Butterflies and *Bt* corn

Allowing Science *to* Guide Decisions

Agricultural Research Service, USDA

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ALLOWING

Controversy and media attention aside, the Bt *corn and monarch butterfly case was unique because an attitude prevailed, even among groups with differing agendas, that decisions about transgenic plants should be based on the weight of scientific evidence.*

Figure 1: Monarch/*Bt* corn timeline

August 1995:
EPA registers the first genetically modified *Bt* corn.

May 1999:
A note appears in *Nature* claiming that transgenic corn pollen harms monarch caterpillars, Losey et al.

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November 1999:
First monarch/*Bt* corn workshop is held.

December 1999:
EPA begins a data call-in, a review of *Bt* corn data.

Science *to* Guide Decisions

Monarch butterflies are familiar to most people as a welcomed garden visitor. They also have become a poster insect for the need to preserve wildlife and wildlife habitat.

But in May 1999, the monarch took on another role – as the center of a tornado of controversy when a small laboratory trial was published that appeared to indicate that the pollen of genetically modified *Bt* corn presented a threat to monarch caterpillars. Although this experiment in no way duplicated natural conditions under which monarch caterpillars may come in contact with corn pollen, mainstream media reported that monarch butterflies were threatened.

Considering the popularity of monarch butterflies, the public outcry that followed media reports that pollen from transgenic corn was killing monarch butterflies was not surprising. These media reports had the effect of heightening public awareness and increasing scrutiny of transgenic plants in terms of potential environmental impact. The reports also intensified one of the most controversial and polarizing issues to face agricultural scientists in recent memory.

Often, such a public outcry drives decision making in advance of true scientific evidence. But in this case of *Bt* corn and monarch butterflies, an attitude prevailed, even among groups with differing agendas, that decisions about transgenic plants should be based on the weight of scientific evidence.

In response to the furor in the media and the public, a group of public and private scientists designed multi-faceted studies to answer questions about *Bt* corn pollen and the monarch butterfly. They published their findings as a group after rigorous scrutiny of their work by the scientific community.

Monarch Watch, www.MonarchWatch.org

Their scientific evidence shows that the risk to the monarch butterfly by *Bt* corn pollen is negligible. The steps taken by these scientists represent a model for risk assessment research of genetically modified plants. Further, the work represents an important example of allowing science to guide decision making.

July 2000: Scientifically reviewed paper is published showing the absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions, Wraight et al.

Arthur Zangerl

October 2000: EPA sponsors a Scientific Advisory Panel, Washington, D.C.

Oct. 9, 2001: A special collection of scientific papers highlighting cooperative research is published in the *Proceedings of the National Academy of Sciences.*

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Oct. 16, 2001: EPA extends registration of *Bt* corn.

"Although the EPA tested Bt *corn or pollen on 'representative organisms' rather than all non-target organisms, we believe the EPA based its decision to register this product on sound science and well-reasoned assumptions."*

Shelton & Sears, 2001

Prologue

EPA approves *Bt* corn commercialization

The U.S. Environmental Protection Agency (EPA) regulates all pesticides and pest control agents to ensure that their use causes no unreasonable adverse effects on the environment or non-target organisms. This includes crop varieties genetically modified to express proteins that protect the plant from insects or other pests. Such "registration" is granted by EPA only after the agency has data to answer specific questions about safety.

Bt corn is enhanced through biotechnology to protect against insect pests. Its built-in insect protection comes from a naturally occurring microorganism called *Bacillus thuringiensis* or "*Bt.*" The protein produced by *Bt* corn selectively targets caterpillars within the order of Lepidoptera. This order includes several moth species harmful to corn, as well as other non-target butterfly species such as the monarch. The primary target is the European corn borer, a moth caterpillar that feeds on cornstalks and ears. Yield losses and the cost of controlling the European corn borer are staggering, estimated at more than $1 billion annually.

