2004

Effects of Bt plants on non-target herbivores

Richard L. Hellmich
U.S. Department of Agriculture, rlhellmi@iastate.edu

Jarrad R. Prasifka
U.S. Department of Agriculture

Patricia L. Anderson
Iowa State University

Effects of Bt plants on non-target herbivores

Abstract
INTRODUCTION

Transgenic (genetically modified) crops producing insecticidal toxins derived from Bacillus thuringiensis (Bt) bacteria have been scrutinised by government agencies, scientists and the public for their potential to negatively impact non-target organisms in or surrounding agricultural habitats. While several crops have been engineered to express one or more Bt toxins, it is such com (= maize) varieties that have had commercial success. Many strains of Bt exist, each producing one or more toxic crystalline (Cry) proteins, but the deleterious effects of each toxin are usually confined to a few related species in a single order of insects. The species to which an ingested Bt toxin may be harmful is defined by the specific pH levels, enzymes, and gut receptors required to solubilise, activate and bind the toxin. The first commercial Bt-com varieties were produced to control the European com borer (Ostrinia nubilalis) and other closely related pest moths, making any organism outside this group a non-target species. However, the specific requirements for a toxin to function have caused non-target research to be focused on herbivores, specifically non-pest moths and butterflies. The possibility that use of Bt crops negatively impacts predators and parasitoids that help regulate pest populations is considered elsewhere in this volume.

Disciplines
Agriculture | Agronomy and Crop Sciences | Entomology | Plant Breeding and Genetics

Comments
This chapter is from Hellmich, R. L., *Prasifka, J. R.* & *Anderson, P. L.* Effects of Bt plants on non-target herbivores. *In* GM Crops: Ecological Dimensions, edited by H.F. van Emden & A. J. Gray. Association of Applied Biologists, Wellesbourne, UK, pp. 75–80. 2004.

7
EFFECTS OF Bt PLANTS ON NON-TARGET HERBIVORES

RICHARD L. HELLMICH[1], JARRAD R. PRASIFKA[1] AND PATRICIA L. ANDERSON[2]

[1]*USDA-ARS, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, c/o Insectary, Iowa State University, Ames, IA 50011, USA*

[2]*Department of Entomology, Insectary, Iowa State University, Ames, IA 50011, USA*

INTRODUCTION

Transgenic (genetically modified) crops producing insecticidal toxins derived from *Bacillus thuringiensis* (Bt) bacteria have been scrutinised by government agencies, scientists and the public for their potential to negatively impact non-target organisms in or surrounding agricultural habitats. While several crops have been engineered to express one or more Bt toxins, it is such corn (= maize) varieties that have had commercial success. Many strains of Bt exist, each producing one or more toxic crystalline (Cry) proteins, but the deleterious effects of each toxin are usually confined to a few related species in a single order of insects. The species to which an ingested Bt toxin may be harmful is defined by the specific pH levels, enzymes, and gut receptors required to solubilise, activate and bind the toxin. The first commercial Bt-corn varieties were produced to control the European corn borer (*Ostrinia nubilalis*) and other closely related pest moths, making any organism outside this group a non-target species. However, the specific requirements for a toxin to function have caused non-target research to be focused on herbivores, specifically non-pest moths and butterflies. The possibility that use of Bt crops negatively impacts predators and parasitoids that help regulate pest populations is considered elsewhere in this volume.

NON-TARGET IMPACTS OF Bt

It is difficult to make generalisations about the non-target effects of *B. thuringiensis* because of the number of strains and toxins that have been isolated for insect control.

Most Bt-corn varieties that target European corn borer come from the same strain, but the process of inserting genes that produce Bt toxins into plant DNA adds another level of complexity. Each unique gene insertion into corn is called a transformation event or simply 'event'. With each event, a Bt gene, promoter, and marker are inserted into a unique location in the corn plant DNA. Variation in the

Bt gene, promoter or location of the insertion may change the amount and location of toxin expressed in the corn. Events that are or have been commercially available are shown in Table 1.

In the United States, the regulatory authority for Bt crops falls to the Environmental Protection Agency (EPA). As part of an overall assessment of the environmental impact of Bt crops, tests on insects not closely related to European corn borers (e.g., ladybirds, honey bees, springtails, and parasitic wasps) were fed amounts of Bt toxins far higher than those in transgenic plants with no adverse effects (US EPA, 2001). Additional data on the effects of Bt on non-target moths come from the use of the Bt strain *kurstaki* as a microbial insecticide, where applications of Bt are the most common insecticide to control gypsy moth, *Lymantria dispar*, a defoliating pest of North American forests. Studies assessing mortality of non-target moths indicate increased mortality of several species following Bt applications (Miller, 1990; Johnson *et al.*, 1995). However, species whose larvae conceal themselves in plant tissues ('shelter-forming' larvae) apparently avoid exposure to toxins by feeding only in areas not reached by Bt sprays (Navon, 1993; Wagner *et al.*, 1996). This emphasises the importance of direct exposure to Bt toxins through feeding. Because only moths feeding on corn tissues (i.e. primary or secondary pests) should be exposed to the Bt toxins produced by corn, little risk was perceived for non-target moths and butterflies. That is, their feeding habits were not expected to expose them to significant amounts of the Bt toxins inside transgenic corn (US EPA, 2001). Consequently the EPA approved the sale of Bt corn in the United States, anticipating only targeted crop pests would be harmed by Bt expressed in plant tissues.

