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# Effects on Monarch Butterfly Larvae (Lepidoptera: Danaidae) After Continuous Exposure to Cry1Ab-Expressing Corn During Anthesis

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**ABSTRACT** Effects on monarch butterfly, *Danaus plexippus* L., after continuous exposure of larvae to natural deposits of *Bacillus thuringiensis* (Bt) and non-Bt pollen on milkweed, were measured in five studies. First instars were exposed at 3–4 and 6–7 d after initial anthesis, either directly on milkweed plants in commercial cornfields or in the laboratory on leaves collected from milkweeds in corn plots. Pollen exposure levels ranging from 122 to 188 grains/cm$^2$/d were similar to within-field levels that monarch butterfly populations might experience in the general population of cornfields. Results indicate that 23.7% fewer larvae exposed to these levels of Bt pollen during anthesis reached the adult stage. A risk assessment procedure used previously was updated with a simulation model estimating the proportion of second-generation monarch butterflies affected. When considered over the entire range of the Corn Belt, which represents only 50% of the breeding population, the risk to monarch butterfly larvae associated with long-term exposure to Bt corn pollen is 0.6% additional mortality. Exposure also prolonged the developmental time of larvae by 1.8 d and reduced the weights of both pupae and adults by 5.5%. The sex ratio and wing length of adults were unaffected. The ecological significance of these sublethal effects is discussed relative to generation mortality and adult performance.

**KEY WORDS** monarch butterfly, Bt corn, chronic effects, risk assessment, nontarget

THE POTENTIAL NONTARGET RISKS to monarch butterfly, *Danaus plexippus* L., of transgenic corn transformed with a gene from the bacterium *Bacillus thuringiensis* (Bt) have been the focus of much scientific research and debate after a laboratory study by Losey et al. (1999) revealed toxicity to monarch butterfly larvae consuming Bt corn pollen deposited on milkweed plants (*Asclepias* spp.). Subsequent studies indicated that the acute effect of Bt corn pollen expressing lepidopteran-active Cry protein on monarch butterfly populations was negligible (Sears et al. 2001). Larval exposure to pollen on a population-wide basis is low, given the proportion of larvae in cornfields during pollen shed, the proportion of fields planted in Bt corn, and the levels of pollen within and around cornfields that exceed the toxicity threshold (Oberhauser et al. 2001, Pleasants et al. 2001). Conservatively, the proportion of the monarch butterfly population exposed to Bt corn pollen was estimated to be <0.8% (Sears et al. 2001). Laboratory bioassays also showed that acute toxic and sublethal effects of pollen from the most widely planted Bt corn hybrids (events MON810 and BT11) are unlikely, even at peak levels of pollen shed (Hellmich et al. 2001). The only transgenic corn pollen that consistently affected monarch butterfly larvae was from the Cry1Ab event 176 hybrids, which have been phased out of commercial use in the United States. Furthermore, field studies performed in Iowa, Maryland, New York, and Ontario, Canada, reported that growth to adulthood or survival of monarch butterfly larvae was unaffected after exposures for 4–5 d to milkweed leaves with natural deposits of Cry1Ab-expressing (events BT11 and MON810) corn pollen (Stanley-Horn et al. 2001). These results indicated negligible effects of Bt pollen to monarch butterfly larvae from short-duration exposures in field settings.

All scientific information on acute toxicity and exposure supports the conclusion that Bt corn poses a limited risk to monarch butterfly populations (Sears et al. 2001). What risk exists is caused by the limited exposure of monarch butterfly populations to Bt pollen in nature. Nevertheless, the studies to date examined acute and sublethal effects after 4–5 d of expo-

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**Table 1. Specific hybrids planted and numbers of Bt events tested in each study**

| Location | Year | Bt events/hybrids | Non-Bt hybrids |
|---|---|---|---|
| Maryland | 2001 | BT11: GSS0966 (4), Syngenta Seeds, Golden Valley, MN | Prime Plus (4), Syngenta Seeds |
| | 2002 | BT11: N65-A1 (4), Syngenta Seeds | N6423 (4), Syngenta Seeds; DKC61-24 (4), Monsanto, St. Louis |
| | | MON810: DKC61–25 YG (4), Monsanto, St. Louis | |
| Ontario | 2001 | BT11: N3030Bt (4) and N2555Bt (3), Syngenta Seeds | DeKalb4064 (2), Bayside87 (2), DeKalb359 (1), N2555 (1), and Pride196 (1), Syngenta Seeds |
| | 2002 | BT11: N2555 (1), N27M3 (1), N29F1 (1), N2555/ N27M3 (1), N3030/ N29F1 (1) and N3030/N2555 (1), Syngenta Seeds | 36H75 (1) and 3893 (1), Pioneer Hi-Bred; Hyland 2333 (1) AND Hyland 2292 (1), Hyland seeds; 25Y2 (1), Syngenta Seeds; and Hyland seeds/ Pride 157 (1), Pioneer Hi-bred). |
| | | MON810: 38W36 (2) and 39F06 (4), Pioneer Hi-Bred, Johnston, IA | |
| Iowa | 2002 | BT11: N2555Bt (8) and N58-D1 (8), Syngenta Seeds | N2555 (8) and P38A24 (8), Syngenta Seeds; N58-F4 (8), Syngenta Seeds, and P34M94 (8), Pioneer Hi-Bred International |
| | | MON810: P38A25 (8) and P34M95 (8), Pioneer Hi-Bred International | |

