Traits for Insect Control with Transgenic *Bt* Corn: What, Why, and How . . . Now and in the Future

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The impact of transgenic *Bt* corn hybrids on insect populations and their control has been dramatic, transforming the way we think about and implement insect management strategies. Some of the first *Bt* corn hybrids, commercialized in the mid-1990s, were extremely effective against corn borers (both European and southwestern), giving corn growers a glimpse of the future potential for transgenic technology in agriculture. However, the commercialization of *Bt* corn hybrids also introduced the somewhat complex concept of insect resistance management (IRM) with non-*Bt* corn refuges, placing further emphasis on management in contrast to in-the-moment decision making.

Early in this decade, the commercialization of transgenic *Bt* corn hybrids for control of corn rootworms generated even more interest and excitement among corn growers who enthusiastically adopted the technology to control the most important insect pests of corn in North America. In due course, transgenic traits for control of both corn borers and corn rootworms were "stacked" in elite corn hybrids with traits for herbicide tolerance, resulting in double-, triple-, and quad-stacked hybrids. Concurrently, "new and improved" versions of transgenic *Bt* corn hybrids were introduced. And on the near horizon are the anticipated products Optimum® AcreMax™1 Insect Protection (so-called "refuge in a bag" from Pioneer) and SmartStax™ (cross-licensed by Monsanto and Dow AgroSciences), an eight-gene stacked combination in corn that will express more than one protein for control of the same target insect, as well as provide herbicide tolerance.

All of these past and proposed future transgenic corn developments promise a dizzying array of choices for corn growers. It is likely that the IRM guidelines and requirements will change, too, creating even more uncertainty about implementation and compliance. The thrust of the discussion will be a review of the currently available and projected traits and their combinations for insect management in corn hybrids and how integrated pest management (IPM) and IRM will be influenced by their widespread use. Results from applied research efforts will supplement the discussion. Where appropriate, case studies will be included to help elucidate some of the complexities and potential drawbacks of using transgenic technology for insect management.

"Stacks"

Corn hybrids with multiple transgenic traits are where the action is now. "Stacks" have caught on rapidly in the United States and even more rapidly over the past two years in the Midwest. According to the USDA's Economic Research Service, the percentages of all corn acres planted to stacked gene hybrids in Illinois and Indiana have increased from less than 20% planted to all genetically engineered hybrids in Illinois, Indiana, and the United States (U.S.) was nearly or at 80% in 2008 (Figure 2). In a display of confidence in this technology, on August 14, 2008, the USDA's (Risk Management Agency) Federal Crop Insurance Corporation Board of Directors approved reductions in insurance premium rates for a wide spectrum of transgenic *Bt* hybrids. In 2009, producers who receive rate reductions in insurance premiums will be required to plant at least 75% of their insured acres with transgenic hybrids that qualify according to specific guidelines. The effectiveness of these products with their high-yield expectations has convinced most people associated with corn production that host plant resistance in the form of transgenic *Bt* corn hybrids is just what we need for insect management.

With such a great demand for transgenic *Bt* corn hybrids, it's not surprising that competition among the major seed companies is fierce, resulting in an ever-increasing number of choices for corn growers. The information in Table 1 lists the types of transgenic *Bt* corn products currently available, as well as a couple of types of products that are expected to be registered for widespread commercial use in the near future. As one would expect, there are similarities and differences among these products, some of which we will elaborate during the discussion.

New Industry Initiatives

In 2008, Pioneer Hi-Bred International, Inc., introduced a new product/program called Optimum® AcreMax™ 1 Insect Protection, which offers corn growers a convenient way to deploy a refuge for corn rootworms — the "refuge in a bag." However, Optimum AcreMax 1 is not yet available for sale or use, and is still subject to regulatory approval. According to Technical Bulletin 08-1743, 08-2020 (page 2): "Pending Environmental Protection Agency (EPA) approval, Optimum AcreMax 1 products would feature a combination of two versions of a hybrid in a single bag. Each bag would contain not more than 98% of a Pioneer® brand hybrid with Herculex XTRA (CRW/CB/LL) insect protection — a combination of the Herculex RW and Herculex I (CB/LL) traits. Each bag also would contain no less than 2% of a hybrid with the Herculex I trait that will satisfy the corn rootworm refuge requirement for the field."