Prior to the registration of *Bt* corn, EPA examined risk assessment data to evaluate the potential effects on a wide range of organisms, including birds, aquatic invertebrates, honey bees, ladybird beetles, earthworms and other non-target organisms.

EPA concluded that "the Agency can foresee no unreasonable adverse effects" to non-target organisms, including butterflies (U.S. EPA, 1995).

This EPA conclusion – that non-target butterflies would not be adversely impacted – was based on knowledge that butterfly or caterpillar exposure to *Bt* corn in the environment would be low. Exposure would be limited to caterpillars developing on weeds within cornfields or very near to cornfields during pollen shed. The fact that pollen moves only a short distance away from cornfields also would limit exposure, as well as the low concentration of milkweeds typically found in cornfields.

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Study appears to indicate harm

A note in *Nature* in May 1999 about a small laboratory feeding trial appeared to show monarch butterfly caterpillars fed milkweed leaves coated with *Bt* corn pollen grew slower and suffered a higher death rate than caterpillars that consumed milkweed leaves free of corn pollen. Monarch caterpillars only eat a diet of milkweed leaves, and milkweed often grows near and in cornfields.

By design, researchers did not mimic natural conditions. These caterpillars were given no choice but to feed on leaves heavily covered with *Bt* corn pollen. The actual dose of *Bt* corn pollen used was not measured.

The lead researcher of the study cautioned that it would be inappropriate to draw any conclusions about the risks to monarchs in the field based solely on these initial results.

Major U.S. print and broadcast outlets picked up on the note without considering the caution. The media presented the idea that monarchs were being killed by pollen from *Bt* corn planted by farmers.

This misimpression fueled a public outcry as publicity spread. The European Commission reacted by placing a freeze on the approval process for *Bt* corn, and activists in the United States called for a moratorium on the further planting of *Bt* corn.

Researchers respond

Scientists responded quickly to the alleged threat to monarchs. In the summer of 1999, in the midst of the media tempest, public and private researchers began studies to explore whether *Bt* corn pollen truly affects monarchs in their natural habitat.

The first of these results were presented at a Monarch Butterfly Research Workshop in Chicago in November 1999, attended by researchers and concerned groups including environmental organizations, industry and regulators. Although the data were preliminary and, in many cases, not yet fully analyzed, the results presented provided evidence that the monarch was *not* likely to be harmed by pollen from *Bt* corn.

Repeatedly, researchers urged that any decisions made about *Bt* corn be based on studies that had undergone rigorous peer review to ensure that the data and the conclusions were of the highest scientific validity, rather than formulating regulations as a reaction to media coverage.

Nine meetings, open to the public, were held between 1999 and 2001, where *Bt* corn/monarch data were discussed. One of these meetings was a workshop coordinated by the Agricultural Research Service held in Kansas City, Mo., in February 2000. About 40 scientists from universities, government, industry and the environmental community participated. They established research priorities needed to firmly answer whether *Bt* corn pollen presented a significant risk to monarchs.

Projects were selected and funded through a grant process, overseen by a steering committee with diverse interests, including those with concerns about the application of biotechnology to agriculture. More than $200,000 in grant funding was provided by the Agricultural Research Service and the industry group the Agricultural Biotechnology Stewardship Technical Committee. Environment Canada, with the approval of the Canadian Food Inspection Agency, provided funding for similar research in Ontario.

The studies focused on exploring what effect, if any, *Bt* corn pollen had on monarchs in fields under typical growing conditions.

"Many lessons have been learnt from the 'monarch saga' not least that it is imperative that conclusions concerning environment or nontarget effects of transgenic crops be based on appropriate methods of investigation and sound risk-assessment procedures."

Gatehouse et al., 2002

An important feature was the coordination and cooperation among the researchers. They agreed to use consistent methods so that the data generated by separate studies would be directly comparable. This allowed information from separate studies across the corn-growing areas in the United States and Canada to be pooled, speeding the research and providing a more complete picture.

