

Impact of *Bt* corn pollen on monarch butterfly populations: A risk assessment

Mark K. Sears^{*†}, Richard L. Hellmich[‡], Diane E. Stanley-Horn^{*}, Karen S. Oberhauser[§], John M. Pleasants[¶], Heather R. Mattila^{*}, Blair D. Siegfried^{||}, and Galen P. Dively^{**}

^{*}Department of Environmental Biology, University of Guelph, Guelph, ON, Canada N1G 2W1; [†]United States Department of Agriculture–Agricultural Research Service, Corn Insects and Crop Genetics Research Unit and Department of Entomology, Iowa State University, Ames, IA 50011; [‡]Department of Ecology and Evolutionary Biology, University of Minnesota, St. Paul, MN 55108; [§]Department of Zoology and Genetics, Iowa State University, Ames, IA 50011; [¶]Department of Entomology, University of Nebraska, Lincoln, NE 68583; and ^{**}Department of Entomology, University of Maryland, College Park, MD 20742

Edited by M. R. Berenbaum, University of Illinois at Urbana–Champaign, Urbana, IL, and approved August 17, 2001 (received for review June 28, 2001)

A collaborative research effort by scientists in several states and in Canada has produced information to develop a formal risk assessment of the impact of *Bt* corn on monarch butterfly (*Danaus plexippus*) populations. Information was sought on the acute toxic effects of *Bt* corn pollen and the degree to which monarch larvae would be exposed to toxic amounts of *Bt* pollen on its host plant, the common milkweed, *Asclepias syriaca*, found in and around cornfields. Expression of Cry proteins, the active toxicant found in *Bt* corn tissues, differed among hybrids, and especially so in the concentrations found in pollen of different events. In most commercial hybrids, *Bt* expression in pollen is low, and laboratory and field studies show no acute toxic effects at any pollen density that would be encountered in the field. Other factors mitigating exposure of larvae include the variable and limited overlap between pollen shed and larval activity periods, the fact that only a portion of the monarch population utilizes milkweed stands in and near cornfields, and the current adoption rate of *Bt* corn at 19% of North American corn-growing areas. This 2-year study suggests that the impact of *Bt* corn pollen from current commercial hybrids on monarch butterfly populations is negligible.

Concern regarding nontarget effects of transgenic crops containing transgenes from the organism *Bacillus thuringiensis* (*Bt*) arose after the publication by Losey *et al.* (1) on the potential risk of corn pollen expressing lepidopteran-active Cry protein to the monarch butterfly, *Danaus plexippus* L. However, the U.S. Environmental Protection Agency (EPA) concluded in an earlier report that the potential impact of *Bt* corn pollen, which contains variable amounts of Cry protein, on sensitive larvae of Lepidoptera was negligible because of factors that limit environmental exposure (2). Clarification of the risk posed by *Bt* corn pollen to monarch butterflies can now be undertaken because of the data reported in this issue of PNAS (3–6) that address exposure and toxic effects of *Bt* corn pollen.

The research contributions reported here represent a collaborative effort established to specifically address the question of risk associated with *Bt* corn pollen to the monarch butterfly. In December 1999, the EPA issued a data call-in requesting industry, researchers and all interested parties to submit information and comments by March 2001 for use in evaluation and potential reregistration of corn hybrids containing Cry proteins (<http://www.epa.gov/pesticides/biopesticides/otherdocs/bt.dci.htm>). The U.S. Department of Agriculture Agricultural Research Service (USDA–ARS) sponsored a Monarch Research Workshop in February 2000 to identify research priorities regarding *Bt* corn and monarch butterflies, to establish cooperation among researchers, and to respond to the EPA request for data. A request for proposals based on workshop priorities was announced in April, after which a steering committee, including Adrianna Hewings (USDA–ARS), Eldon Ortman (Purdue University), Mark Scriber (Michigan State University), Eric Sachs (Monsanto), and Margaret Mellon (Union of Concerned Scientists) selected projects to be funded. Funding came from a grant

pool provided by ARS and the Agricultural Biotechnology Stewardship Technical Committee (7). The guiding principles for problem formulation followed by the consortium were the elements of risk assessment that underlie the approach by EPA to ecological risk assessment (<http://www.epa.gov/NCEA/ecorsk.htm>).