Pioneer scientists maintain that unique antifeedant properties of Herculex RW hybrids justify this refuge-in-a-bag approach to resistance management (page 3): "The antifeedant mechanism prevents corn rootworm larvae from developing to larger, more destructive stages. Larvae languish on Herculex RW roots. Ultimately, most die from the many naturally occurring mortality factors that suppress corn rootworm populations in the field. These natural factors — starvation, predators and disease — work in combination with the Herculex RW trait to provide exceptional crop protection. The few that do survive Herculex RW contribute to susceptible beetles produced in the refuge."

One of the key premises for Pioneer's request for a reduction in the amount of refuge corn is the assumption that selection pressure is less intense due to the antixenosis (non-feeding preference, indirect lethality) feature of Herculex RW hybrids, as opposed to the selection pressure resulting from more direct, lethal (antibiosis) responses of insects feeding on *Bt*-expressing tissue (e.g., European corn borers feeding on leaves of Herculex I). In an article in the *Journal of Applied Entomology* (vol. 132, pages 189–204), scientists with Pioneer (Lefko et al. 2008) concluded that "putative or major resistance to 59122 [authors: event number of Herculex RW Cry34/35Ab1] is rare in U.S. populations of WCR." They explained further that the low level of survival of western corn rootworm adults on Herculex RW corn was partially explained by a tolerance trait with complex inheritance, a trait they consider minor as it relates to efficacy of Herculex RW corn. The article is accessible, for a fee, on the Internet at http://www3.interscience.wiley.com/cgi-bin/fulltext/119408370/PDFSTART.

Although we are convinced that producers will be supportive of the convenience and increased profitability associated with a reduced refuge (2 to 5%) that could be poured out of a bag into a planter and planted, we are uncertain about a refuge reduction of this proposed magnitude. We wonder whether selection pressure over time would result in the evolution of a resistant western corn rootworm strain that is less discriminating in selecting roots (*Bt* or non-*Bt*). In time, a non-discriminating corn rootworm strain might be able to feed and survive on Herculex RW roots and develop into a breeding population. Would the reduction of the current refuge requirement of 20% to a smaller percentage (2 to 5%) of non-transgenic plants hasten this potential development? In an article published in the *Journal of Economic Entomology* (vol. 99, pages 1407–1414), Onstad (2006) cautioned industry about proposing small fractions of nontransgenic seeds for refuge, based on the assumptions of his modeling effort. Further scientific scrutiny and debate are warranted.

In 2010, Monsanto Company and Dow AgroSciences LLC, via a cross licensing agreement, hope to commercialize (pending US EPA approval) SmartStax™ corn hybrids that express multiple Cry proteins for insect control — Cry3Bb1 and Cry34/35Ab1 for rootworms; Cry1A.105, Cry2Ab2, and Cry1F for Lepidoptera). In addition, SmartStax hybrids will have traits that provide tolerance to two herbicides, glyphosate and glufosinate. This significant technological achievement promises to provide corn growers with an excellent tool for wide-spectrum insect control. A significant reduction in required refuge (maybe to as low as 5%) for insect resistance management is anticipated.

SmartStax hybrids will introduce the concept of "gene pyramiding" to corn growers, a concept already familiar to cotton growers who have planted Monsanto's Bollgard II with two genes derived from *Bt*. The concept is discussed more thoroughly as part of the following discussion about insect resistance management.