EPA acts

EPA issued a monarch butterfly data call-in notice in December 1999 to the biotech industry. A data call-in is a list of questions for which EPA seeks research results before considering a regulatory response. In this case, the questions the EPA asked for the data call-in were similar to the research priorities established by scientists at previous meetings. EPA set a deadline for answering these questions.

Public and private researchers summarized all of the available data from studies already under way that covered monarch butterfly distribution and behavior; corn pollen dispersal; milkweed distribution; and toxicity testing of *Bt* proteins and *Bt* corn pollen on the monarch.

EPA followed the data call-in by forming a Scientific Advisory Panel (SAP), a group of experts, to examine the scientific issues surrounding the pending amendments to the re-registration of *Bt* corn. The panel was asked to review the newly available monarch data from 1999 and 2000.

Researchers reach conclusions

SWALLOWTAIL STUDY


Arthur Zangerl

In the summer of 2000, University of Illinois researchers reported a study showing the absence of toxicity of *Bt* corn pollen to black swallowtails under field conditions (Wraight et al., 2000). Unlike the note in *Nature*, this study considered mortality factors *under field conditions* where environmental factors (sun, wind and rain) may influence pollen dispersal and deposition, and consumption by larvae given free choice of feeding location. In laboratory assays – with the exception of *Bt* corn event 176 – researchers did not see mortality even at *Bt* pollen doses fivefold higher than those typically found in the field.

MONARCH STUDIES


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Twenty-nine scientists in the United States and Canada conducted laboratory and field studies in 1999 and 2000 to evaluate in detail any impact by biotech corn pollen on monarch butterflies. Weed scientists, corn researchers, entomologists and other specialists shared and compared data, and pooled expertise from different fields of science to make a complete assessment of the potential impact of *Bt* corn pollen on monarch butterflies. Such widespread cooperation was important in developing the complete picture quickly.

Not only were data shared during the studies, the scientists also grouped their data when it came time to write the scientific journal articles that would subject the research to rigorous scientific review. This allowed the research that answered all of the basic questions to be reviewed at the same time and published together, rather than stringing out the reports over a period of months and in a number of journals. This would have made it harder to put the complete picture together, especially for the public.

On October 9, 2001, the *Proceedings of the National Academy of Sciences* published six comprehensive studies in an issue that featured this research.


Monarch Watch, www.MonarchWatch.org

"These studies represent a concerted effort to study the impact of GM crops on the monarch butterfly, based on sound science and proven methods of risk assessment, and exemplify an approach that is a laudable model for future endeavors."

Irwin & Krishna, 2002

Examination of the natural habitat of monarch butterflies indicates several events must coincide for the possibility to even exist that Bt *pollen could cause harm to the monarch population.*

Understanding the Science:

Key Findings of the Monarch Risk Assessment

Risk assessment overview

A risk assessment has two basic components:

1) What is the potential for *toxicity* for a particular species?

2) What is the likelihood of *exposure* to the toxicant?

This is the standardized approach for estimating risk posed by pesticides, industrial byproducts and other potential toxicants to many non-target species. The scientific community considers it the most credible method for determining actual risk and it is also the method accepted by the EPA.

Examination of the natural habitat of monarch butterflies indicates several events must coincide for the possibility to even exist that *Bt* pollen could cause harm to the monarch population. First, monarchs have to have laid eggs on milkweed plants and the caterpillars must emerge from the eggs just as the *Bt* corn is producing pollen. Corn, including genetically modified corn, only produces pollen during a narrow window of seven to 10 days each year. Next, the caterpillars must feed on milkweed leaves with *Bt* corn pollen. Third, the caterpillars must consume enough *Bt* pollen to reach potentially toxic levels. The *Bt* corn/monarch risk assessment assembled the likelihood of all of these circumstances occurring.

The risk assessment's key finding: The potential risk to monarch butterfly populations from *Bt* corn pollen is negligible.