Only three papers concerning the impact of *Bt* corn pollen on nontarget Lepidoptera have been published (1, 8, 9), and they are limited in their application to risk assessment (7). For example, the dose of pollen was not specified in the exposure study by Losey *et al.* (1), and the study by Jesse and Obrycki (8) used pollen collection and handling techniques that probably resulted in contamination from corn anthers or tassel fragments, which contain significantly higher levels of Cry protein than the pollen (3). Finally, neither study addressed the spatial or temporal potential for exposure by monarch larvae to pollen in cornfields, thereby precluding a risk assessment.

In this paper, we develop a weight-of-evidence approach to the risk of exposure of monarch larvae to *Bt* corn pollen and the impact of such exposure on populations of the monarch butterfly in eastern North America by using recently published information based on collaborative research by scientists in the U.S. and Canada (3–6). We use an approach to risk assessment that has been performed for many nontarget species in relation to pesticides (10–14), industrial by-products (15, 16), and other potential toxicants found in the environment (17). The approach to this process is consistent, well documented, and standardized (<http://www.epa.gov/NCEA/ecorsk.htm>). It requires consideration of both the expression of toxicity and the likelihood of exposure to the toxicant as the basic components for a risk assessment procedure.

Materials and Methods

Hazard Identification. Toxicity of purified *Bt* proteins to larval stages of butterflies and moths is well known (18, 19). Studies conducted on the use of *Bt* sprays in forests for gypsy moth control have shown that Cry proteins can adversely affect nontarget Lepidoptera (20, 21). Field data from these studies indicated a temporary reduction in lepidopteran populations during prolonged *Bt* use, although widespread irreversible harm was not apparent (22). Lepidopteran-active *Bt* protein expressed in pollen of *Bt* corn hybrids may pose a risk to sensitive species, such as monarch butterflies, in or near cornfields during anthesis (1, 8). Milkweeds, *Asclepias* spp., and especially common milk-

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: *Bt*, *Bacillus thuringiensis*; USDA–ARS, U.S. Department of Agriculture Agricultural Research Service; EPA, U.S. Environmental Protection Agency; LOEC, lowest-observable-effect-concentration.

[†]To whom reprint requests should be addressed. E-mail: msears@evb.uoguelph.ca.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

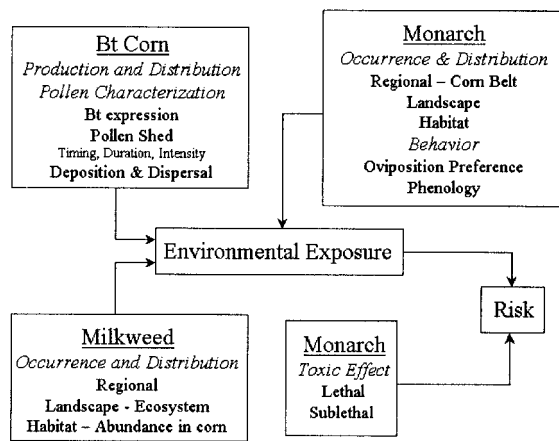


Fig. 1. Conceptual model of components of risk assessment of the impact of *Bt* corn pollen on populations of the monarch butterfly.

weed, *Asclepias syriaca* (L.), are the sole larval food source for monarch butterfly larvae and are abundant throughout the corn-growing regions of North America (23). As such, hazard from *Bt* corn pollen deposited on milkweed leaves warrants consideration of its ecological risk to monarch populations.

Conceptual Model. Risk assessment requires knowledge of four essential components: (i) hazard identification, (ii) nature of dose response to a toxin, (iii) probability of exposure to an effective dose, and (iv) characterization of risk (24). Components of a risk assessment approach as applied to the case of *Bt* corn and monarch butterfly are depicted in Fig. 1. *Bt* proteins expressed in corn plant tissues can bring about specific reactions in the gut of lepidopteran larvae (25), including nontarget larvae that consume *Bt* corn pollen. The magnitude of the reaction will depend on the type of protein produced by various transgenic events of hybrid *Bt* corn, the amount of protein expressed in pollen grains from different events, the amount of pollen consumed by larvae of different developmental stages, and the susceptibility of larvae to the *Bt* protein. That a hazard may exist was suggested by Losey *et al.* (1). Characterization of toxic effects is necessary to establish the first component of risk. Laboratory and field assays of lethal and sublethal toxicity resulting from exposure to doses of *Bt* pollen are required to establish toxicity thresholds for comparison against the dose encountered within the environment. These toxicity thresholds will vary based on expression levels for individual *Bt* corn events in conjunction with environmental factors determining ecological exposure.