MONARCH BUTTERFLIES IN THE NEWS

Just as planting of Bt corn was becoming common, the conclusion that Bt crops presented little danger to non-target herbivores was challenged. Losey *et al.* (1999) exposed larvae of the monarch butterfly, *Danaus plexippus*, to leaves of tropical milkweed, *Asclepias curassavica*, dusted with unquantified amounts of pollen from Bt corn. When compared to larvae fed leaves with no pollen or leaves with pollen from non-Bt corn, larvae consuming leaves treated with Bt-corn pollen ate

Table 1. Commercialised Bt-corn events targeting European corn borer in the United States.

Event	Availability	Toxin	Bt strain	Company	Trade name
176	1995-2001	Cry1Ab	*kurstaki*	Dow AgroSciences Syngenta	NatureGard Maximizer
Bt11	1996-present	Cry1Ab	*kurstaki*	Syngenta	YieldGard
Mon810	1996-present	Cry1Ab	*kurstaki*	Monsanto	YieldGard
DBT418	1997-2001	Cry1Ac	*kurstaki*	Monsanto	Bt-Extra
CBH351	1997-2000	Cry9c	*tolworthi*	Aventis	StarLink
TC1507	2001-present	Cry1F	*aizawai*	Dow AgroSciences	Herculex

less, weighed less, and had higher mortality. Losey *et al.* (1999) suggested that corn pollen drifting onto the monarch's primary host plant, common milkweed (*Asclepias syriaca*), could pose a danger to the monarch population in areas of the United States where Bt corn is grown. A second study further supported the premise that pollen from Bt-corn varieties could harm monarch butterflies. Jesse & Obrycki (2000) showed 20% mortality over two days when monarch larvae consumed sections of leaves with field-deposited pollen from one Bt variety, *versus* no mortality in a similar non-Bt treatment. Laboratory results of one non-Bt and two Bt varieties again showed higher mortality when monarch larvae were fed pollen from Bt corn.

The monarch is one of a few insects that the public both recognises and values; their wintering sites in Mexico are a tourist attraction, and their annual migration across the United States is also of public interest. This level of interest combined with three facts to create attention. First, the monarch breeding area in the U.S. overlaps with the major corn-growing states (Wassenaar & Hobson, 1998). Second, the primary food of monarch larvae, common milkweed, is a familiar plant both in and around corn fields. Lastly, by 1999 almost 30% of the corn planted in the USA were Bt-corn varieties. As a result, the EPA requested that companies producing Bt corn submit data to clarify possible effects of Bt-corn pollen on monarch butterflies, with the possibility that future planting of Bt corn in the United States might be limited if further evidence of risk to monarch butterflies were shown.

THE SCIENTIFIC RESPONSE

The public reaction to the monarch issue and the EPA request for data from industry representatives signalled a need for a complete and scientifically rigorous exploration of the relationship between Bt pollen and monarch butterflies. The scientific response to this was a collaboration of scientists from government, industry, and academia that formally began in early 2000. Scientists prioritised research objectives and resolved to undertake a more complete assessment of the risk that Bt-corn pollen might pose to monarch butterflies. Two main issues related to evaluating risk were the degree to which the monarch population might be exposed to Bt-corn pollen and how different amounts of pollen from various corn varieties impact monarch larvae under both field and laboratory conditions.

The degree to which monarch populations might be exposed to Bt-corn pollen is a function of several variables. These include the length of time that monarch larvae and corn pollen co-occur, the proportion of monarch larvae developing in or near corn fields, and the amount of pollen commonly found on milkweed leaves consumed by larvae. Results from Oberhauser *et al.* (2001) showed that monarchs use milkweed plants in corn throughout their breeding season, but that the amount of time that larvae might be exposed to pollen varies among different areas in the Midwestern USA and Canada. Areas near the northern limit of monarch breeding have a longer period of overlap between larvae and corn pollen due to differences in plant maturity. Estimates of the proportion of monarch adults produced in different types of habitats suggested that corn fields were an important source of monarch butterflies and that the majority of monarch adults in the upper Midwest originated in agricultural habitats (i.e. including crops other than corn). The natural deposition of corn pollen onto milkweeds in and around corn fields was addressed by Pleasants *et al.* (2001). The amount of pollen to which monarch larvae are exposed is limited by rain, which can remove over half the pollen from leaves. Over several sites a very broad range of pollen densities was detected, with average pollen levels inside corn fields about 170 grains/cm^2. But simple estimates of pollen exposure levels may be too high because the upper milkweed leaves, on which monarch larvae prefer to feed, have 50–70% less pollen than leaves from the middle section. Corn pollen drifting onto milkweed plants outside the field extended at least 4–5 m, but the amount of pollen on leaves decreased rapidly at and beyond the edge, falling more than 10-fold just 2 m from the field edge.

