Monsanto Petition (11-202-01p) for Determination of Non-regulated Status of MON 87712 Soybean

OECD Unique Identifier: MON-87712-4

Plant Pest Risk Assessment

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A. Introduction

Monsanto Company has petitioned APHIS (APHIS # 11-202-01p) for a determination that genetically engineered (GE) soybean (*Glycine max* (L.) Merr.) event MON 87712 is unlikely to pose a plant pest risk (Monsanto 2011) and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000[1]. This plant pest risk assessment was conducted to determine if MON 87712 is unlikely to pose a plant pest risk.

MON 87712 utilizes the *BBX32* gene from *Arabidopsis thaliana* that results in production of a protein that interacts with one or more endogenous transcription factors to regulate the plant's day/night physiological processes. This results in increased availability of assimilates, an extended period of photosynthetic activity, changes in diurnal metabolism during the reproductive phase of the soybean plant, and significantly increased yield compared to control plants (Holtan 2011; Monsanto 2011).

Potential impacts to be addressed in this risk assessment are those that pertain to the use of MON 87712 and its progeny in the absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 87712 is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is not a plant pest, then APHIS has no regulatory authority over that organism.

Potential impacts in this plant pest risk assessment are those that pertain to plant pest risk characteristics. APHIS regulation 7 CFR 340.6(c) specifies the information needed for consideration in a petition for nonregulated status. APHIS evaluates information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impacts on the weediness of any other plant with which it can interbreed, potential changes to agricultural or cultivation practices, potential effects on non-target organisms, potential indirect plant pest effects on other agricultural products, and transfer of genetic information to organisms with which it cannot interbreed.

B. Development of MON 87712 Soybean

In the U.S. soybean is grown on over 78.9 million acres (Figure 1, next page) with a value of $31.7 billion in 2009 (USDA ERS 2010). Growers select soybean lines adapted to the different environmental and climatic features, weed and disease pressures, cost of

[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(14)) defines plant pest as:
"Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

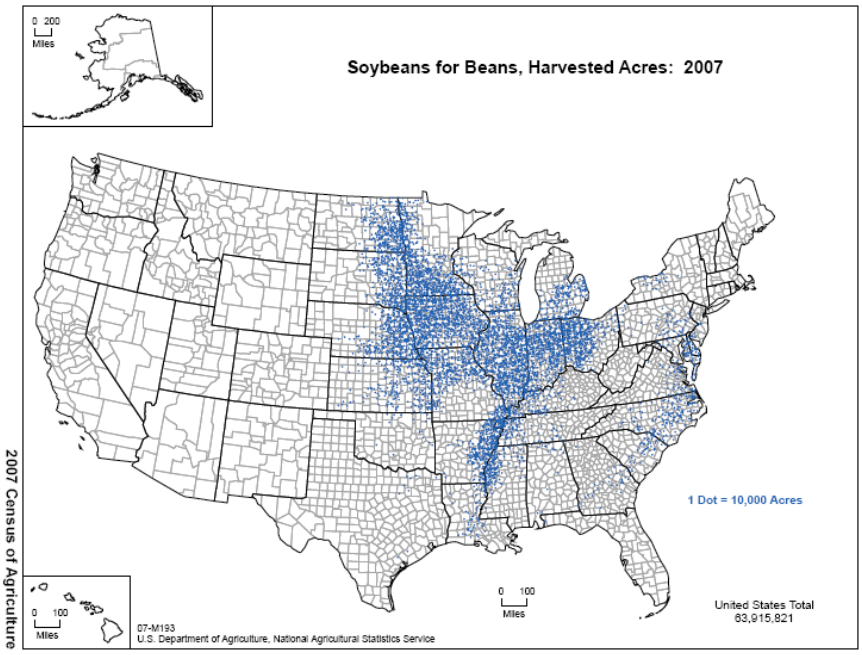

Figure 1. Soybean production areas in the U.S. (USDA NASS 2010b).

seed and other inputs, technology fees, human safety, ease and flexibility of the production system and marketing reasons (Gianessi 2006; Reddy 2001).