Consideration of risk as a function of exposure and effect requires that lines of evidence be established in four areas of inquiry: (i) Is there some density of *Bt* pollen on milkweed leaves that represents a lethal or sublethal threat to monarch larvae or later stages of development? (ii) What proportion of *Bt* pollen deposited on milkweed leaves in and around cornfields exceeds the toxicity threshold for larvae of monarchs? (iii) What proportion of monarch populations use milkweed in and near cornfields? (iv) What is the degree of overlap between the phenological stages of monarch larvae and corn anthesis over the shared range of these species? Answers to these questions are possible on the basis of data summarized in the four companion papers (3–6).

Results

Characterization of Effects of *Bt* Corn Pollen. The Cry1A proteins expressed in most commercial *Bt* corn hybrids are toxic to the monarch butterfly (3). Mortality, expressed as LD₅₀, was estimated at 3.3 ng of protein/ml diet, whereas growth inhibition (EC₅₀) was estimated to be 0.76 ng/ml (2). However, the

expression of Cry1Ab endotoxin within pollen of various events varies considerably depending on the promoter gene involved (26). Expression is greatest in event 176 *Bt* corn (1.1–7.1 μg/gm pollen) (<http://www.inspection.gc.ca/english/plaveg/pbo/dd9609e.shtml>, <http://www.epa.gov/pesticides/factsheets/fs006458t.htm>), a line that is being phased out through 2003. This event exceeds, by nearly two orders of magnitude, protein expression in events Bt11 and Mon810 (0.09 μg/gm pollen) (<http://www.epa.gov/scipoly/sap/2000/october/>), which is near the current level of detection by immunoassay.

Laboratory bioassays of pollen fed to first instar monarchs for 4 days on leaf discs or whole detached leaves of common milkweed, *A. syriaca*, indicate that pollen from event 176 *Bt* corn causes mortality and sublethal effects, such as growth inhibition, at very low concentrations (3). Mortality was evident, even at low densities of pollen, but was variable because of the typical reaction by larvae to *Bt* proteins of feeding cessation followed by extended periods of time before death (3). Growth inhibition, a more sensitive measure of protein intoxication, could be detected at 5–10 grains/cm². Pollen from all other events, including Mon810 and Bt11 corn hybrids as well as events not presently grown, such as Dbt418, Cbh351, and Tc1507 (expressing Cry1Ac, Cry9C, and Cry1F proteins, respectively) did not demonstrate lethal or sublethal effects, even at densities above 1,000 pollen grains/cm² (3). These data were used to establish a no-observable-effect-level for growth inhibition of larvae for event 176 pollen and for Bt11 and Mon810 pollen.

Five field bioassays were undertaken to determine the outcome of exposure of larvae under field conditions on milkweed plants growing or placed in the field. Field studies performed in Iowa, Maryland, New York, and Ontario incorporating natural levels of pollen from *Bt* corn plants (6) demonstrated no acute effects of Bt11 and Mon810 corn pollen on survival or growth of monarch larvae. However, impacts of event 176 pollen were observed. In Iowa, reduced weight gain was noted for larvae exposed to event 176 pollen on milkweeds within cornfields at a density of 23 pollen grains/cm². Both survival and weight gain were affected in Maryland, where a series of assays using milkweed leaves collected from plants in an event 176 cornfield were carried out over the pollen-shed period. After 6 and 9 days of accumulation of pollen within the cornfield, pollen concentrations on milkweed leaves reached an average of 67 and 161 grains/cm². Survival of first instars was reduced by 60 and 51%, respectively, compared with larvae exposed to milkweed leaves collected from outside *Bt* cornfields. Weight gain of survivors was reduced because of consumption of *Bt* pollen, but only significantly so after exposure to pollen accumulated over a 6-day period (6).

In a separate field trial in Maryland, effects on survival and growth of first instar monarchs on leaves of milkweed within a field of a sweet corn hybrid expressing Bt11 endotoxin were evaluated and compared with the effects of residues after applications of a pyrethroid insecticide. Survival of larvae that fed on insecticide-treated milkweed leaves from within the cornfield was low (0–10%). Survival also was influenced significantly (65–79%) by insecticide that drifted onto milkweeds leaves 3 m outside the field. In contrast, survival of larvae exposed to leaves taken from within both *Bt* and non-*Bt* corn plots ranged from 80 to 93%, and there were no significant differences in larval survival between these two plots (6).