Separate experiments continued to investigate the toxicity of pollen from different varieties of Bt corn, including two types (events 176 and Bt11) implicated as causing mortality of larvae by Jesse & Obrycki (2000). Laboratory toxicity and dose-response studies by Hellmich *et al.* (2001) concluded that only pollen from event 176 hybrids consistently affected monarch larvae. Even at low levels (11–20 grains/cm^2), pollen from event 176 reduced feeding, weight gain, and survival of monarch larvae. This effect is due to the pollen-specific promoter in event 176, which causes high levels of Bt toxin to be produced in corn pollen. In the USA, event 176 is no longer commercially available and never represented

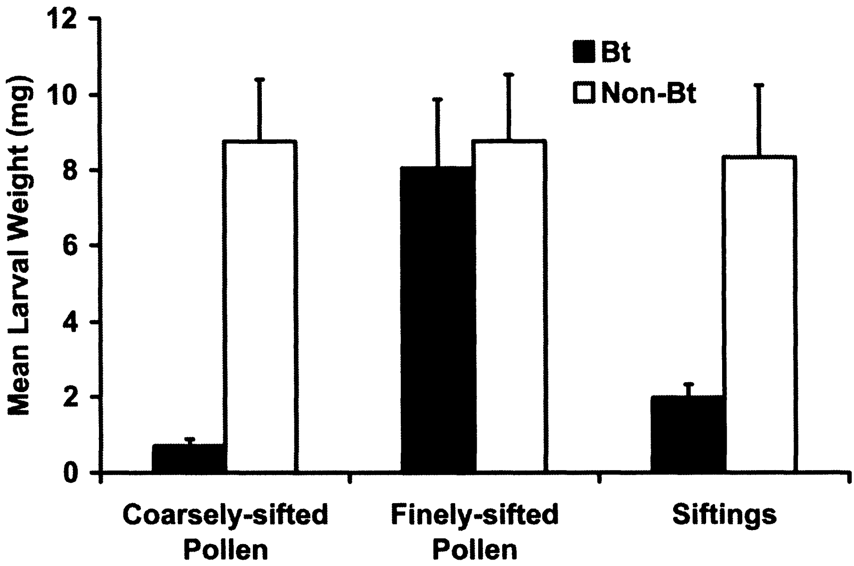

Fig. 1. Mean ± SE weight (mg) of monarch larvae fed for 96 h on pollen-treated discs of milkweed. Treatments included: pollen collected from event Bt11 (Cry1Ab) N7070Bt or N7070 (non-Bt) processed with a coase sieve (250-(m), fine sieve (90-(m), and the siftings remaining after pollen was passed through the fine sieve. Mean pollen levels were (900 grains/cm^2. Figure adapted from Hellmich *et al.* (2001).

more than 2-3% of the corn planted in the USA Hellmich *et al.* (2001) found Bt pollen from other varieties did not harm larvae at densities up to 1000 grains/cm^2, levels more than five times the average seen in natural pollen deposition studies. This result contradicts the finding by Jesse & Obrycki (2000) that Bt11 pollen could cause larval mortality at 135 grains/cm^2. However, Jesse & Obrycki (2000) note considerable non-pollen contamination (mostly fractured anthers) in the pollen used to conduct their tests. Hellmich *et al.* (2001) showed that such contamination was the most likely cause of mortality attributed to Bt11 pollen, as finely sifted Bt pollen did not adversely impact larvae, though the siftings collected caused reduced larval weights. In the first report implicating Bt-corn pollen as a hazard to monarchs, Losey *et al.* (1999) did not measure the density of Bt11 pollen on milkweed leaves or test for the presence of non-pollen contaminants. Consequently, their results cannot be effectively re-assessed with the benefit of more recent data on the effect of Bt pollen on monarch larvae.

Sears *et al.* (2001) compiled and analysed data from collaborators on the toxicity of Bt-corn pollen and probable exposure to pollen in different areas where corn and monarchs co-occur to quantify the risk that Bt pollen poses to monarch butterflies. The risk assessment concluded that pollen from currently available Bt varieties will have no acute effects on monarch butterfly larvae in field settings. Although effects of chronic exposure have not yet been documented, the risk assessment still concludes that the impact on monarch populations will be low because the overall exposure to Bt pollen is low.