Numbers in parentheses indicate the number of replicate plots or fields of each hybrid.

sure of developing larvae to Bt pollen. In cornfields, larvae hatching at the onset of anthesis may be exposed to biologically active Cry1Ab protein for periods of 12 d or more (Russell and Hallauer 1980). This worst-case scenario could potentially impact the ~0.8% of the monarch butterfly population exposed to Bt corn pollen in cornfields. For this reason, the EPA-amended registration document for Bt corn (Environmental Protection Agency 2001) states that studies on longer-term exposure of monarch butterfly larvae to Bt pollen should be considered. Reported here are studies that measured the potential effects on monarch butterfly larvae feeding continuously on milkweeds with naturally deposited levels of Cry1Ab pollen and anthers that occur within Bt cornfields during anthesis. These studies analyze the hazard associated with the exposure scenario that may occur under field conditions and allow determination of a more precise estimate of the overall risk to monarch butterfly populations.

## Materials and Methods

**Experimental Design.** Five studies were conducted in either field plots or commercial fields of Cry1Ab-expressing hybrids and nontransgenic corn over 2 yr (from 2001 to 2002) in Maryland, Iowa, and Ontario, Canada. Each study measured standardized endpoints of monarch butterfly fitness after continuous exposure of larval cohorts to milkweed leaves with natural deposits of corn pollen. Corn hybrids were planted at the normal time at each study location and grown according to recommended commercial practices. Plant growth was monitored to determine the beginning of anthesis (day 0), which was defined by the shed of pollen on 50% of corn tassels.

Experimental designs varied among studies with respect to replication, specific hybrids planted, and numbers of events tested (Table 1). For the 2001 study in Maryland, four replicated plots of BT11 sweet corn and its near non-Bt isoline were planted in a randomized block design at the University of Maryland Research and Education facility in Upper Marlboro, MD. Each plot contained 16 rows, 0.75 m in width and 39 m in length, separated by a 10-m noncropped buffer. In the 2002 study, four replicated plots of grain corn were planted in a 2 by 2 split-plot design at the University of Maryland Research and Education facility in Beltsville, MD. A BT11 hybrid and its near non-Bt isoline were arranged as subplots in one main plot, and a MON810 hybrid and its near non-Bt isoline were arranged as subplots in the second main plot. Each subplot measured 12 rows (0.75 m wide by 30 m long). In Ontario, Canada, field bioassays in 2001 were conducted in seven BT11 and seven non-Bt fields of grain corn ranging from 2.4 to 23 ha in Wellington County, Ontario, Canada. A similar 2002 study was conducted in 18 commercial fields of corn (2.4–24 ha each) planted within Wellington County. Sites consisted of three corn types: six BT11 fields, six MON810 fields, and six conventional hybrid fields. In Iowa, replicated plots of Bt events and related near nontransgenic isolines were established in two 2.2-ha fields at the Iowa State University Johnson Farm in 2002. The first field was planted with 90-d BT11 and MON810 hybrids and 90-d near-isoline hybrids. The second field was planted with 108-d BT11 and MON810 hybrids and 108-d near-isoline hybrids. Hybrids were randomly assigned to eight blocks within each field, giving a total of 16 replicates of each event and their isolines. Plots contained 24 rows and measured 18.3 by 16.7 m. Specific hybrids planted at each location are given in Table 1.

In each study, common milkweed plants, *Asclepias syriaca* L., were grown in the greenhouse from rhizome pieces in plastic containers to a height of 50–60 cm. Before anthesis, groups of potted plants were placed either at a distance of 20–50 m from the field edge within each commercial field or near the center of each plot, at least 3 m or more from the outside edge. Six to 12 plants were spaced 1 m apart within each group.

**Bioassays.** A monarch butterfly colony was established for each study from field-collected larvae and reared in the laboratory at 25°C and 70% RH with a photoperiod of 14:10 (L:D) h. Eggs produced were collected daily and stored at 10°C to synchronize development and provide a large pool of larvae for bioassays. Cohorts of first instars (<12 h old) were initially exposed to naturally deposited levels of Bt and non-Bt pollen beginning at two times during anthesis. In all studies, bioassays were initiated at 3–4 d after the onset of anthesis. With the exception of the Iowa study, a second set of larval cohorts was exposed at 6–7 d after anthesis. These bioassay timings exposed the most sensitive first, second, and third instars (Hellmich et al. 2001) to fresh Cry1Ab-expressing pollen during 50–75% of the anthesis period. Two rearing methods were used to expose larvae continuously to pollen on milkweed leaves during their development. In the Maryland studies, two cohorts of five larvae were reared in the laboratory until they pupated on leaves collected from the same group of milkweed plants in each hybrid plot. Individual leaves or whole terminal sections of leaves were removed from the upper third of plants, placed upright in either chilled petri dishes or waxed cardboard buckets with chilled water, and brought to the laboratory. At each bioassay time, cohorts were held on excised leaves in petri dishes until most larvae reached fourth instar, after which they were transferred to waxed cardboard buckets containing plant terminals of milkweeds. Additional leaf material from the same plot was added to provide enough food for larvae to develop to pupation. In the Iowa and Ontario studies, either one or two cohorts at each assay time were reared directly on potted plants in each hybrid plot or commercial field. Cohorts consisted of five or six larvae depending on the study. Larvae were placed on the upper half of one milkweed plant covered with a fine mesh cage to prevent predation. The mesh cages contained circles of wire fastened inside at both ends to keep the cage from touching the plant. Wooden stakes (1 m in height) were placed beside each plant with an arm extending to suspend the mesh cage over the plant. Every 3 d for the first 9 or 12 d (varied with the study) and every 2 d thereafter, larvae were moved to a new milkweed plant to continue to feed. When larvae became prepupae and stopped feeding, they were brought to the laboratory and held in an environmental chamber at 24°C until pupation.

