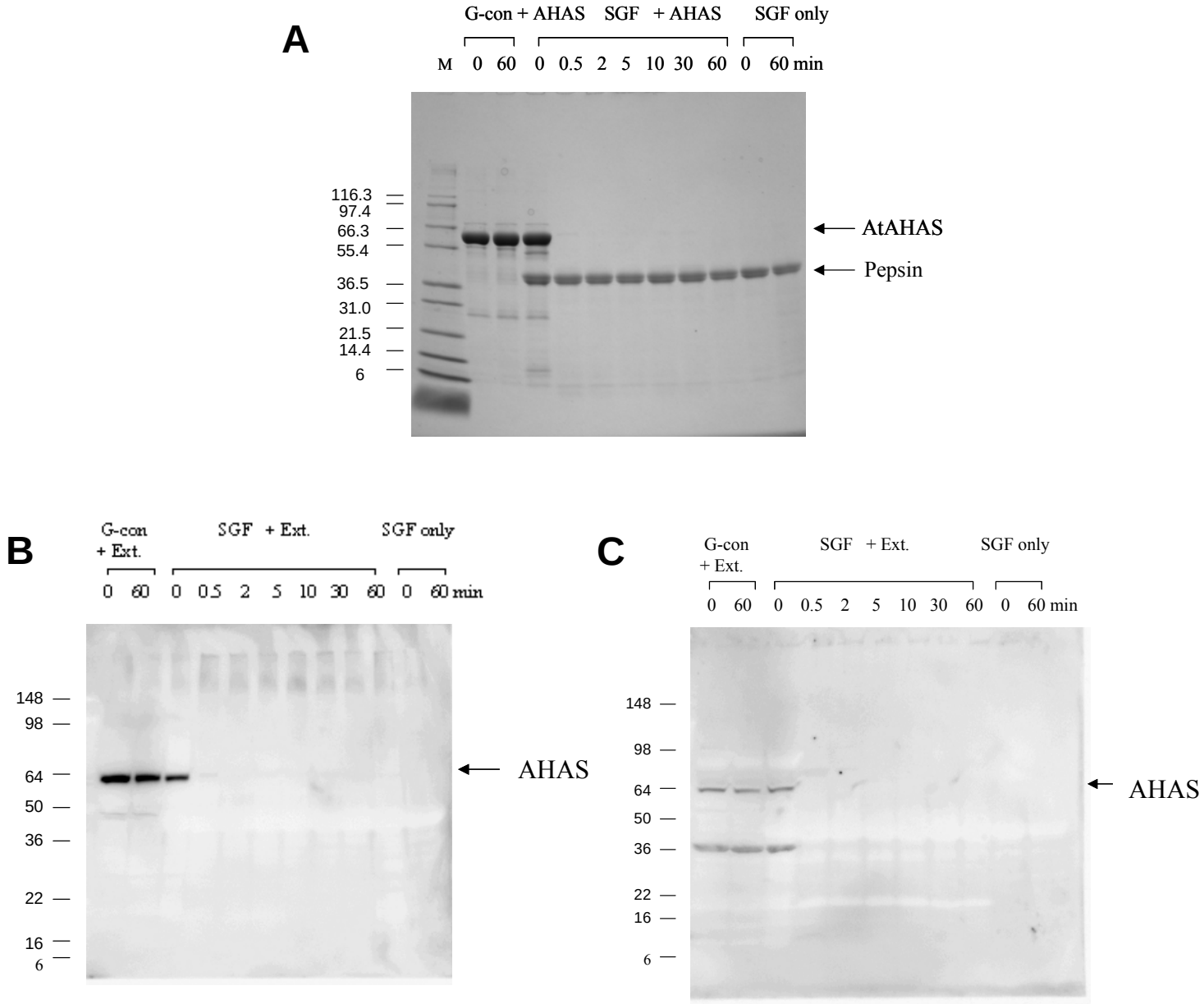


Figure H-2. Susceptibility of AtAHAS to Digestion by Simulated Mammalian Intestinal Fluid (SIF).

Incubation time at 37ºC is indicated in minutes. "SIF" is simulated mammalian intestinal fluid containing pancreatin; "I-con" is SIF without pancreatin. "SIF only" indicates no AtAHAS sample was included. **Panel A**. Coomassie blue stained 4-20% polyacrylamide gel containing digestion reactions carried out with the sample AtAHAS-0107. **Panel B**. Western blot analysis of digestion reactions carried out with leaf extract from CV127 soybean. **Panel C**. Western blot analysis of digestion reactions carried out with grain extract from CV127 soybean. AtAHAS is *ca.* 64,000 mol wt. and molecular weight (x 10^{-3}) markers are indicated.