Insect Resistance Management (IRM)

Currently, corn growers who plant hybrids with *Bt* traits must implement insect resistance management (IRM) strategies. Key among the IRM strategies is deployment of structured refuges of corn without *Bt* toxins for the target insect. The refuge is intended to ensure an ample population of *Bt*-susceptible insects to mate with *Bt*-resistant insects (presumed to be rare) that might emerge from *Bt* corn. Currently, a corn grower who plants *Bt* corn must plant at least 20% of his or her acres to refuge hybrids that do not include the *Bt* trait(s) for the target insect(s). Compliance with IRM strategies is required by the US EPA and seed companies selling *Bt* corn and is strongly endorsed by the National Corn Growers Association.

To date, there has been no documented occurrence of populations of European corn borers and corn rootworms that have developed resistance to *Bt* in the field. We believe that IRM strategies, most notably non-*Bt* corn refuges, have helped greatly in this regard. However, the escalating use of *Bt* corn hybrids will place increasingly more selection pressure on the target insects. A case study of populations of European corn borers over time clearly demonstrates the level of selection pressure being placed on this species by *Bt* corn. In Illinois in 2008, the density of European corn borers determined from our annual fall survey was 0.093 larva per plant, the lowest density recorded since the survey was begun in 1943. Furthermore, 81% of the 504 cornfields surveyed had no evidence of corn borer infestations, and no corn borer larvae were found in 86% of the fields. Such a dramatic reduction in European corn borer populations attributable to *Bt* corn implies that failure to deploy refuges could accelerate the onset of resistance to *Bt* in populations of corn borers, decreasing the length of time that *Bt* corn is expected to be effective.

It's important to note a current debate about the alleged discovery of the first case of field-evolved resistance to a *Bt* toxin produced by a transgenic crop. In an article published in *Nature Biotechnology* (vol. 26, February 2008, pages 199–202), a team of scientists from the University of Arizona (Tabashnik et al.) published evidence that some bollworms (*Helicoverpa zea*, known to us as corn earworms) have become resistant to *Bt* toxin Cry1Ac expressed in *Bt* cotton in fields in Arkansas and Mississippi. Comparisons of bollworm populations collected in 1992 and 1993 (before *Bt* cotton) with bollworm populations collected from 2002 through 2004 suggested that susceptibility to Cry1Ac had declined. The authors claimed that bollworm resistance to Cry1Ac expressed in *Bt* cotton resulted in part due to resistance not being recessive, a major assumption of the high-dose refuge resistance strategy. In effect, the Cry1Ac protein is not being expressed in a dose sufficiently high within *Bt* cotton plants to kill the heterozygous progeny of susceptible and resistant bollworms.

The authors also emphasized that the resistance of *H. zea* to *Bt* toxin Cry1Ac in transgenic cotton has not caused widespread crop failures.

However, not all scientists agree with the conclusions reached by Tabashnik et al. (2008). In correspondence published in *Nature Biotechnology* (vol. 26, October 2008, pages 1072–1074), another team of scientists (Moar et al.) “emphatically” disagreed with the interpretations of Tabashnik et al. (2008) concerning the evolution of field-level resistance in bollworms to the Cry1Ac protein expressed in *Bt* cotton. They criticized Tabashnik et al. (2008) for their over reliance on laboratory-based data and argued that resistance confirmation depends on the evaluation of larval survival (putative resistant and susceptible larvae) on *Bt* plants in the field. They also pointed out that no change in the efficacy of *Bt* cotton to bollworms has occurred within the past decade. In their correspondence, Moar et al. (2008) claimed that the first case of field-evolved resistance occurred in fall armyworms found in Puerto Rico *Bt* cornfields expressing the Cry1F protein. This incident was reported by A. Reynolds at the annual meeting of the Entomological Society of America in 2007. Failures of *Bt* corn to prevent fall armyworm injury were documented, and the use of *Bt* corn expressing the Cry1F protein was discontinued in Puerto Rico.