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"It doesn't look like you're ever going to have a pollen density in the field where you would have some kind of detrimental effect."

Dr. John Pleasants, Iowa State University, Department of Zoology and Genetics

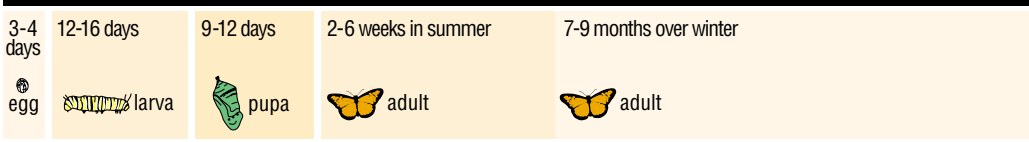
Answering the questions one by one

How much *Bt* corn pollen does it take to have a toxic impact on monarchs?

There actually are a number of different types of *Bt* corn, each expressing a slightly different *Bt* protein. For the three most common types of *Bt* corn – Bt11, MON810 and TC1507 – doses of more than 1,000 *Bt* corn pollen grains/cm² of milkweed leaf surface were required – sometimes much more – to see significant negative effects on caterpillar development (Hellmich et al., 2001). Caterpillars were exposed to pollen on milkweed leaves for up to five days at doses ranging from 100 to more than 1,000 pollen grains/cm² without any observed effects in terms of weight gain or mortality.

Pollen levels measured on milkweed leaves in cornfields during pollination do not commonly exceed 1,000 grains/cm². In fact, the amount of pollen was significantly less than 1,000 grains/cm², with means ranging from 10 to 425 grains/cm² (Pleasants et al., 2001).

Figure 2: Monarch life history



An adverse effect on caterpillars was seen at a lower dose with one rarely planted *Bt* corn: Event 176. It took 10 grains/cm² to affect the larvae. But event 176 was the earliest developed *Bt* corn and was quickly supplanted by other types. It has never been planted on more than 2 percent of all the acres planted with corn, and the biotech company marketing event 176 hybrids did not seek EPA re-registration in 2001.

Do monarch caterpillars and corn pollen overlap?

Monarchs migrate north and east from their overwintering grounds in the fir forests of the Sierra Madre Mountains near Mexico City and establish populations in the southern United States east of the Rocky Mountains. This first generation of monarchs produces adults that move into the northern United States and southern Ontario, including Corn Belt states such as Nebraska, Iowa, Illinois and Minnesota, where the majority of *Bt* corn is grown. Two to three generations of monarchs are then produced in these areas. Adults from the last generation migrate to Mexico to overwinter.

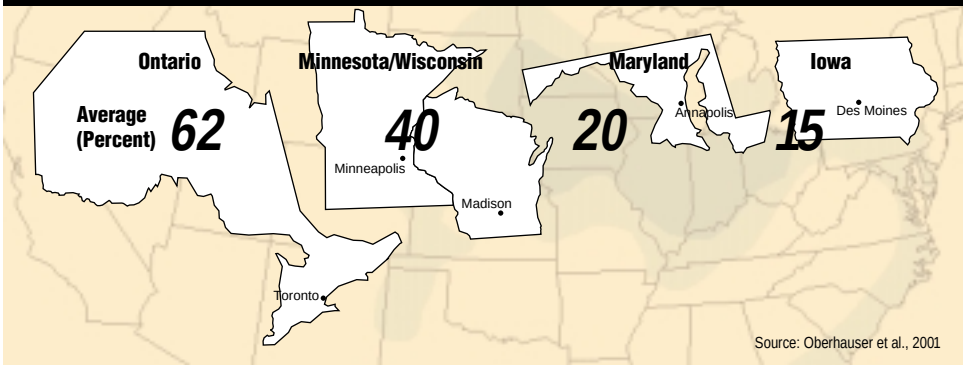
Monarchs undergo complete metamorphosis with four distinct stages: Egg, larva (caterpillar), pupa (chrysalis) and adult (see **Figure 2**). Caterpillars go through five "instars," meaning they shed their skin five times as they grow progressively larger. Monarchs go from egg to adult in about a month. The adults live another two to six weeks in summer, and those that overwinter in Mexico can live more than six months.