In all studies, standardized data on eight fitness parameters were recorded for each cohort. Larval survival and instar stage were recorded on each day that a cohort was moved or provisioned with new leaves. Developmental time and survival were expressed as the number of days to pupation and eclosion and proportion of individuals reaching pupation and eclosion. Each pupa was weighed 24 h after pupation and hung along the inside wall of the container by its cremaster to allow adults to emerge properly in a vertical position. After eclosion, each butterfly was sexed, weighed, and measured for expanded wing length from tip to tip.

**Pollen and Anther Counts.** The density of pollen on the excised leaves or potted plants fed to each cohort was estimated by counting grains per unit surface area under a stereomicroscope. In the Maryland studies, counts were made within two 0.33-$cm^2$ viewing areas on each excised leaf or on randomly selected upper and lower leaves of each terminal provided at each change of food. In the Iowa and Ontario studies, either all leaves or a random sample of leaves were removed from the potted plant after each larval transfer and brought back to the laboratory, where pollen grains were counted in randomly selected 0.25- or 1-$cm^2$ sections of each leaf. In the Ontario studies, each leaf was also carefully enclosed in strips of contact paper to ensure that no pollen was lost and counts in comparable viewing areas were made on the contact paper as per the study by Stanley-Horn et al. (2001). In all studies, the number of anthers was recorded for each leaf viewed for pollen. Because food was not provided to each cohort on a strict daily basis, the density of pollen grains per square centimeter to which each cohort was exposed was calculated as a weighted average of each pollen count multiplied by the number of days each leaf, terminal, or potted plant was exposed to larvae.

**Data Analysis.** All data sets were tested before analysis for normality and homogeneity of variances using Spearman's rank correlation and Shapiro-Wilk's $W$ test. Analysis of variance (ANOVA) using the mixed model procedure (SAS Institute 1996) was performed for each bioassay time on the combined data set without transformation. The model included the fixed factor for hybrid type (non-Bt, event BT11 hybrids, event MON810 hybrids). Respective nonexpressing lines for Bt events were pooled into one non-Bt group. The data from each study were treated as a completely randomized design because different layouts of replicated plots were used. The study location was added to the model as a random effect to provide a broader inference relevant to the general population of cornfields similar to those studied. A cohort-within-replicate random effect was also added to account for subsampling. The Satterthwaite option was used to compute degrees of freedom of the unbalanced design of the combined data. Contrast tests were conducted to examine response differences between each Bt event and non-Bt hybrids, BT11 and MON810 hybrids, and the combined Bt events and non-Bt hybrids.

## Results

**Pollen and Anther Deposition.** Pollen deposition on milkweed leaves fed to individual cohorts ranged from 8 to 651 grains/$cm^2$. Mean densities for the Ontario 2001, Maryland 2001, Iowa 2002, Maryland 2002, and Ontario 2002 studies were 188, 249, 158, 122, and 169 pollen grains/$cm^2$, respectively. Pollen deposition from sweet corn (Maryland 2001) was ~57% higher than the pollen deposition from field corn pooled over the other hybrids. Densities during larval development in bioassays initiated at 3–4 and 6–7 d after initial

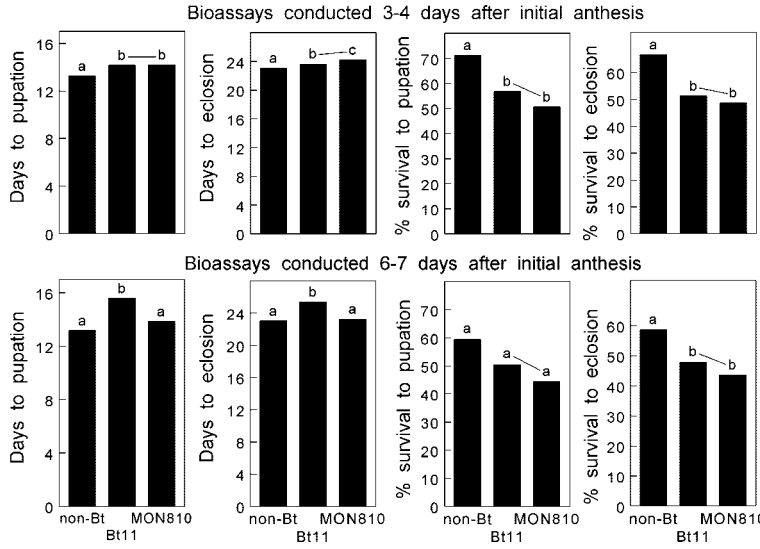


**Fig. 1.** Developmental time and percentage survival to pupation and eclosion of larval cohorts reared on leaves or whole milkweed plants exposed to BT11, MON810, and non-Bt corn during anthesis. Means are based on data pooled over five studies (Maryland, 2001 and 2002; Ontario, 2001 and 2002; Iowa, 2002) conducted on days 3–4 and 6–7 after initial anthesis. Mean bars within each graph with a different letter are significantly different ($P < 0.05$). Bt hybrid bars connected by a line between letters denote that the pooled Bt effect was significantly different from the non-Bt response.