In correspondence published immediately following the Moar et al. correspondence in *Nature Biotechnology* (vo. 26, October 2008, pages 1074–1076), Tabashnik et al., reiterated their conclusions regarding the field-development of resistance of bollworms to Cry1Ac and their contention that their findings represented the first example of such an occurrence. The papers comprising this scientific debate are accessible via the Internet at http://www.nature.com/nbt/index.html.

Gene pyramiding for insect resistance management

Gene pyramiding in transgenic *Bt* crops can be defined as the simultaneous expression in the same plant of two or more different *Bt* traits targeting the same insect. Theoretically, gene pyramiding would significantly delay the onset of development of insect populations resistant to the different *Bt* traits. Scientists have indicated that the amount of refuge necessary to delay resistance for an extended period can be reduced with pyramiding in the same hybrids. The first pyramided transgenic *Bt* crop registered for use in the U.S. was Bollgard II in 2002, with two genes derived from *Bt* — Cry1Ac and Cry2Ab2. These pyramided cotton plants have been very effective (due in part to different binding sites of the Cry proteins in the insect midgut) against pink bollworms (*Pectinophora gossypiella*), including laboratory strains resistant to the Cry1Ac protein.

Research published in *Nature Biotechnology* (vol. 21, December 2003, pages 1494–1497) by Zhao et al., revealed that gene pyramiding (Cry1Ac and Cry1C) in transgenic *Bt* broccoli had the potential to delay resistance to diamondback moth (*Plutella xylostella*) more effectively than single-toxin plants. They stated, “Our experiments showed that allowing the concurrent release of cultivars with the two *Bt* genes in separate plants, each with one *Bt* gene, is not the best way to delay resistance. Even sequential release would result in control failure of at least one cultivar sooner than if pyramided varieties were used.” Key to the long-term durability of pyramided *Bt* plants is the absence of cross resistance to Cry proteins expressed within transgenic plants.

Cross resistance is a well documented phenomenon for several classes of insecticides, enabling resistant insect species to survive when exposed to related compounds. A review of the gene pyramiding and *Bt* resistance management literature by Manyangarirwa et al., in the *African Journal of Biotechnology* (vol. 5, May 2006, pages 781–785) indicated that the success of pyramided *Bt* plants for preventing or delaying resistance is based upon three fundamental assumptions:

- “ Insects resistant to only one toxin can be effectively controlled by a second toxin produced in the same plant.”
- “Strains resistant to two toxins with independent actions cannot emerge through selection pressure with one toxin alone.”
- “A single gene will not confer resistance to two toxins that are immunologically distinct and that have different binding targets.”

Obviously, if these assumptions are not met, cross resistance to more than one *Bt* gene may occur. The article is accessible on the Internet at http://www.academicjournals.org/AJB/PDF/pdf2006/16May/Manyangarirwa%20et%20al.pdf.

A review article published in *Trends in Biotechnology* (vol. 26, October 2008, pages 573–579) by Bravo and Soberón provides an excellent overview of the mode of action of *Bt* Cry toxins and the mechanisms of resistance to *Bt* Cry toxins in insects selected for resistance. The mode of action of *Bt* Cry toxins is primarily pore formation that occurs after several biochemical steps after larvae have ingested the toxins. Pore formation causes the insect’s midgut cells to burst, resulting in death of the larva. Laboratory colonies of several insects have been selected for resistance to *Bt* Cry toxins, and several mechanisms of resistance have been identified. However, the most commonly observed mechanism of resistance to *Bt* Cry toxins has involved mutations in toxin receptors, which reduces the binding of the toxin to receptors in the midgut cells.

Bravo and Soberón (2008) discussed the concept of gene pyramiding, including reference to SmartStax, and suggest, as others have, that pyramided *Bt* crops should not be introduced while other *Bt* crops expressing only a single *Bt* Cry toxin are grown simultaneously. They also cited studies that have demonstrated cross resistance and that resistance to different Cry toxins that bind to different receptors is possible. The authors offered suggestions for strategies to cope with insect resistance. The article is accessible, for a fee, on the Internet at http://www.sciencedirect.com/science/journal/01677799.

