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Effects of transgenic Bacillus thuringiensis corn pollen on the monarch butterfly

Abstract
Transgenic Bt corn has been widely planted in Iowa. This study considered whether plant tissues released by Bt corn (pollen and anthers) have an effect on monarch butterfly larvae.

Keywords
Entomology, Biocontrol and Integrated Pest Management, GMOs, Wildlife and recreation

Disciplines
Agriculture | Entomology
Effects of transgenic *Bacillus thuringiensis* corn pollen on the monarch butterfly, *Danaus plexippus*

**Abstract:** Transgenic Bt corn has been widely planted in Iowa. This study considered whether plant tissues released by Bt corn (pollen and anthers) have an effect on monarch butterfly larvae.

**Background**

The speed with which transgenic crops have become widespread has caused controversy about the assessment and management of their environmental risks. Transgenic *Bacillus thuringiensis* (Bt) corn was approved by the Environmental Protection Agency in 1995. The foundation for the regulation of transgenic Bt crops is a history of safe use of Bt sprays. However, previous studies examining the effect of Bt insecticide sprays on non-target organisms have documented negative effects on non-target moth and butterfly (Lepidoptera) species. The expression and dispersal of Bt toxin in tissues from transgenic corn plants may pose a risk to non-target Lepidoptera similar to that observed from Bt sprays. When Bt corn pollen landed on the leaves of plants in and around cornfields, it exposed non-target Lepidopteran larvae feeding on these plants to Bt toxins.

The monarch butterfly, *Danaus plexippus*, is a butterfly species likely to be impacted by widespread planting of Bt corn. Cornfields constitute 19 percent of the *D. plexippus* effective breeding habitat; the size of the wintering adult population in Mexico makes it likely that the monarchs are utilizing *A. syriaca* (milkweed) growing in cornfields. It is estimated that 43 times more *D. plexippus* adults are being produced from milkweeds in and around cornfields than in non-agricultural land in Iowa.

Previous studies have shown that high densities of corn pollen and anthers accumulate on milkweeds in cornfields. *D. plexippus* larvae ingest the pollen and anthers, and may be adversely affected by the Bt toxin expressed in the pollen and anthers. The increasing use of Bt corn in row crop agriculture makes it valuable to determine the possible negative impacts on monarch larvae.

The objectives for the research were to:

1. Determine the sub-lethal effects of Bt corn pollen exposure on monarch larval development and adult characteristics,
2. Quantify the use of milkweeds adjacent to Bt and non-Bt cornfields by monarchs, and

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**Budget:**
$9,453 for year one
$25,500 for year two
3. Compare the survival of experimental cohorts and natural populations of monarchs adjacent to Bt and non-Bt cornfields.

**Approach and methods**

Objective 1: Three densities of transgenic and non-transgenic pollen, representative of observed field densities, were used to saturate filter paper in Petri dishes. Leaf disks were placed on the paper, and monarch larvae were placed on leaf disks. Following 48 hours of exposure to pollen, each larva was observed and development times and adult characteristics were analyzed to determine treatment effects.

Objective 2: In July 2000, three sites in central Iowa, each with Bt and non-Bt cornfields, were selected. Each site had milkweeds growing within and adjacent to the fields. Each field site was surveyed weekly for monarch eggs and larvae. The number of milkweed plants examined and the distance surveyed in each of the three locations was recorded. The occurrence of monarchs was analyzed by examining the number of monarch life stages. This provided a comparison of the numbers of monarchs at different field sites and locations, regardless of how many milkweed plants were present. The proportion of milkweed plants with a monarch also was examined. In this analysis, researchers determined if the position of a plant in relation to the cornfield influenced the probability of a milkweed harboring eggs or larvae.

Objective 3: In 2000, three one-acre sites at ISU research farms were planted as paired plots with Bt and non-Bt corn. Milkweed plants were transplanted to three locations at each field site. A week later, five first instar monarchs were placed on each milkweed plant. Researchers recorded the number and life stage of monarch larvae every 24 hours for seven days. Leaves were collected and pollen densities were estimated at three to eight days after larval introduction.

*Two monarchs on milkweed in a ditch.*

![Two monarchs on milkweed in a ditch.](image)
In 2001, three one-acre sites at ISU research farms were planted as paired plots with Bt and non-Bt corn. Milkweed plants were transplanted at different locations in each field. Some first instar monarchs were placed on non-caged milkweeds, while others were placed on milkweed plants encased in cylindrical mesh tomato cages. Researchers recorded the number and life stage of each monarch larva every 24 hours until pupation. Further measurements were taken after the adults emerged. In addition, corn pollen densities and anthers were counted on each milkweed plant.