Questions remain about the potential effects of anthers from Bt corn. Fractured anthers caused adverse effects on monarch larvae in the laboratory but are not abundant on milkweeds in the field (Hellmich *et al.*, 2001). However, whole anthers are commonly found on milkweeds in corn fields (Jesse & Obrycki, 2000; Hellmich *et al.*, 2001; P. L. Anderson, *unpublished*). Data on Bt-corn anthers similar to those collected for Bt-corn pollen will be useful in determining whether toxins expressed in anthers pose any risk to monarchs. Research on the milkweed tiger moth, another non-target herbivore, showed no adverse effects of Bt anthers (Jesse & Obrycki, 2002). Using the studies on the monarch butterfly and Bt pollen as a standard for a scientifically rigorous risk assessment, ongoing research at Iowa State University and the USDA-ARS Corn Insects Research Unit will help assess the risk anthers may pose to monarch butterflies.

CONCLUSIONS

The events surrounding the commercialisation of Bt corn and the ensuing controversy regarding monarch butterflies underscore the need for methodical investigation in sensitive or public-interest areas of science. The resolution of the issues surrounding pollen also suggests that collaborative research is an effective way thoroughly to address a complex issue with results that are scientifically rigorous. Although Bt-corn pollen does not pose an acute risk to monarch butterflies, research is ongoing to determine whether there are chronic effects due to Bt pollen or Bt anthers.

ACKNOWLEDGEMENTS

The use of trade, firm, or corporation names in this article is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

REFERENCES

Hellmich, R. L., Siegfried, B. D., Sears, M. K., Stanley-Horn, D. E., Daniels, M. J., Mattial, H. R., Spencer, T., Bidne, K. E. & Lewis, L. E. (2001) Monarch larvae sensitivity to *Bacillus thuringinesis*-purified proteins and pollen. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 11925–11930.

Jesse, L. C. H. & Obrycki, J. J. (2000) Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia*, **125**, 241–248.

Jesse, L. C. H. & Obrycki, J. J. (2002) Assessment of the non-target effects of transgenic Bt corn pollen and anthers on the milkweed tiger moth, *Euchatias egle* Drury (Lepidoptera: Arctiidae). *Journal of the Kansas Entomological Society*, **75**, 55–58.

Johnson, K. S., Scriber, J. M., Nitao, J. K. & Smitley, D. R. (1995) Toxicity of *Bacillus thuringiensis* var. *kurstaki* to three nontarget Lepidoptera in field studies. *Environmental Entomology*, **24**, 288–297.

Losey, J. E., Rayor, L. S. & Carter, M. E. (1999) Transgenic pollen harms monarch larvae. *Nature (London)*, **399**, 214.

Miller, J. C. (1990) Field assessment of the effects of a microbial pest control agent on nontarget Lepidoptera. *American Entomologist*, **36**, 135–139.

Navon, A. (1993) Control of lepidopteran pests with *Bacillus thuringiensis*. In Bacillus thuringiensis, *an Environmental Biopesticide: Theory and Practice* (Entwistle, P. E., Cory, J. S., Bailey, M. J. & Higgs, S., eds), pp. 125–146. Wiley, New York.

Oberhauser, K. S., Prysby, M. D., Mattila, H. R., Stanley-Horn, D. E., Sears, M. K., Dively, G. P., Olson, E., Pleasants, J. M., Lam, W. K. F. & Hellmich, R. L. (2001) Temporal and spatial overlap between monarch larvae and corn pollen. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 11913–11918.

Pleasants, J. M., Hellmich, R. L., Dively, G. P., Sears, M. K., Stanley-Horn, D. E., Mattila, H. R., Foster, J. E., Clark, P. & Jones, G. D. (2001) Corn pollen deposition on milkweeds in or near cornfields. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 11919–11924.

Sears, M. K., Hellmich, R. L., Stanley-Horn, D. E., Oberhauser, K. S., Pleasants, J. M., Mattila, H. R., Siegfried, B. D. & Dively, G. P. (2001) Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 11937–11942.

US EPA (2001) Environmental assessment. In *Biopesticides Registration Action Document* - Bacillus thuringiensis *Plant-Incorporated Protectants* (www.epa.gov/pesticides/biopesticides/pips/bt_brad2/3-ecological.pdf).

Wagner, D. L., Peacock, J. W., Carter, J. L. & Talley, S. E. (1996) Field assessment of *Bacillus thuringiensis* on nontarget Lepidoptera. *Environmental Entomology*, **25**, 1444–1454.

Wassenaar, L. I. & Hobson, K. A. (1998) Natal origins of monarch butterflies at wintering colonies in Mexico: new isotopic evidence. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 15436–15439.