Characterization of Exposure to *Bt* Corn Pollen. Exposure depends on (i) phenological overlap between monarch populations and corn anthesis, (ii) spatial overlap between milkweeds used by monarchs and cornfields, and (iii) pollen densities encountered on leaves of milkweed plants in and near cornfields.

Phenological Overlap. Pollen from corn plants within a particular field is shed over a period of 1–2 weeks between mid-July and

mid-August during the season, whereas larvae develop over a more prolonged period. Potential for exposure of susceptible stages of monarch larvae to corn pollen depends on synchrony of their development with pollen shed of corn plants. Locations in four corn-growing regions were monitored for phenological development of monarch populations and anthesis (4). These locations were established in Iowa, Maryland, Minnesota/Wisconsin, and Ontario. Overlap of the more susceptible stages of monarchs, primarily first and second instars, with pollen shed was considered for purposes of risk assessment.

The presence of susceptible larvae at the time of corn anthesis varied considerably across the regions studied (4). In the more northern locations (Minnesota/Wisconsin and Ontario), about 40 and 62% of the larvae overlapped with pollen shed, respectively, whereas in areas further south (Iowa and Maryland), about 15 and 20% of the larval stages overlapped, respectively. Data from a computer simulation of monarch phenology and corn development support the general observation that overlap increases at higher latitudes across the Corn Belt (D. Calvin, Department of Entomology, Pennsylvania State University, University Park, PA, personal communication). Projected overlap from these simulations is likely overestimated because in the model, 30-year average temperature data were used, whereas our in-field measurements were made during 2000 in three to five specific fields at each of four locations.

Spatial Overlap. Density of milkweed stands in cornfields compared with nonagricultural lands and data on the proportion of the landscape in corn and nonagricultural lands provided a basis on which to determine the proportion of the milkweed population that was in cornfields (4). In all locations, densities were higher in nonagricultural lands than in cornfields, but the range of difference was considerable. In Minnesota/Wisconsin and Iowa, the density of milkweed was approximately four to seven times greater in nonagricultural fields than in cornfields, whereas in Ontario, the density was up to 115 times greater. In areas where corn is more intensively cultivated, as in Iowa and southern Minnesota/Wisconsin, less nonagricultural land exists, and the overall proportion of milkweed on a landscape basis is higher in cornfields and other crop lands than in nonagricultural land. In regions of the corn-growing area where mixed habitats are more common, such as in Maryland and Ontario, milkweeds are more abundant in the nonagricultural landscape and provide proportionately greater habitat than those in cornfields (4).

The likelihood of monarch larvae feeding on milkweed plants in cornfields depends not only on what proportion of milkweeds are in cornfields but also on the relative usage by monarchs of milkweeds in cornfields relative to milkweeds in other habitats. Observations from the four regions studied indicated that monarch butterflies locate and lay eggs on milkweeds in corn despite the canopy of the crop obscuring the milkweeds. Sampling done in cornfields and in nonagricultural land in these areas suggests that egg densities per plant are higher in corn in Iowa and Minnesota/Wisconsin but are the same in cornfields and nonagricultural lands in Maryland and Ontario (4). In Iowa, egg densities were higher also in soybean fields than in nonagricultural areas.

Pollen Densities Encountered. Dispersal of corn pollen was described by Raynor *et al.* (27), who demonstrated deposition of pollen as much as 60 m from field edges. Because of the rapid decline in concentration of pollen from the field margin outward, risk assessment concerns are focused on the concentration of pollen on milkweed leaves within the cornfield and those leaves found outside the field up to 5 m from the field edge. During the period of pollen shed, samples of pollen were collected on sticky trap surfaces and on milkweed leaves (5). Samples were taken at various distances within and beyond the margins of cornfields to estimate the concentration of pollen that could be encountered