For the past ~28 years soybean yields in the U.S. have increased 65% at an annual rate of ~2.3% (Figure 2, next page). In order to meet the domestic and export demands, soybean productivity in the U.S. has been accomplished by both increasing the area under cultivation and by these constant yield increases per unit area. For example, from 1924 to 2010, soybean acreage increased almost 50-fold, but in the last decade the increase in planted area had been only 6% (USDA-NASS 2011). The annual improvement in U.S. soybean yield can be attributed to genetic and agronomic innovations and better control of pests and diseases that provide producers better tools to meet production demands (Specht et al. 1999), depending also on continuing infusions of genetic resources for yield stability and growth (USDA ERS 2011).

Monsanto has developed a high yielding variety MON 87712 through the insertion of the gene *BBX32* from *Arabidopsis thaliana* that altered the conventional soybean variety A3525 to modulate aspects of its diurnal biology to enhance growth and reproductive development. The *BBX32* gene is known to produce a protein that interacts with one or more endogenous transcription factors that regulate day/night processes resulting in increased availability of assimilates (Monsanto 2011, pp 200-224; Holtan et al. 2011). The increased yield results from higher canopy-level assimilate availability, as observed by factors associated with higher photosynthetic rate and altered diurnal plant metabolism. These diurnal processes involve carbon and nitrogen metabolism which increase the amount of assimilates that is available to potentially produce more and/or heavier seeds.

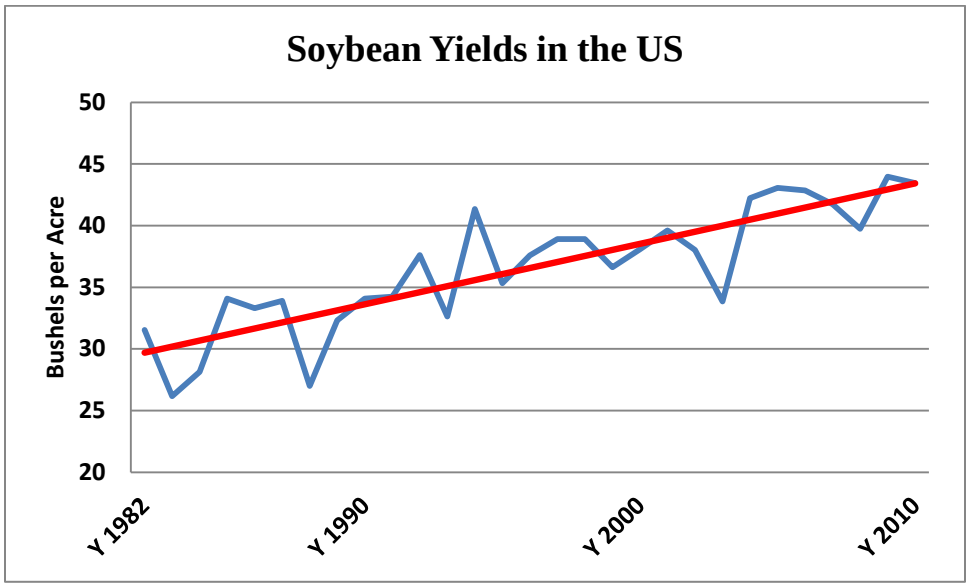

Figure 2. Soybean yield trend in the U.S. (USDA ERS 2011).

Monsanto has initiated a food/feed safety consultation on MON 87712 with the Food and Drug Administration (FDA) (Monsanto 2011, p. 29). A final decision from FDA is pending.

Description of the Modification

MON 87712 was developed through *Agrobacterium*-mediated transformation of soybean variety A3525 using the plasmid vector PV-GMAP5779 to inoculate soybean meristem tissue.