Concluding Remarks

Planting transgenic *Bt* corn has become the foundation of pest management strategies for some of our most economically important insect pests of corn. However, failure to implement and integrate IRM and IPM strategies will likely trigger negative economic and ecological consequences. Unrealistic expectations for *Bt* corn also may trigger knee-jerk reactions that would threaten the durability of the current technologies. (Some case studies from Illinois will emphasize the expected efficacy of current and future *Bt* corn products.) Pyramiding two or more *Bt* genes within plants for control of the same target insect offers enormous potential for delaying development of insect populations resistant to *Bt*. But we should not assume that we can abandon other important resistance management strategies such as the use of refuges, albeit reduced in size. Instead, integration of IRM tactics (pyramided plants deployed with a mosaic of refuges and plants expressing a diversity of Cry proteins) is warranted, as is the implementation of sound IPM strategies (e.g., use of scouting and economic thresholds to influence the temporal and spatial use of *Bt* hybrids). Adherence to integration of IRM and IPM strategies will give us the best bang for our buck in the long run.

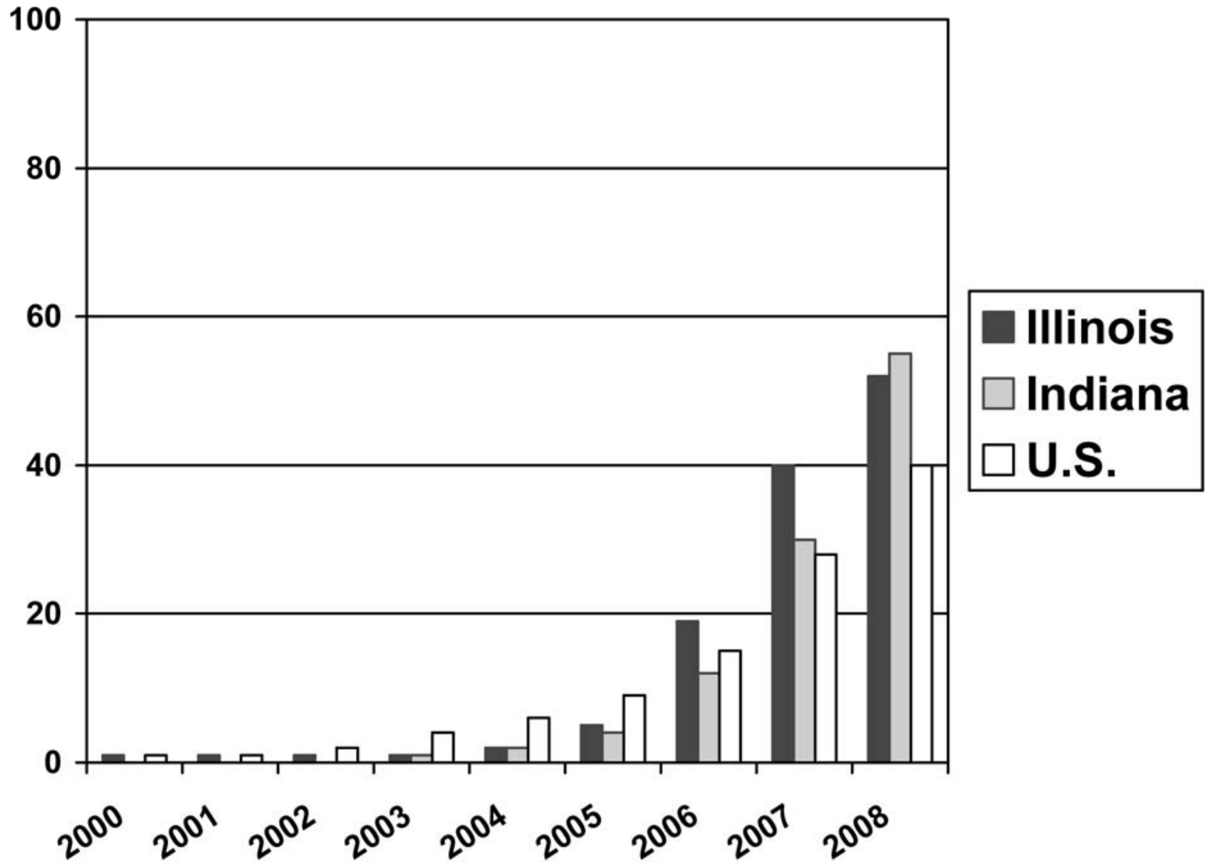

Figure 1. Percentage of all corn acres planted to stacked gene varieties. Source: United States Department of Agriculture Economic Research Service. Data Sets — Adoption of Genetically Engineered Crops in the U.S. http://www.ers.usda.gov/Data/BiotechCrops/.