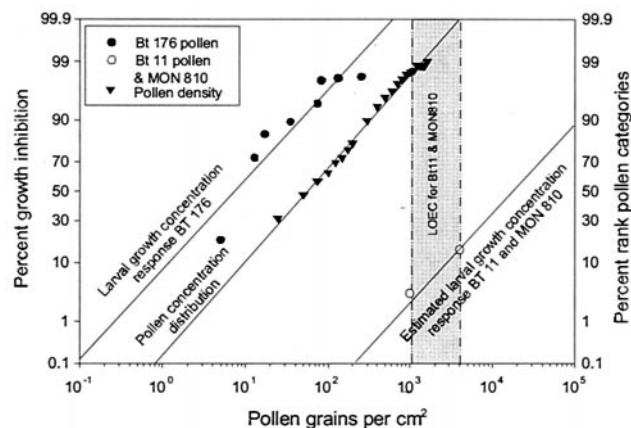


Fig. 2. Percent growth inhibition at 96 h for monarch larvae exposed to pollen from event 176 corn hybrids and for larvae exposed to pollen from Bt11 corn hybrids. These two response curves are compared with the average cumulative proportion of pollen on milkweed leaves as recorded within Iowa and Ontario cornfields during 1999–2000.

by monarch larvae. Data from three locations, Iowa, Maryland, and Ontario (5), demonstrated a 5-fold reduction in concentration of pollen from just within the edge of the cornfield to about 2–3 m distant. Within-field densities across the different studies averaged between 65 and 425 pollen grains/cm² on milkweed leaves at the peak of corn anthesis, with an average of 171 grains/cm². From these data, frequency histograms were derived to determine the likelihood of encounter by first or second instars of different concentrations of pollen within and outside cornfields. These frequency distributions can be used to determine the probability of a larva encountering a toxic pollen dose.

Risk Characterization. To determine risks to monarch larvae associated with *Bt* corn pollen, two components of greatest significance are: (i) the frequency with which effective environmental concentrations exceed the thresholds for mortality or sublethal effects, such as growth inhibition, of each *Bt* pollen type, and (ii) the proportion of monarch larval populations in eastern North America that are exposed to toxic levels of *Bt* pollen.

Probability of Toxicity. It is clear from both laboratory and field-based studies (3, 6) that pollen from the dominant commercial *Bt* corn hybrids (Mon810 and Bt11) does not express Cry1Ab protein to a level that will impact monarch populations to any significant degree. Hellmich *et al.* (3) suggested a conservative lowest-observable-effect-concentration (LOEC) be established for these hybrids at 1,000 pollen grains/cm² of milkweed leaf surface on the basis of a combined analysis of laboratory bioassays exposing larvae to 1,000–1,600 of pollen grains/cm². Growth inhibition was evident for larvae exposed to event 176 pollen at 5–10 grains/cm², the lowest dose where activity was noted by Hellmich *et al.* (3), therefore the effective environmental concentrations for event 176 corn pollen will frequently exceed this threshold in fields where it is planted.

Probabilities of toxicity for events 176, Bt11, and Mon810 pollen are depicted in Fig. 2 as a dose–effect relationship for exposure of larvae to pollen plotted on log-probability scales following methods accepted by the EPA (<http://www.epa.gov/NCEA/ecorisk.htm>). Growth inhibition of first instar monarchs in response to increasing concentrations of event 176 pollen, as reported by Hellmich *et al.* (3), is illustrated, with a no-observable-effect-level at 5–10 pollen grains/cm². In comparison, a hypothetical response curve for Bt11 and Mon810 pollen is depicted by using the same slope parameter for the event 176

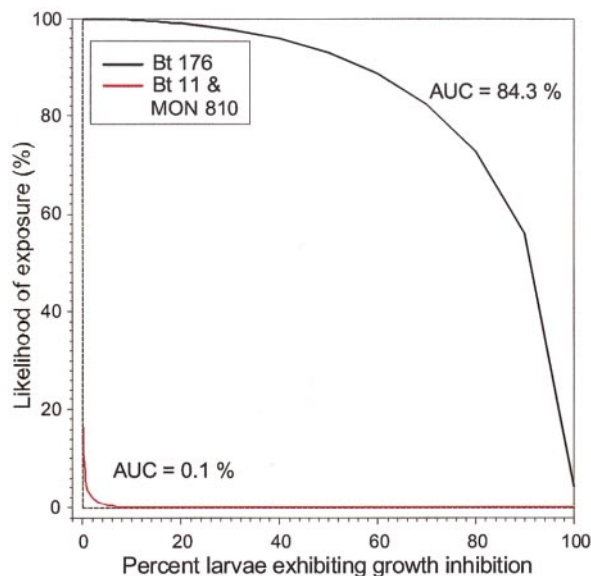


Fig. 3. Joint probability curves that compare the likelihood of exposure of monarch larvae to events 176 or Bt11 corn pollen with the percent of larvae demonstrating inhibition of growth. The estimated area under the curves provides a comparative measure of the impact that exposure to each type of pollen poses.

response (the Cry1Ab protein is identical in each event) and with a LOEC established, for sake of argument, as a range between 1,000 and 4,000 grains/cm². Pollen deposition on milkweed leaves during 1999–2000 (5) is represented on a separate scale in a cumulative frequency occurrence curve.