Plasmid Vector PV-GMAP5770 - (Monsanto 2011, p. 36):

- PV-GMAP5770 is approximately 11.4 kb and contains two T-DNA which are delineated by a Left and Right Border regions to facilitate this transformation. The first T-DNA (I) contains the *BBX32* coding sequence under the regulation of the *e35S* promoter and the *E6 3'* untranslated region. The second T-DNA (II) contains the *cp4 epsps* coding sequence regulated by the *FMV/EF-1α* promoter, *EF-1α* leader, *EF-1α* intron, *CTP2* targeting sequence, and the *E9 3'* untranslated region.

- The backbone region of PV-GMAP5779, located outside of both T-DNAs, contains two origins of replication for maintenance of the plasmid vector in bacteria (*ori V*, *ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer protein for maintenance of plasmid vector copy number in *E. coli* (*rop*).

The *BBX32* Coding Sequence and the BBX32 Protein (T-DNA I)

- The expression cassette in MON 87712 contains the coding region for BBX32 protein from *Arabidopsis thaliana* (Monsanto 2011, page 45). This protein regulates the plant's day and night processes resulting in an increased availability of assimilates.

- The second T-DNA (T-DNAII) contains the cp4 epsps coding sequence with its associated promoter, leader, intron, targeting sequence, 3 'UTR-functioning as a marker gene. Post-transformation breeding work removed this T-DNA from MON 87712 (Monsanto 2011, p. 7).

C. Expression of the Gene Product and Changes to Plant Metabolism

The BBX32 protein regulates the activity of specific transcription factors that allow plants to fine-tune a transcriptional response to specific endogenous or environmental inputs. This protein specifically modulates light signaling by acting antagonistically with another protein (ELONGATED HYPOCOTYL5 (HY5)), resulting in maintaining a dark-mediated pattern of gene expression and hypocotyl elongation.

D. Potential Impacts on Disease and Pest Susceptibilities

APHIS assessed whether MON 87712 is likely to have significantly altered disease and pest susceptibility. This assessment encompassed consideration of the introduced trait and disease and pest susceptibility data from MON 87712 field trials.

Soybean is not a plant pest in the United States (USDA 2011). The *Agrobacterium* transformed plants used in the generation of MON 87712 were treated with an antibiotic to kill the *Agrobacterium* cells. Additionally, DNA sequences derived from plant pests that were incorporated in MON 87712 do not result in the production of infectious agents or disease symptoms in plants, and so it is unlikely that MON 87712 could pose a plant pest risk. The genetic modifications, including genetic elements, expression of the gene products and their functions have been summarized above.

MON 87712 has been field tested in the United States since 2006. Agronomic data was collected in 2009 in 19 locations that represented a diverse range of environmental conditions where MON 87712 is expected to be grown. No statistically significant differences were observed for germination, emergence, seedling vigor, days to flower, plant height, lodging, pod shattering, grain moisture and weight (Monsanto 2011, p. 130), disease incidence and insect damage (Monsanto 2011, Appendix H). No qualitative or quantitative observations indicated any biologically meaningful differences from the comparator A3525 and the control lines or differences outside the range of conventional soybean norms.

Given the interactions between the environment, the genetic backgrounds of the cultivars used and some inherent genetic variability within soybean varieties, APHIS did not

identify any results presented by Monsanto that would constitute an increased plant pest risk. Expression of *BBX32* in event MON 87712 soybeans is not expected to cause plant disease or influence susceptibility of MON 87712 or its progeny to diseases or other pests. Therefore, pest and disease control methods are expected to be similar and no direct or indirect plant pest effect on raw or processed plant commodity is expected.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

MON 87712 was similar to its parental line A3525 in the interaction of these plants with the symbiotic nitrogen-fixing bacterium *Bradyrhizobium japonicum* (Monsanto 2011, p. 137) under laboratory conditions. The field cultivation of MON 87712 compared with A3525 did not have a detrimental effect on the abundance of beneficial arthropods such as lacewings, ladybird and carabid beetles, spiders, parasitic wasps, and beneficial Heteroptera (Monsanto 2011, pp. 382-384), therefore the cultivation of MON 87712 is not expected to have a negative effect on organisms that are directly related with soybean cultivation and that are beneficial to agriculture.