anthesis averaged 163 and 170 pollen grains/cm$^2$, respectively. Pollen deposition on milkweeds within the non-Bt, BT11, and MON810 hybrids averaged 163, 155, and 174 grains/cm$^2$ during the first bioassay and 172, 173, and 158 grains/cm$^2$ during the second bioassay, respectively. There were no statistically significant differences in pollen densities between assays or among hybrid types. The number of anthers was positively correlated with the amount of pollen deposited on leaves ($r = 0.286$; $P < 0.001$). Anther densities were statistically the same during both assay times but significantly different among hybrids ($F = 5.25$; df = 2,50.9; $P = 0.008$). Deposition on milkweeds within the non-Bt, BT11, and MON810 hybrids averaged 1.3, 1.1, and 1.8 anthers/leaf, respectively. Specifically, the only significant difference among hybrids was between the two Bt events.

**Development Time.** The number of days required for first instars to develop to eclosion averaged 22.7 d in the Maryland studies, where larval cohorts were reared in the laboratory at 24°C. Mean developmental time was slightly longer (24.2 d) for cohorts reared in the field on potted plants in the Iowa and Ontario studies. Continuous exposure to Bt pollen during larval development had a significant effect on developmental time to pupation ($F = 6.12$; df = 2,36.2; $P = 0.005$) and to eclosion ($F = 9.43$; df = 2,121; $P < 0.001$), when first instars were initially exposed at 3–4 d after the onset of anthesis (Fig. 1). Development to adult emergence was prolonged by 0.6–1.2 d compared with larval growth on milkweed leaves with non-Bt pollen. Both Bt events had a significant effect on development time, although larvae exposed to MON810 pollen were significantly slower to develop to adults than those exposed to BT11 pollen ($F = 4.33$; df = 2,122; $P = 0.04$). Developmental time was similarly affected when larvae were initially exposed to Bt pollen at 6–7 d during anthesis (Fig. 1). Results of this second bioassay showed average delays of 0.7–2.4 d to pupation ($F = 3.55$; df = 2,27.8; $P = 0.04$) and 0.2–2.4 d to eclosion ($F = 4.34$; df = 2,25.7; $P = 0.02$). Unlike the first assay results, however, contrast tests revealed no significant development effects on larvae exposed to event MON810 pollen but significant delays to pupation ($F = 6.77$; df = 1,26; $P = 0.015$) and eclosion ($F = 7.5$; df = 1,22.7; $P = 0.012$) for larvae exposed to BT11 pollen. The Ontario 2001 study included only cornfields of event BT11, and developmental time was consistently 2–2.5 d longer because of cooler temperatures. Furthermore, the Iowa study did not include the second assay. Thus, the development response to BT11 pollen during the second bioassay may be exaggerated relative to the slight delays from the MON810 pollen.

**Survival.** Overall survival of larval cohorts reared to eclosion under laboratory conditions in the Maryland studies was significantly higher (66.1%) compared with survival of cohorts reared on caged plants in the field (44.6%). Natural mortality factors such as weather and exposure to pathogens and predators probably accounted for the difference, even though the potted plants were caged in the field. Continuous exposure of first instars to naturally deposited densities of Bt pollen beginning at days 3–4 of anthesis had a significant effect on survival to pupation ($F = 8.49$; df = 2,72.8; $P < 0.001$) and eclosion ($F = 7.22$; df = 2,72.4; $P < 0.001$; Fig. 1). Mean survival to pupation was 71.2, 56.9, and 50.6% for larval cohorts exposed to