It is apparent from Fig. 2 that significant overlap of commonly encountered concentrations of pollen from event 176 hybrids with doses necessary for growth inhibition (and mortality) would likely occur within or near cornfields. For example, over 50% of a cohort of first instars exposed to 10 pollen grains/cm² on milkweed leaves would be expected to exhibit growth inhibition. When compared with the pollen deposition curve, 90% of the samples of milkweed leaves examined in our study of pollen deposition had a density at or above this level. By contrast, the LOEC range for Bt11 and Mon810 of 1,000–4,000 pollen grains/cm² would be encountered by larvae in only 0.7–0.1% of natural in-field situations. Pollen deposition estimated from any single sample over two seasons never exceeded 1,600 grains/cm (3, 5). Even if the LOEC for Bt11 and Mon810 were established at the conservative concentration of 1,000 grains/cm², 99.3% of encounters by monarch larvae of pollen would be below this concentration (5).

To illustrate the potential impact of each pollen type on first instar monarchs exposed to event 176, Bt11, and Mon810 pollen within cornfields, a joint probability curve (Fig. 3) was constructed (<http://www.epa.gov/NCEA/ecorisk.htm>; ref. 14). The likelihood of exposure is plotted against the incidence of growth inhibition as derived from data in Fig. 2. For event 176 pollen, the impact would be significant, as depicted by the area under the curve (AUC, 84%). In contrast, the impact for Bt11 corn pollen (AUC, 0.1%) would be considered negligible.

Probability of Exposure. Milkweeds exist in cornfields across most of the North American corn-growing regions, and monarch butterflies use this resource as a host for their offspring during the period of pollen shed (4). Quantification of the proportion of monarch populations in each region potentially exposed to *Bt* corn pollen is difficult to ascertain. Our data, collected over a single season at four locations throughout the corn-producing area of North America, illustrate the various factors influencing potential exposure to *Bt*

corn pollen (4). These data are insufficient to provide a definitive estimate of exposure in most cases, but a bounding estimate is possible. Wassenaar and Hobson (28), by using isotope analysis of overwintering monarchs in Mexico, estimated that 50% of the monarch population originates within all or part of 15 states and one province that represent the central core of the North American Corn Belt (Table 1). More than 93% of North American corn is grown in this area, which extends from eastern Kansas/Nebraska to western New York. By using USDA statistics, we estimate that about 28% of crop and pasture land within this area, which together constitute the monarch breeding habitat, consists of corn (Table 1). Adoption of *Bt* corn across this area encompassing 50% of the monarch-breeding habitat was about 19% of the corn crop in 2000 (29).

Distribution of milkweeds within and around cornfields is variable across the Corn Belt. In Iowa and southern Minnesota, milkweed in cornfields represents a large proportion of the total abundance in those areas, but in Ontario and Maryland, where corn represents a smaller proportion of the landscape, milkweed in cornfields constitutes only a small proportion of its overall abundance (4). These data provide a variable picture, but by using data from Iowa, a suitable estimate of exposure to *Bt* corn pollen by monarchs can be obtained. In addition to the fact that a significant proportion of land in Iowa is devoted to corn production, a milkweed plant in cornfields was 1.7 times more likely to receive a monarch egg than a milkweed plant in nonagricultural land (4). Even though milkweed densities are approximately seven times higher in nonagricultural land than in cornfields, the proportion of monarch larvae contributed by milkweed from within cornfields is 45 times greater than that of nonagricultural land. If all of the landscape within Iowa that represents breeding habitat for monarchs is coupled with the higher per-plant egg densities on milkweeds within cornfields, the relative density of milkweeds in different habitats and the predominance of corn as a proportion of the total habitat, 56% of monarchs in Iowa are estimated to originate from within cornfields (4). This value probably differs considerably from region to region, but we do not have comparable data for other locations. From the data available (Table 1), we consider the situation in Iowa to represent the upper end of the exposure scale.