F. Potential for Enhanced Weediness of MON 87712 Soybean

APHIS assessed whether MON 87712 soybeans are any more likely to become a weed than the non-transgenic recipient soybean line, or other soybean currently cultivated. The assessment encompasses consideration of the basic biology of soybean and an evaluation of unique characteristics of MON 87712 soybean.

In the U.S., soybean is not listed as a weed in the major weed references (Crockett 1977; Holm 1979; Muenscher 1980) nor is it listed as a noxious weed species by the federal government (USDA 2011). Soybeans are not frost tolerant, do not survive freezing winter conditions (OECD 2000), and do not reproduce vegetatively. After crop harvest, soybean may germinate as a volunteer in the succeeding crop due to lack of dormancy (Padgette 1996). If any seeds remain viable and germinate the following year, mechanical methods or herbicides can be used to control volunteers. Based on the familiarity of soybean as the parent plant, there has been no report of soybean escaping cultivation and becoming established as a weed in United States (Holm 1979; Monsanto 2011). In managed ecosystems, soybean does not effectively compete with other cultivated plants or primary colonizers (OECD 2000).

Weediness for the purposes of this part of the plant pest risk assessment is an attribute which causes a crop to act as a weed due to the addition of genes when compared to the non-GE comparator. If the fitness of MON 87712 soybean improves in natural or agricultural ecosystems due to the inserted DNA, the potential for weediness could increase. The following analysis of the inserted DNA is intended to document that MON 87712 soybeans have a negligible likelihood of increased weediness.

In 2009 Monsanto conducted field trials to evaluate phenotypic characteristics comparing MON 87712 with the non-transgenic variety A3525 lacking the *BBX32* gene. Agronomic performance characteristics, including germination, dormancy, emergence, vegetative growth, reproductive development, seed retention and lodging, plant-environment

interactions, plant-symbiont interactions, volunteer potential characteristics, and persistence outside of cultivation characteristics were evaluated (Monsanto 2011, pp. 117-141, appendix G). There were no significant differences among all the parameters listed above between MON 87712 and A3525, except that MON 87712 had a ~1.6% higher earlier plant stand count, reached 50% senescence 2 days later and physiological maturity 2.5 days later, had a ~3.3% higher final plant stand and a 6.8% higher yield than A3525 (Monsanto 2011, p. 128, appendix G). Based on analysis of data on all these parameters, soybean MON 87712 is unlikely to pose any more of a plant pest risk from weediness than the conventional soybean from which it was derived.

APHIS also assessed whether MON 87712 is any more likely to become a weed than its parental comparator line A3525 or other soybean varieties currently under cultivation. The assessment encompasses consideration of the basic biology of soybean and an evaluation of the unique characteristics of MON 87712 under field conditions. To increase weediness of the soybean plant there would have to be selection pressure on the line (Tiedge 1989). Because the *BBX32* gene will not affect the survival of MON 87712 and because soybean is not itself weedy, this type of selection pressure does not now and is unlikely ever to exist.

Results on growth characteristics, seed production and germination indicate that MON 87712 is not significantly different from its comparators (Monsanto 2011, pp. 117-141, appendix G). There is no indication that MON 87712 possesses a selective advantage that would result in increased weediness. MON 87712 lacks the ability to persist as a troublesome weed and it is highly unlikely that typical weed management practices for soybeans will change significantly.