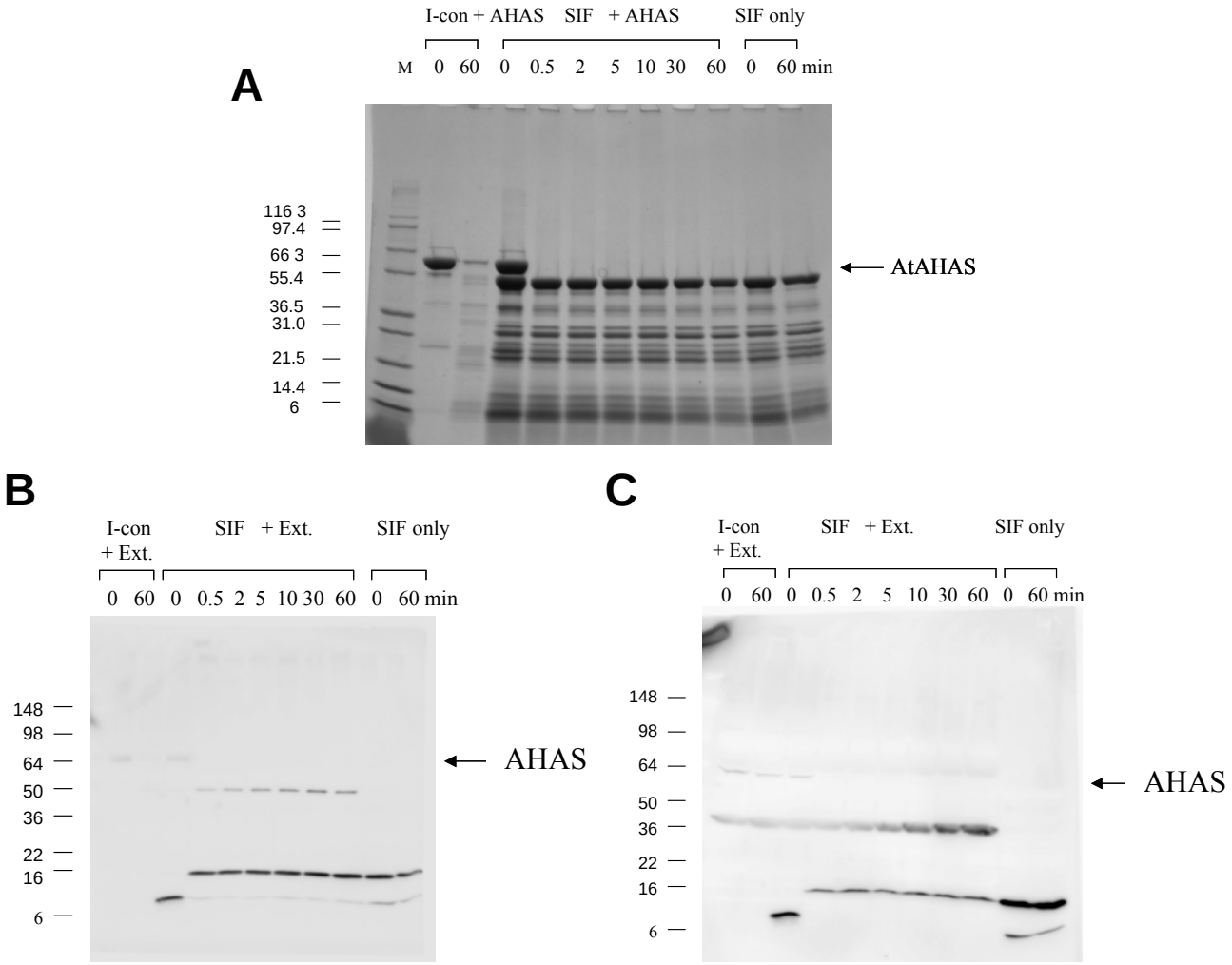
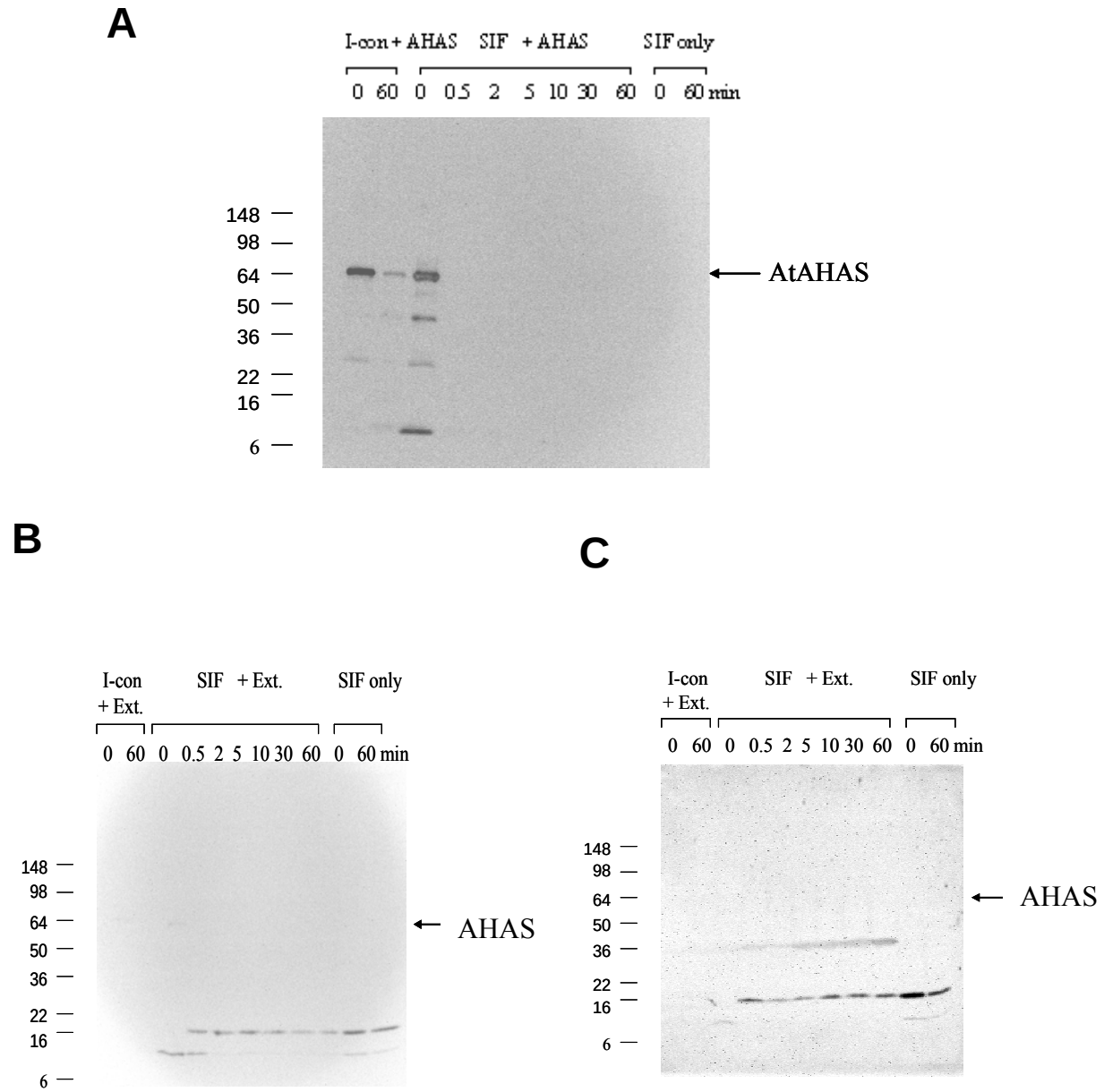