Results and discussion

Objective 1: Total development time of larvae exposed to Bt pollen when less than 12 hours old or from 12 to 36 hours old was similar for all pollen concentrations and types. Developmental time and adult characteristics of larvae surviving the 48-hour exposure showed no sub-lethal effects of transgenic corn pollen exposure. Additional studies are required to determine if continuous larval exposure to Bt pollen influences developmental time or adult characteristics. A reduction of adult lipid levels could indicate that a larva fed less, or didn’t digest nutrients as efficiently due to ingestion of Bt toxins. Migratory adult monarchs rely on lipids for energy; thus, a lower level of lipids carried over from the larval stage could reduce their ability to reach Mexico.

Objective 2: The mean number of monarch eggs or larvae and the probability of a milkweed having an egg or larva were similar on the three sites, so the site data were combined. Similar numbers of eggs were observed in or near Bt cornfields compared to non-Bt cornfields and the average numbers of eggs within cornfields were similar to numbers in the road-sides. The highest number of eggs was observed during Week 4 of sampling. The majority of the eggs and larvae were observed on the underside of the milkweed leaves.

Objective 3: Survival curves for monarch larvae on non-caged milkweed plants were similar for monarch larvae in the Bt corn compared with the larvae in the non-Bt corn. On caged milkweed larvae in 2001, researchers observed a trend of higher survival to the adult stage among monarch larvae (56 percent) exposed to non-Bt corn pollen and anthers compared to survival of larvae (23 percent) in the Bt fields. Researchers observed no additional mortality in the larvae in the non-Bt cornfields after seven days, whereas mortality in the Bt cornfields continued to occur until day 22. However, the difference in the survival of monarch larvae in the Bt corn compared to the non-Bt corn was not significant at the 0.05 level.

Conclusions

Investigators observed no sub-lethal effects of Bt corn pollen exposure on monarch larval development and adult characteristics. They saw no sub-lethal effects on monarch larval development time, pupal weight, adult dry weight, adult wing length, or adult lipid content. Short duration exposure early in development did not affect overall larval development time or the adult characteristics that were measured. A study investigating the effect of chronic Bt tissue exposure is needed to more fully gauge the impact of Bt corn tissues on monarchs.

Similar numbers of monarch eggs and larvae were found in Bt and non-Bt cornfields. Investigators noted similar numbers of eggs on milkweed plants growing within the corn compared to milkweed plants growing in the nearby roadside. However, the probability of a milkweed having at least one larva was higher if the plant was growing in a cornfield. These results show that immature stages of monarchs occur within the corn ecosystem and will be affected by changes in agricultural practices.
After minimizing predation through mechanical exclusion, researchers quantified mortality due to exposure to Bt corn pollen and anthers. They observed trends toward higher rates of mortality of monarch larvae in Bt cornfields. They also demonstrated that anthers from Bt corn plants are frequently deposited on milkweed plants. Additional studies on the effects of Bt corn need to quantify the effects of pollen and anther tissues on non-target lepidopterans.

Impact of results

Bt corn has been promoted as a safer alternative for humans and the environment, compared to the use of broad-spectrum insecticides. However, most field corn in the U.S. Corn Belt is not treated for above-ground insect pests and most corn hybrids have substantial resistance to corn borers. Thus, Bt corn is not used to replace insecticide use. Instead it poses another mortality factor for non-target lepidopterans feeding on other plants within the agroecosystem.

This research demonstrated that monarch larvae are present in and near Bt and non-Bt cornfields, that there is a trend toward decreased larval survival on caged milkweed in Bt cornfields, and that short-term exposure to Bt corn causes no sub-lethal effects on adult monarchs.

Three possible strategies exist for a farmer who wants to reduce the risks from Bt corn pollen for non-target species. The simplest tactic would be to use only those Bt-corn hybrids that do not express the Bt toxin in the pollen. The drawback is that Bt hybrids lacking the Bt toxin in the pollen may not be as effective against first instar European corn borers that feed on corn pollen prior to feeding on plant leaf tissues.

Another solution would be the creation of “buffer zones” of non-Bt corn around Bt cornfields. It has been demonstrated that very little corn pollen goes beyond three or four rows from the original plant. A “buffer zone” of at least four rows would trap most transgenic pollen. It is suggested that 20 to 30 percent of the corn planted by a grower be non-Bt varieties to serve as a refuge for resistance management. Thus, it may be possible to make the refuge into a buffer zone for Bt pollen collection. The size and shape of the non-Bt areas would have to be designed carefully to insure that they effectively served both purposes.

A third option, not planting transgenic corn hybrids, would eliminate the potential risks to non-target species.

Education and outreach

Eight scholarly journal articles have appeared based on this project. Presentations on the project results were given at several events, including the Entomological Society of America’s 2000 meeting. The project and its findings received considerable news media coverage.