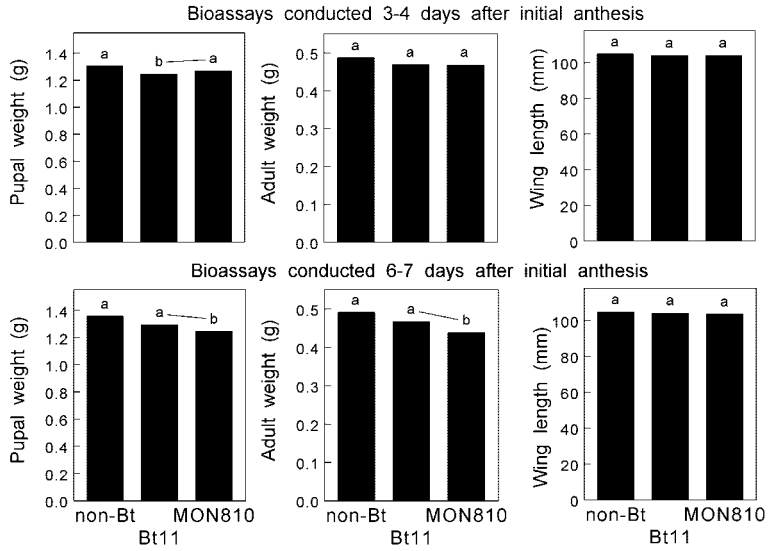


**Fig. 2.** Pupal weights, adult weights, and wing lengths of monarch butterflies reared from larvae on leaves or whole milkweed plants exposed to BT11, MON810, and non-Bt corn during anthesis. Means are based on data pooled over five studies (Maryland, 2001 and 2002; Ontario, 2001 and 2002; Iowa, 2002) conducted on days 3–4 and 6–7 after initial anthesis. Mean bars within each graph with a different letter are significantly different ($P < 0.05$). Bt hybrid bars connected by a line between letters denote that the pooled Bt effect was significantly different from the non-Bt response.

non-Bt, BT11, and MON810 pollen, respectively. Mean survival to eclosion was 66.7, 51.3, and 48.5% for larval cohorts exposed to non-Bt, BT11, and MON810 pollen, respectively. Pupal death contributed only a small proportion to the total mortality and was not significantly different among hybrid types. This suggests that larvae exposed to Bt pollen have the same chance as unexposed larvae to reach eclosion if they are able to pupate. There were no statistical differences between Bt events ($P = 0.65$). Exposure to Bt pollen beginning at days 3–4 of anthesis resulted overall in 25% fewer larvae surviving to adults. Similar reductions in survival were observed when larvae were exposed to Bt pollen during the second bioassay initiated at 6–7 d. Mean survival to pupation was 59.3, 50.4, and 44.4% for larval cohorts exposed to non-Bt, BT11, and MON810 pollen, respectively. Although the main hybrid effect and contrasts with individual events were not significant, contrast testing of the pooled effect of Bt hybrids on development to pupation was significant ($F = 4.79$; df = 1,31.6; $P = 0.04$). Mean survival to eclosion was 58.6, 47.8, and 43.6% for larval cohorts exposed to non-Bt, BT11, and MON810 pollen, respectively. The main hybrid effect ($F = 3.14$; df = 2,82.1; $P = 0.049$) and tests contrasting each Bt event with non-Bt hybrids were significant. However, effects on survival were not statistically different between Bt events. Together, 22% fewer larvae reached the adult stage when they were continuously exposed to Bt pollen starting at 6–7 d after the onset of anthesis.

**Pupal and Adult Size.** Exposure to Bt pollen during larval development produced consistently smaller pupae and adults by weight (Fig. 2). Main hybrid effects were statistically significant only for pupal weight at the first bioassay time ($F = 4.08$; df = 2,133; $P = 0.019$). However, contrast tests of the pooled effect of Bt pollen were significant for pupal weight of cohorts exposed during both assays (3–4 d: $F = 6.47$; df = 1,133; $P = 0.012$; 6–7 d: $F = 5.85$; df = 1,75; $P = 0.018$) and for adult weight when larvae were exposed at 6–7 d of anthesis ($F = 5.85$; df = 1,75; $P = 0.018$). Adults reared from Bt pollen-exposed larvae weighed 7.9% less than those exposed to non-Bt pollen. In accordance with adult weight, larvae exposed to Bt pollen developed into adult butterflies with numerically shorter wing length but neither the main hybrid effect nor contrast tests revealed statistically significant differences. Average sex ratio was slightly in favor of females (1.1:1, female:male) but not significantly affected by hybrid type ($P = 0.40$).

## Discussion

Results of the five studies conducted over 2 yr at three locations were consistent for all endpoints, even though experimental designs and bioassay methods varied with each study. The temporal overlap of anthesis with cohorts of developing larvae was constant for each corn hybrid, but the amount of pollen and anthers shed was not controlled in the field studies. With the exception of the Maryland 2001 study, pollen densities ranging between 122 and 188 grains/cm$^2$ on milkweed plants were in agreement with the average within-field density of 170.6 grains/cm$^2$ based on several studies from different localities reported in the study by Pleasants et al. (2001). Pollen densities were not significantly different among the non-Bt and Bt hybrids during both bioassay times. Thus, larvae were exposed to within-field pollen den-

sities that are relevant to those that monarch butterfly populations would experience in the general population of cornfields. Bt corn anthers deposited on milkweeds present an additional risk to monarch butterfly larvae if they encounter and actually consume these plant parts. In this study, anther densities averaged less than two per leaf and were highly variable within a milkweed plant. A recent study (Anderson et al. 2004) showed no significant adverse effects when monarch butterfly larvae were exposed to five Bt anthers per leaf in field-cage studies. The amount of anther material eaten by a typical larva is highly variable compared with the consumption of pollen, which is distributed more evenly over the leaf surface. Thus, the presence of anthers probably contributed little to the observed effects, even though there were slightly more anthers found on milkweed leaves within MON810 hybrids. Because the long-term effects were statistically the same for both Bt events in most cases, it is reasonable to discuss the results pooled over both events.