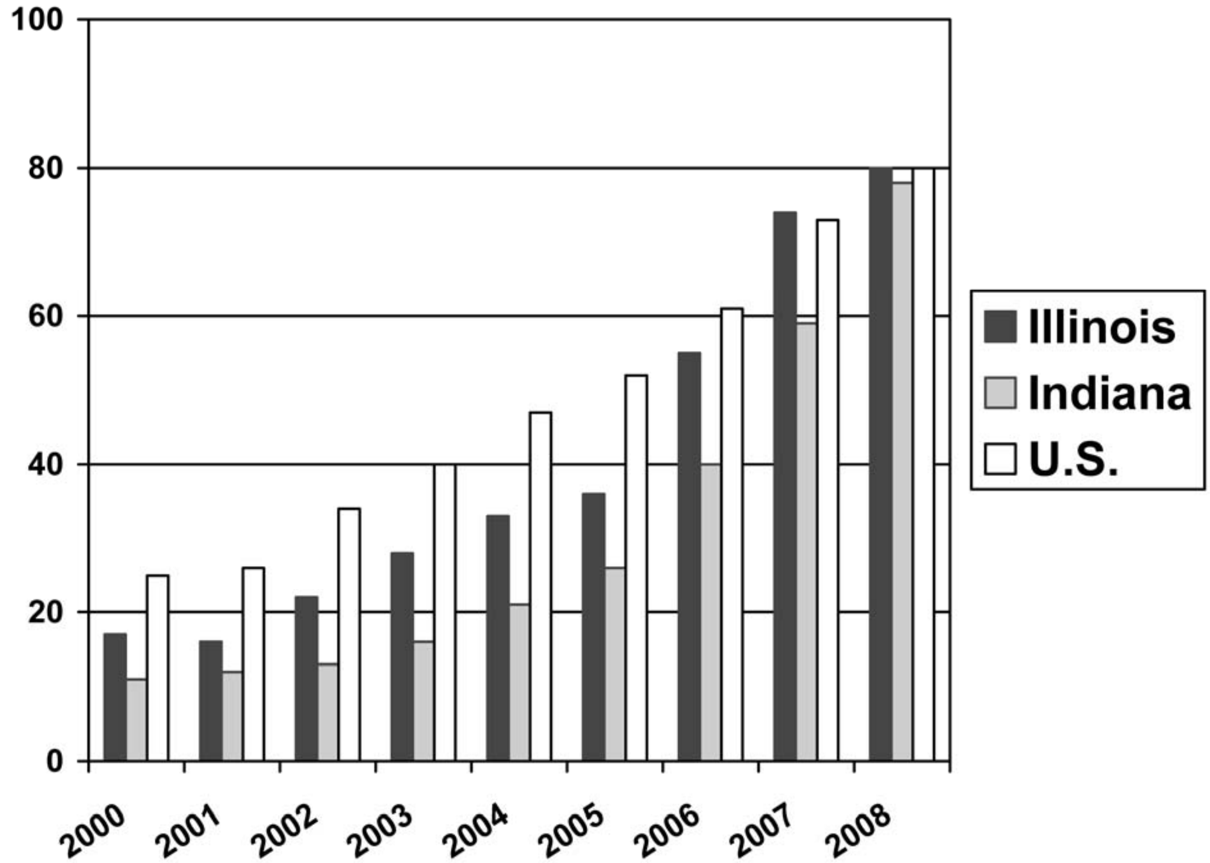

Figure 2. Percentage of all corn acres planted to all GE (genetically engineered) varieties. Source: United States Department of Agriculture Economic Research Service. Data Sets — Adoption of Genetically Engineered Crops in the U.S. http://www.ers.usda.gov/Data/BiotechCrops/.

Table 1. ***Bt*** **corn traits (events and Cry [crystalline] proteins), registrants, product trade names, and target organisms.**

Event	Cry protein(s)	Registrant(s)	Product tradenames[1]	Target organisms[1,2]
Bt-11	Cry1Ab	Syngenta Seeds, Inc.	Agrisure CB	Lepidoptera complex
MIR604	mCry3A	Syngenta Seeds, Inc.	Agrisure RW	Corn rootworm
Bt-11 + MIR604 (stack)	Cry1Ab + mCry3A	Syngenta Seeds, Inc.	Agrisure CB/RW	Lepidoptera complex + corn rootworm
TC 1507	Cry1F	Mycogen Seeds/ Dow AgroSciences LLC, and Pioneer Hi-Bred Intl. Inc./ DuPont	Herculex I	Lepidoptera complex
DAS-59122-7	Cry34/35Ab1	Pioneer Hi-Bred Intl. Inc./Dupont, and Mycogen Seeds/ Dow AgroSciences	Herculex RW	Corn rootworm
DAS-59122-7 + TC 1507 (stack)	Cry34/35Ab1 + Cry1F	Pioneer Hi-Bred Intl. Inc./Dupont, and Mycogen Seeds/Dow AgroSciences	Herculex XTRA	Lepidoptera complex + corn rootworm
MON810	Cry1Ab	Monsanto Co.	YieldGard Corn Borer	Lepidoptera complex
MON863	Cry3Bb1	Monsanto Co.	YieldGard Rootworm	Corn rootworm
MON810 + MON863 (stack)	Cry1Ab + Cry3Bb1	Monsanto Co.	YieldGard Plus	Lepidoptera complex + corn rootworm