G. Potential Impacts on the Weediness of Any Other Plants with which It Can Interbreed

Soybean is highly self-pollinating (Ahrent 1994). When soybean plants are grown directly adjacent to other soybean plants, the amount of natural cross pollination has generally been found to be 0.5 - 1 percent (Fehr 1980; OECD 2000; Ray 2003) although higher values (2.5 percent) occur in some varieties (Abud 2007). Outcrossing can be reduced to 0 – 0.01 percent with a separation distance of 10 meters (Abud 2007). At greater distances from the pollen source, cross pollination rates decrease rapidly. Based upon these factors, it is highly unlikely for MON 87712 to naturally outcross or hybridize with other soybean varieties in agricultural settings.

In assessing the risk of gene introgression form MON 87712 soybeans into its sexually compatible relatives, APHIS considered two primary issues: 1) the potential for gene flow and introgression and, 2) the potential impact of introgression.

Glycine has approximately 9 species, with *G. max* being placed in the subgenus *Soja* along with one other species, *G. soja* (previously *G. ussuriensis*). *Glycine max* is only sexually compatible with *G. soja* and no other *Glycine* species. *Glycine max* is the only species located in the United States other than a few *G. soja* plants in research plots. *Glycine max* has never been found in the wild in the U.S. (Hymowitz 1987). Therefore, it

is not likely that gene flow and introgression will occur between MON 87712 and other species of soybean. APHIS has determined that any adverse consequences of gene flow from MON 87712 soybean to wild or weedy species in the United States are highly unlikely.

H. Potential Changes to Agriculture or Cultivation Practices

MON 87712 soybeans were field-studied in a wide range of environmental conditions in 2009. In 19 different sites that represent most of the soybean growing environments, MON 87712 was cultivated and compared with its parental line (A3525) and another 18 commercial varieties under the same agronomic practices (Monsanto 2011, pp. 346-347). Most of the agronomic performance characteristics, including germination, dormancy, emergence, vegetative growth, reproductive development, seed retention and lodging, plant-environment interactions, plant-symbiont interactions, volunteer potential characteristics, and persistence outside of cultivation characteristics (Monsanto 2011, Appendix G), demonstrated that there were no significant differences when MON 87112 was grown in the field compared with commercial varieties. However, MON 87712 had some characteristics that were different than control and/or commercial varieties: a ~1.6% higher earlier plant stand count, reached 50% senescence 2 days later and physiological maturity 2.5 days later, had a ~3.3% higher final plant stand and a 6.8% higher yield than A3525 (Monsanto 2011, p. 128, Appendix G). None of these characteristics would likely require MON 87712 to be cultivated using different agronomic practices than other soybeans.

MON 87712 was not more susceptible to a wide-range of soybean diseases than its parental line A3525 (Monsanto 2011, p. 375), nor more susceptible to pest arthropods (Monsanto 2011, pp. 376-381), therefore it is not expected that cultivation of MON 87712 would require different amounts or types of pesticides for its cultivation than other soybean varieties.

I. Potential Impacts from Transfer of Genetic Information to Organism with which MON 87712 Soybeans Cannot Interbreed

Horizontal gene transfer and expression of DNA from a plant species to bacteria is unlikely to occur (Keese 2008). First, many bacteria (or parts thereof) that are closely associated with plants have been sequenced, including *Agrobacterium* and *Rhizobium* (Kaneko 2000; Kaneko 2002; Wood 2001). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Brown 2003; Koonin 2001). Third, the FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans, animals, or in the environment is remote (FDA 1998). Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant plant pest risk.

J. Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if event MON 87712 is likely to pose a plant pest risk. Based on the information provided by the applicant and the lack of plant pest risk from the inserted genetic material, weedy characteristics, atypical responses to disease or plant pests in the field, effects on non-targets or beneficial organisms in the agro-ecosystem, changes to agricultural practices, and horizontal gene transfer, APHIS has concluded that soybean event MON 87712 is unlikely to pose a plant pest risk.

K. References

Abud, S., de Souza, P.I.M., Vianna, G.R., Leonardecz, E., Moreira, C.T., Faleiro, F.G., Júnior, J.N., Monteiro, P.M.F.O., Rech, E.L., Aragão F.J.L. Gene flow from transgenic to nontransgenic soybean plants in the Cerrado region of Brazil. Genetics and Molecular Research 6:445-452.