Combined data show that continuous exposure of monarch butterfly larvae to natural deposits of Bt pollen on milkweed plants within cornfields had significant effects on larval development, larval survival, and body size of resulting pupae and adults. Because survival of larval cohorts during both bioassay times showed similar responses, the combined results indicate that 23.7% fewer first instars developing on milkweed leaves with Bt pollen during the first week of anthesis reached the adult stage. The actual outcome of this effect may not be the same if larvae were exposed to Bt pollen in the presence of natural enemies. Mortality is very high in natural monarch butterfly populations, and 92–98% of the eggs do not reach the pupal stage (Orrell 1971, Borkin 1982, Zalucki and Kitching 1982). Many causes of egg and larval mortality have been reported including plant mediated physical and chemical factors, weather events, and predation by spiders, ants, coccinellids, lacewing larvae, predaceous bugs, and wasps (Zalucki et al. 2001, Zangerl et al. 2001, Koch et al. 2003). Mortality over the entire larval developmental period averages around 80% (Zalucki and Brower 1992, Lynch and Martin 1993, Oberhauser et al. 2001, Prysby and Oberhauser 2004) compared with the 44–55% of larvae that died in these studies. Any mortality from exposure to Bt pollen would be in addition to the overall percentage if the probability of death is an independent and mutually exclusive event from all other sources of mortality. However, intoxication by ingesting Bt pollen does not cause an immediate death and reduction in total survival of monarch butterfly larvae. Toxicity to prolonged exposure from Bt pollen interacts with the joint probability of larvae exposed to all other mortality factors experienced by natural populations. Many intoxicated larvae would die anyway of some other cause. For example, assuming that the combined mortality effects were constant over all instars, the resultant larval mortality would be 84.7% if the chances of larvae dying were 80% by other mortality factors and 23.7% by Bt pollen exposure.

$$0.847 = 0.8 + 0.237 - (0.8 \times 0.237)$$

Outcomes of multiple sources of mortality can be nonadditive and result in either synergistic or antagonistic interactions in mortality (Johnson and Gould 1992, Soluk 1993, Losey and Denno 1998, Roy et al. 1998, James 2003). Intoxicated monarch butterfly larvae might be exposed to a higher risk of predation caused by their delayed development and possible reduced defense behavior. This could lead to greater mortality than the expected combined mortality illustrated in the above example. Conversely, intoxicated larvae might be less available to natural enemies because of reduced movement and altered distribution on milkweed plants (Jensen et al. 2001). For risk assessment purposes in this study, however, it is assumed that the portion of larvae of the natural population that survive to adulthood will be proportionally reduced by 23.7% if exposed continuously to Bt pollen.

Exposure to Bt pollen prolonged the developmental time of larvae by an average of 1.8 d (i.e., it took 7.8% longer time to eclose). Delays in development can have indirect adverse effects on survival of both larval and adult butterflies. In many insects, prolonged growth exposes developing larvae and pupae to natural enemies for a longer period, which could lead to increased generation mortality (Clancy and Price 1987, Benrey and Denno 1997). Late-emerging adults can affect the fitness of the migratory population by increasing the risks of mortality because of early freezes and the lack of nectar sources en route to Mexico. Also, there is anecdotal evidence that butterflies on the peripheries of the overwintering colonies exhibit lower survival (K. S. Oberhauser, personal communication), and these individuals may be the ones that arrive late. These effects are not likely to occur in migratory populations if newly eclosed butterflies remain in the breeding area for a period of time before migration southward commences. Weights of both pupae and adults reared from larvae exposed to Bt pollen were significantly reduced by an average 5.5%. This difference is small compared with an 11% coefficient of variation in adult body size observed in monarch butterfly populations (Oberhauser 1997). Nonetheless, smaller body size of adult monarch butterflies has been correlated with shorter life spans and reduced fecundity (Peters 1983, Oberhauser 1997), and it also may have a negative effect on dispersal capacity, even though wing length was unaffected. Migrating monarch butterflies with larger wingspans or greater lipid reserves may be more successful in migrating to and from overwintering sites and surviving the winter (Masters et al. 1988, Van Hook 1993, Alonso-Meija et al. 1997). All of these fitness disadvantages could negatively impact monarch butterfly populations, but their ecological significance must be considered in light of the actual exposure risk and small magnitude of differences observed.

According to the study by Wolt et al. (2003), a quantitative risk assessment provides an objective way to account for the ecological risk of Bt corn pollen on the monarch butterfly. The worst-case characteriza-