MON88017	Cry3Bb1	Monsanto Co.	YieldGard VT Rootworm	Corn rootworm
MON88017 + MON810 (stack)	Cry3Bb1 + Cry1Ab	Monsanto Co.	YieldGard VT Triple	Lepidoptera complex + corn rootworm

Table 1. (continued)

Event	**Cry protein(s)**	**Registrant(s)**	**Product trade names**[1]	**Target organisms**[1,2]
MON88017 + MON89034 (stack)	Cry3Bb1 Cry1A.105 + Cry2Ab2	Monsanto Co.	YieldGard VT Triple Pro (limited commercialization in U.S. anticipated for 2009)	Lepidoptera complex + corn rootworm
MON88017 + MON89034 + DAS-59122-7 + TC 1507 (stack)	Cry3Bb1 Cry1A.105 + Cry2Ab2 Cry34/35Ab1 Cry1F	Monsanto Co. + Dow AgroSciences LLC (cross – licensing agreement)	SmartStax (targeted commercialization in 2010)	Lepidoptera complex + corn rootworm

[1] Emphasis in this table is on insect-control traits, although product trade names may also include reference to herbicide tolerance (e.g., tolerance to glyphosate or glufosinate), for which weeds are the target organisms.

[2] The Lepidoptera complex in corn includes several species. However, different products for control of Lepidoptera have different levels of efficacy against some of the species. Refer to the registrant's information about the Lepidoptera controlled by specific products. The Lepidoptera complex of significance in the Midwest includes black cutworm, corn earworm, European corn borer, fall armyworm, southwestern corn borer, and western bean cutworm.