The larval stage, when caterpillars feed exclusively on milkweeds, is of greatest interest to researchers, because milkweeds are found within and around *Bt* cornfields throughout the Corn Belt.

Considering the vast amount of land on which corn is grown, it is not surprising that data suggest that a significant proportion of monarchs produced in the Midwest come from agricultural areas. To evaluate the potential impact of *Bt* corn on monarch populations, researchers studied the spatial and temporal overlap between the monarch populations and corn pollen production (Oberhauser et al., 2001).

Researchers found in northern areas of the Corn Belt (Ontario, Minnesota and Wisconsin), the peak numbers of caterpillars of the final monarch generation did coincide with pollen shed in the summer of 2000. This meant there was a large overlap of first and second instars with pollen shed, as shown in **Figure 3**. The overlap of the two windows was much less in the south (Iowa) and east (Maryland), where pollination occurred before peak population buildup. Although Ontario had the greatest overlap, it also had the greatest proportion of non-agricultural monarch habitats. Milkweed in Ontario cornfields was found not to be an important contributor to monarch production.

Figure 3: Overlap of small monarch larvae & corn pollen shed



Where is *Bt* corn grown?

About 28 percent of the crop and pastureland within the North American Corn Belt – an area extending from eastern Kansas/Nebraska to western New York – is planted in corn (Sears et al., 2001). In 2001, 26 percent of North American cornfields were planted with *Bt* varieties, and the vast majority of those were planted with events MON810 and Bt11 (see **Figure 4**).

Figure 4: Commercial *Bt* corn products

Product	Registrant	Trade name	Cry protein	Commercial status
Bt11	Syngenta	YieldGard®	Cry1Ab	Available
MON810	Monsanto	YieldGard®	Cry1Ab	Available
TC1507	Mycogen	Herculex™	Cry1F	Available
	Pioneer Hi-Bred	Herculex™	Cry1F	Available
176	Syngenta	KnockOut®	Cry1Ab	Phased out
	Mycogen	NatureGuard®	Cry1Ab	Phased out
DBT418	DEKALB	Bt-Xtra™	Cry1Ac	Phased out
CBH351	Aventis	StarLink®	Cry9C	Sales halted September 2000

Where is milkweed found?

Monarch butterflies lay their eggs exclusively on plants in the milkweed family (*Asclepiadaceae*), usually on the underside of a leaf.

Rather than make assumptions about where milkweed occurs, researchers conducted a census. Milkweed densities generally are much higher in nonagricultural habitats than in cornfields, and densities are higher along field edges than within fields (Oberhauser et al., 2001), as shown in **Figure 5**. This is an important finding because milkweeds in nonagricultural habitats would not have harmful levels of *Bt* pollen drifting onto their leaves, because levels of pollen deposition fall off sharply just a few feet from cornfields. Therefore, monarch caterpillars in field margins are less likely than those in cornfields to encounter *Bt* corn pollen. However, a high percentage of monarchs are likely to be found in and around cornfields or other agricultural habitat simply due to the prevalence of agricultural land in some states.