Ahrent, D.K., Caviness, C.E. (1994) Natural cross-pollination of twelve cultivars in Arkansas. Crop Sci. 34:376-378.

Brown, J.R. (2003) Ancient horizontal gene transfer. Nat. Rev. Genet. 4:121-132.

Crockett, L. (1977) Wildly Successful Plants: North American Weeds. University of Hawaii Press, Honolulu, Hawaii. 609 pp.

FDA (1998) Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants. pp. 28.

Fehr, W.R.; Hadley, H.H. (1980) Hybridization of Crop Plants. American Society of Agronomy and Crop Science Society of America, Madison, Wisconsin. (p. 592).

Gianessi, L., Reigner, N. (2006) Pesticide use in U.S. crop protection: 2002 With Comparison to 1992 & 1997. Fungicides & Herbicides. Crop Life Foundation, Washington, D.C.

Holm, L., Pancho, J. V., Herbarger, J. P., Plucknett, D. L. (1979) A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Holtan, H. E. *et al* (2011) BBX32, an Arabidopsis B-Box protein, functions in light signaling by suppressing HY5-regulated gene expression and interacting with STH2/BBX21. Plant Physiology 156: 2109-2123.

Hymowitz, T., Singh, R.J. (1987) “Taxonomy and Speciation” in Soybeans: Improvement, Production, and Uses - Second Edition. American Society of Agronomy.

Kaneko T. *et al.* (2000) Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. DNA Research 7:331-338.

Kaneko T. *et al.* (2002) Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Research 9:189-197.

Keese, P. (2008) Risks from GMOs due to horizontal gene transfer. Environmental Biosafety Research 7:123-149.

Koonin, E.V., Makarova, K.S., Aravind, L. (2001) Horizontal gene transfer in prokaryotes: quantification and classification. Annual Review of Microbiology 55:709-742.

Monsanto (2011) Petition for the determination of nonregulated status for MON 87712 soybean. USDA APHIS petition number 11-202-01p.

Muenscher, W. C. (1980) Weeds. Second Edition. Cornell University Press, New York and London. (p. 545).

OECD (Organization for Economic Co-operation and Development Consensus) (2000) Document on the Biology of *Glycine max* (L.) Merr. (Soybean).

Padgette, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M., Fraley, R.T. (1996) New weed control opportunities: development of soybeans with a Roundup Ready gene. Pp53-84. In S.O. Duke (ed.). Herbicide Resistant Crops-Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. CRC Press, Boca Raton, Florida.

Ray, J. D., Kilen, T. C., Abel, C. A., and Paris, R. L. 2003. Soybean natural cross pollination rates under field conditions. Environmental Biosafety Research 2: 133-138.

Reddy, K.N. (2001) Glyphosate-resistant soybean as a weed management tool: opportunities and challenges. Weed Biol. Manage. 1:193-202.

Specht, J.E., Hume, D. J., and Kumudini, S. V.. 1999. Soybean yield potential - A genetic and physiological perspective. Crop Science 39: 1560-1570.

Tiedje, J.M., Colwell, R.K., Grossman, Y.L., Hodson, R.E., Lenski, R.E., Mack, R.N., Regal, P.J. (1989) The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70:298-315.

USDA (2011) Invasive and noxious weeds. United States Department of Agriculture. Accessed December 01, 2011 at: http://plants.usda.gov/java/invasiveOne?startChar=B

USDA ERS (2010) Adoption of Genetically Engineered Crops in the U.S.: Soybeans Varieties. Accessed May 5, 2010 at: http://www.ers.usda.gov/Data/BiotechCrops/ExtentofAdoptionTable3.htm.

USDA NASS (2011) Quick Stats. Accessed December 01, 2011 at: http://www.nass.usda.gov/index.asp.

Wood, D.W. *et al.* (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science 294:2317-2323.