**Table 2. Parameter estimates for probability of harm of monarch butterfly larvae from Bt corn pollen within corn fields in states and provinces of the Corn Belt that constitute 50% of the eastern North American monarch butterfly population**

| State | Parameter estimates[a] for risk of exposure | | | | | | Probability of harm (*R*) |
|---|---|---|---|---|---|---|---|
| | l | o | a | m | $P_e$ | $P_t$ | |
| IA | 0.560 | 0.253 | 0.37 | 0.193 | 0.010117 | 0.237 | 0.002398 |
| IL | 0.423 | 0.119 | 0.24 | 0.178 | 0.002150 | 0.237 | 0.000508 |
| IN | 0.376 | 0.130 | 0.09 | 0.102 | 0.000449 | 0.237 | 0.000106 |
| KS | 0.119 | 0.001 | 0.30 | 0.038 | 0.000001 | 0.237 | 0.000000 |
| KY | 0.132 | 0.015 | 0.19 | 0.018 | 0.000007 | 0.237 | 0.000002 |
| MI | 0.211 | 0.552 | 0.21 | 0.035 | 0.000856 | 0.237 | 0.000203 |
| MN | 0.287 | 0.559 | 0.38 | 0.083 | 0.005060 | 0.237 | 0.001199 |
| MO | 0.120 | 0.008 | 0.33 | 0.055 | 0.000017 | 0.237 | 0.000004 |
| NE | 0.393 | 0.073 | 0.41 | 0.072 | 0.000847 | 0.237 | 0.000200 |
| NY | 0.135 | 0.412 | 0.19 | 0.003 | 0.000032 | 0.237 | 0.000024 |
| OH | 0.263 | 0.229 | 0.06 | 0.090 | 0.000325 | 0.237 | 0.000077 |
| ON | 0.300 | 0.350 | 0.35 | 0.002 | 0.000074 | 0.237 | 0.000017 |
| PA | 0.220 | 0.313 | 0.19 | 0.019 | 0.000249 | 0.237 | 0.000059 |
| SD | 0.229 | 0.420 | 0.51 | 0.063 | 0.003090 | 0.237 | 0.000733 |
| WI | 0.261 | 0.325 | 0.23 | 0.045 | 0.000878 | 0.237 | 0.000208 |
| WV | 0.026 | 0.105 | 0.19 | 0.005 | 0.000003 | 0.237 | 0.000024 |
| Average | 0.253 | 0.241 | 0.265 | | | 0.237 | |
| Total | | | | 1.000 | 0.024160 | | 0.005763 |

[a] l, proportion of monarch butterflies from corn; o, overlap of pollen shed with susceptible larval stages; a, adoption rate of Bt corn; m, proportion of land area planted to corn in each state; $P_e$, probability of exposure; $P_t$, probability of toxicity.

tion of risk to the monarch butterfly will depend on the proportion of the breeding population that overlaps with anthesis by 50–75% and is exposed continuously as developing larvae to within-field pollen and anther densities similar to those reported here. In a previous study that examined risk associated with short-term exposures (Sears et al. 2001), we used an accepted statement of risk:

$$R = P_e \times P_t$$

where R is risk, $P_e$ is probability of exposure, and $P_t$ is probability of toxicity.

Our previous data indicated no mortality resulting from short-term exposure (4 or 5 d) of small larvae to Bt corn pollen (Stanley-Horn et al. 2001), and larger larvae are relatively tolerant of Bt Cry proteins (Hellmich et al. 2001). Because our current field data consistently showed a survivorship impact from continuous exposure to Bt pollen and anthers on milkweed plants, we can assign a probability of toxic effect ($P_t$) of 23.7% or 0.237.

To develop a measure of exposure for monarch butterfly larvae to Bt corn pollen, the procedure used in a previous study by Sears et al. (2001) has been adopted and updated. Components of exposure (Table 2) include the land base devoted to corn in a given region in which monarch butterflies may develop, the degree to which Bt corn has been adopted by growers in that region, an estimate of the overlap of pollen shed with sensitive larval stages of the monarch butterfly, and a weighting factor for each region that describes its proportional contribution to monarch butterfly populations in the Corn Belt. The values for the estimated proportion of the second generation monarch butterfly population exposed to corn pollen in each region were derived from a modeling system (Calvin et al. 2000). The system consists of three interacting components: a weather model, a corn developmental model, and a monarch butterfly developmental model. The weather model provides spatially interpolated temperature data for each 1-km$^2$ plot across the Corn Belt to calculate degree-days as input into the developmental models. The corn developmental model simulates corn growth progression based on planting date, corn maturity group, and degree-day accumulation at a base threshold of 10°C and an upper threshold of 30°C. Anthesis was estimated to occur when 52% of the total degree-days needed for maturity is reached. The monarch butterfly developmental model consists of a function for arriving migrating adults at each geographic location in North America and a series of developmental equations that are temperature driven to move the population through each development stage. Developmental equations used in this model were derived from Zalucki (1982). The date of arrival of the northward migration at any latitude is dictated by the altitude angle of the sun. This relationship was interpreted from 4 yr (1996–1999) of observations of first monarch butterflies seen by the public. The modeling system was verified by comparing the simulated and observed monarch butterfly stages and pollination periods for selected sites (Calvin et al. 2001). In this study, model simulations overlaid regional periods of corn anthesis and susceptible monarch butterfly life stages (first, second, and third instars) using 30 yr (1973–2002) of weather data from 278 stations across the Corn Belt. The simulations estimated the proportion of monarch butterfly larvae that were exposed to 50% or more of the shed pollen. This estimation was done by partitioning the local anthesis period and monarch butterfly population into weighted daily cohorts, summing the daily corn and monarch butterfly cohorts into an overall percentage, and highlighting the percentage when it equaled or exceeded the 50% exposure threshold. The simulated percentages for individual stations