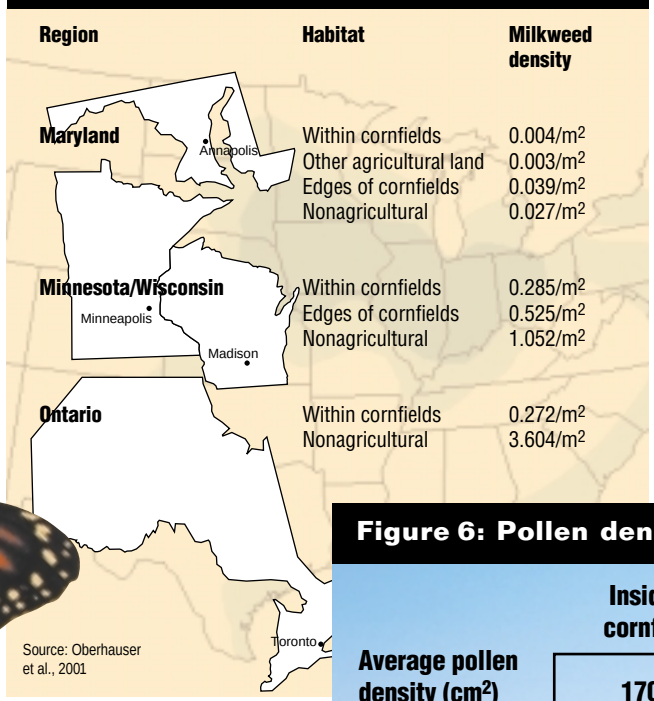
How much *Bt* corn pollen is found on milkweed leaves?

To really understand the risk from *Bt* corn pollen for monarch caterpillars, it is necessary to know how much *Bt* corn pollen reaches milkweed leaves and how long it stays on the leaves. The only place that caterpillars will be in close contact with the pollen is on milkweed leaves.

Researchers (Pleasants et al., 2001) found that pollen densities were highest inside cornfields (averaging 170.6 grains/cm^2) and that levels were progressively lower from field edges outward (see **Figure 6**). They also found 95 percent of all leaf samples had pollen densities below 600 grains/cm^2. This level is significantly less than the pollen density in excess of 1,000 pollen grains/cm^2 required to see adverse effects on monarch caterpillars for Bt11, MON810 and TC1507.

Many factors influence pollen levels. The most important is rainfall, which removes pollen from leaves. In a single rain event, 86 percent of pollen was removed from milkweed leaves on plants outside a cornfield (Pleasants et al., 2001). A second factor is wind direction, which results in higher pollen levels in the downwind direction and lower levels upwind.

Figure 5: Milkweed density in study sites



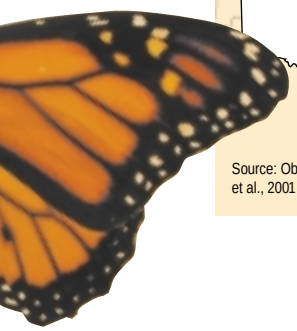
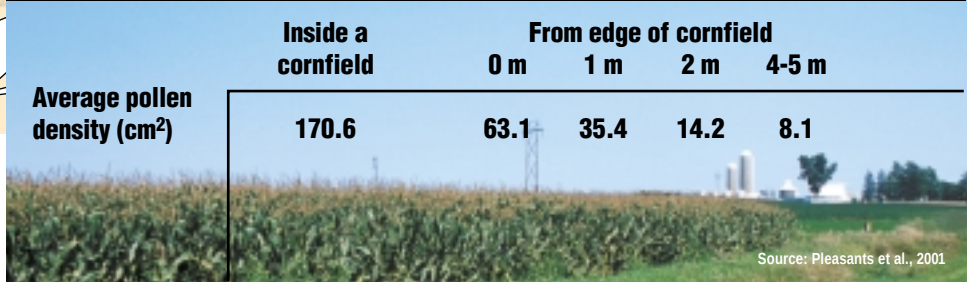


Figure 6: Pollen density levels on milkweed leaves



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Where do monarch caterpillars feed?

The majority of monarch eggs are laid on the underside of milkweed leaves on the upper portion of the plant, and this is where young caterpillars tend to feed. The upper portion of milkweed plants also has been found to most likely have the lowest pollen levels because the leaves often point up. Researchers found that upper leaves had only 30 to 50 percent of the pollen density found on leaves in the middle portion (Pleasants et al., 2001).