Figure H-3. Western Blot Analysis of Simulated Mammalian Intestinal Fluid (SIF) Reactions for AtAHAS-0107, Conventional Soybean Leaf and Conventional Soybean Grain Protein Extracts.

Incubation time at 37ºC is indicated in minutes. "SIF" is simulated mammalian intestinal fluid containing pancreatin; "I-con" is SIF without pancreatin. "SIF only" indicates no AtAHAS sample was included. **Panel A**. Western blot analysis using a 4-20% polyacrylamide gel containing the same digestion as in Figure H-2 panel A for AtAHAS-0107. **Panel B**. Western blot analysis of digestion reactions carried out with leaf extract from conventional soybean leaves. **Panel C**. Western blot analysis of digestion reactions carried out with grain extract from conventional soybeans. AtAHAS is *ca.* 64,000 mol wt. and molecular weight (x 10^{-3}) markers are indicated.



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January 13, 2009

Biotechnology Regulatory Services,
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Attn: Cynthia Eck,
Document Control Officer,
4700 River Road, Unit 147
Riverdale, MD 20737-1236

Re: Request for Determination of Regulatory Status

Dear Ms. Eck,

Please find enclosed an original and two hard copies, plus a copy on a compact disc of a petition for determination of the regulatory status of a soybean, *Glycine max* (L.) Merr., that has been genetically modified to be tolerant to imidazolinone herbicides and that is currently classified as a "regulated article". Based on the data and information presented in the enclosed petition, we believe that there is no longer reason to believe that this modified soybean plant should be deemed to be a regulated article. This modified soybean plant does not present any plant pest risks and it is not otherwise deleterious to the environment. The enclosed petition does not contain confidential business information.

The undersigned certifies that, to the best of his knowledge and belief, this petition includes all data, information, and views relevant to the matter, whether favorable or unfavorable to the position of the undersigned, which is the subject of this petition.

Yours Sincerely,

James M. Ligon, Ph.D.
Regulatory Affairs Manager
BASF Plant Science, L.L.C.