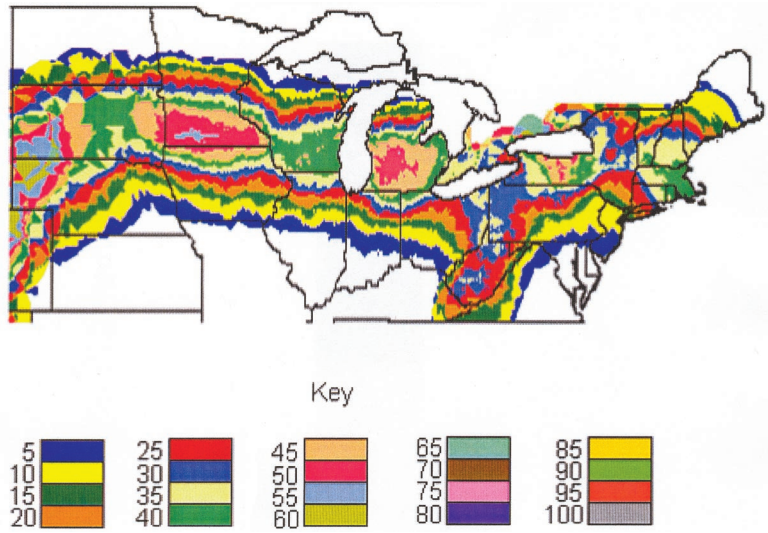


**Fig. 3.** Model predictions of the percentage of the second generation of monarch butterfly exposed as first, second, and third instars to the first 6 d of corn anthesis.

were interpolated into a 10-km-resolution landscape map (Fig. 3). The first monarch butterfly generation in the Corn Belt was not considered, because it does not overlap with anthesis.

The breeding range for monarch butterfly populations is extensive across eastern North America. Wassenaar and Hobson (1998), in a study of the range of monarch butterflies in North America, used isotope analysis of overwintered individuals to show that 50% of the breeding population develops in 15 states and 1 Canadian province. This area, stretching from Kansas/Nebraska to New York, is nearly contiguous with the Corn Belt, in which 88% of North American corn is produced. Corn production varies considerably from state to state, and the proportion of the landscape devoted to corn production also varies considerably (National Agricultural Statistics Service 2003).

The state of Iowa may be considered an upper regional boundary for estimates of the overlap of monarch butterfly larvae and production of Bt corn pollen. It is in the middle of the Corn Belt, much of its agricultural land base is devoted to producing corn, and Bt corn varieties have been widely adopted. From Table 2, the product of three factors (l = land base, o = overlap of pollen shed, and a = adoption rate of Bt corn) equals the risk of exposure for that state. Risk of harm to monarch butterfly populations is then defined as:

$$R = (l \times o \times a) \times P_t$$

or

$$R = (0.560 \times 0.253 \times 0.37) \times 0.237 = 0.0124$$

or 1.2% of the Iowa population would not survive exposure to Bt pollen. If adoption of Bt corn in Iowa increased to the legal limit of 80%, risk to monarch butterflies would increase to 0.0261 (2.6% of the second generation would not survive). Iowa's contribution to the impact of Bt corn pollen on monarch butterflies breeding throughout the Corn Belt is designated as m or 19.3, the proportion of the land planted to corn. When all 15 states and the province of Ontario are considered, the risk to monarch butterfly populations is 0.00576, or 0.6% of the total of monarch butterflies breeding in the Corn Belt (Table 2). These estimates are comparable with those in the study by Sears et al. (2001) and indicate that the overall conclusions concerning risk to monarch butterfly populations are largely unchanged. In a worst-case scenario, assuming that the fitness effects on monarch butterfly development and body size also result in adult toxicity or nonreproduction, 2.4% of the breeding population in the Corn Belt would be at risk. As mentioned previously, these estimates relate to 50% of the breeding population in North America. Monarch butterflies outside the Corn Belt are relatively unaffected because of the low acreage of corn and low percentage of overlap of larvae with anthesis.

Long-term exposure of monarch butterfly larvae throughout their development to Bt corn pollen is detrimental to only a fraction of the breeding population because the risk of exposure is low. When this impact is considered over the entire range of the Corn Belt, the ecological outcome is very small. Moreover, Bt corn adoption is associated with lower insecticide use against target Lepidoptera (Pilcher et al. 2002), and most insecticides are acutely toxic to larvae occurring in corn or in other crops that provide habitat for monarch butterfly populations. In field bioassays, larvae died within hours after feeding on milkweeds exposed to a single application of a pyrethroid insecticide (Stanley-Horn et al. 2001). Monarch butterfly populations also have shown remarkable resiliency to catastrophic mortality events, such as the unusual cold and wet storms in January 2002, which killed an esti-

mated 80% of the wintering adults in the Mexican highlands (Taylor 2002). Also, drought conditions in the Corn Belt in 2003 were predicted to have an adverse effect on the size of the migratory population. However, based on the total area occupied by monarch butterflies in Mexico during the 2003 wintering season (Quinn 2003) and fall sightings in 2003 (Taylor 2003), populations have recovered to near average levels. Considering that monarch butterflies can rebound from such events and produce historically average migratory populations despite high mortality during the breeding season, it is likely that Bt corn will not affect the sustainability of monarch butterfly populations in North America.

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