Similarly, young caterpillars do not tend to eat on the leaf midrib, where pollen densities were found to be higher.

What does this mean when you put it all together?

In the formal risk assessment of *Bt* corn on monarch populations, scientists carefully considered all of the questions of toxicity and exposure. **Their conclusion: *Bt* corn pollen does not pose a risk to monarch populations.**

To reach this conclusion, scientists integrated the following results:

- The density of Bt11, MON810 and TC1507 types of *Bt* corn pollen that overlay milkweed leaves in the environment rarely comes close to the levels needed to harm monarch butterflies. Both laboratory and field studies confirmed this.
- There is limited overlap between the period that *Bt* corn sheds pollen and when caterpillars are present.
- Only a portion of the monarch caterpillar population feeds on milkweeds in and near cornfields.

So toxicity is negligible and exposure under field conditions is low.

The public will be pleased to learn that *Bt* corn pollen does not harm monarchs living in the Corn Belt. Monarchs are threatened by many factors, including harsh weather, predatory insects, and habitat loss in overwintering sites and summer breeding grounds. Monarch caterpillars developing on milkweeds within agricultural areas also may be adversely affected by the application of conventional insecticides targeting crop pests or herbicides that affect weed species including milkweeds.

The harm posed to monarchs by insecticide use, especially in agricultural situations, is the final piece in assembling the true picture of risk assessment. The potential for harm from the use of genetically modified corn cannot be evaluated by itself. It must be considered in light of the alternative to planting *Bt* corn, which is insecticide use by farmers.

An experiment in a sweet corn field (Stanley-Horn et al., 2001) demonstrated that monarchs were adversely affected by treatments of lambda-cyhalothrin, a broad-spectrum insecticide commonly applied to non-*Bt* sweet corn to eliminate pests and damage. It is important to note that *Bt* corn can dramatically reduce the number of insecticide treatments typically applied to sweet corn and field corn (U.S. EPA, 2000; Gianessi et al., 2002). *Bt* corn eliminates the need to apply conventional insecticides for corn borer protection because of its built-in insect protection made possible through biotechnology.

In the future, new *Bt* corn products are expected to transform the way growers control rootworms, the No. 1 insect pest of corn. This biotech corn could help growers significantly reduce overall insecticide use, resulting in much less impact on the environment. This does not mean that corn rootworm *Bt* corn should not be scrutinized for impact on non-target organisms such as the monarch butterfly. But any risk found also should be weighed against harm done to those non-target insects and other animals by unintended exposure to chemical insecticides.

Bt CORN REGISTRATION EXTENDED

Based on a comprehensive review of scientific information by EPA, *Bt* corn registrants received an extended registration for insect-protected corn for an additional seven years.

The monarch research published in the *Proceedings of the National Academy of Sciences* and reviewed by the EPA clearly supports the conclusion that "*Bt* corn does not harm monarch butterfly populations" (EPA, 2001).

Monarch Watch. www.MonarchWatch.org

"The pollen issue – Does Bt corn pollen pose a significant risk? – has been answered. But what other risks are there in this case of monarchs? All of these things need to be assessed in terms of risks, benefits and comparative risks."

Dr. Mark Sears, chair of the Department of Environmental Biology, University of Guelph, Ontario

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Monarch Watch
www.MonarchWatch.org

Additional information

Agricultural Biotechnology Communications
www.agribiotech.info

American Society of Plant Biologists, Genetically Modified Crops: What Do the Scientists Say?
www.aspb.org/publications/plantphys/gmcpub.cfm

ARS-USDA, Research Q&A: *Bt* Corn and Monarch Butterflies
www.ars.usda.gov/is/br/btcorn

Colorado State University, Transgenic Crops: An Introduction and Resource Guide
www.colostate.edu/programs/lifesciences/TransgenicCrops

Cornell University, Informing the Dialogue About Agricultural Biotechnology
www.nysaes.cornell.edu/agbiotech

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