Temporal overlap of pollen shed with the presence of sensitive larvae in Iowa was 15% (4). An estimate of the probability of exposure (P_e) to *Bt* corn pollen by larvae of the final monarch generation arising within Iowa can be expressed as:

$$P_e = l \times o \times a = 0.56 \times 0.15 \times 0.25 = 0.021, \quad [1]$$

or 2.1% of the population, where P_e = probability of exposure, l = proportion of monarchs from corn, o = overlap of pollen shed with susceptible larval stages, and a = adoption rate of *Bt* corn. Because this represents an estimate of the potential exposure for Iowa, similar data would be required to estimate the probability of exposure for other locations in the Corn Belt. We do not have complete data for monarch and milkweed densities across the Corn Belt and cannot assume that relative productivity of crop and nonagricultural habitats is the same in other states. For Iowa, the proportion of the monarch population estimated to come from corn, 56%, was roughly similar to the proportion of the breeding habitat in Iowa that is corn (42%; Table 1). If we assume that this same relationship holds in other areas, we can use the proportion of corn grown relative to the total breeding habitat in other states (from Table 1) as an estimate of relative monarch production.

Estimates of these three exposure factors and the estimated contribution of each state and province to $\approx 50\%$ of the eastern North American monarch population arising from the portion of the Corn Belt, as indicated by Wassenaar and Hobson (28), provides a broad view of potential exposure (Table 2). Our estimates of overlap of the pollen-shed period in each location

Table 1. Crop area for corn production in the Corn Belt states and provinces (2000) and the relationship of *Bt* corn to monarch butterfly breeding range

State	Land area, ha (2000)			Corn as % of crop and pasture	% <i>Bt</i> corn [‡]	% <i>Bt</i> corn of crop and pasture	% crop and pasture area w/in 50% monarch breeding habitat [§]	Area w/in breeding habitat, ha	% area of 50% breeding habitat
	Arable land*	Crop and pasture*	Corn planted [†]						
IA	13,588	11,670	4,982	42.7	26	10.7	1.00	11,670	19.2
IL	12,776	10,724	4,536	42.3	14	5.9	1.00	10,724	17.7
IN	8,077	6,146	2,309	37.6	7	2.6	1.00	6,146	10.1
KS	19,730	11,613	1,377	11.9	26	3.1	0.20	2,323	3.8
KY	8,947	3,241	575	13.2	11	1.5	0.33	1,439	2.4
MI	11,826	4,229	891	21.1	8	1.7	0.50	2,115	3.5
MN	18,292	10,024	2,876	28.7	30	8.6	0.50	5,012	8.3
MO	15,874	9,986	1,195	12.0	22	2.6	0.33	3,295	5.4
NE	19,077	8,666	3,402	39.3	26	10.2	0.50	4,333	7.1
NY	10,730	3,241	43	13.5	11	1.5	0.05	162	0.3
OH	8,856	5,461	1,438	26.3	6	1.6	1.00	5,461	9.0
ON	2,840	2,390	583	24.4	20	4.9	0.05	120	0.2
PA	9,500	2,858	628	22.0	11	2.4	0.40	1,143	1.9
SD	17,954	7,620	1,742	22.9	37	8.5	0.50	3,810	6.3
WI	12,245	5,435	1,418	26.1	14	3.7	0.50	2,717	4.5
WV	5,320	952	24	2.6	11	0.3	0.33	314	0.1
Total	186,685	101,016	27,835	26.5	17.8	4.4		59,346	1.00

*Ref. 30; †, http://usda.mannlib.cornell.edu/mor_start.html; ‡, <http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bba/acrg0600.pdf>; §, Ref. 28.

with the presence of monarch larvae are based partly on the projections of the simulation model described previously and partly on our own observations. In this instance, our estimate for the exposure of monarchs in the Corn Belt states and Ontario is 1.6%. Because monarchs in the Corn Belt represent 50% of the total monarch population, the exposure for the entire monarch population would be no greater than 0.8%.

The proportion of the population of monarchs in Iowa that would be exposed to pollen levels that exceed the no-observable-effect-level for each event (P_t) can be derived from data presented in Fig. 2. For event 176 pollen, monarch larvae would likely encounter pollen densities equal to or exceeding the LOEC in 90% of field situations during anthesis, whereas this would be true in only 0.7% or less of field situations for Bt11 and Mon810 pollen. Overall risk (R) is the combined probability of exposure and toxic effect or:

$$R = P_e \times P_t \quad [2]$$

If we assume that event 176 comprised 5% (a from Eq. 1 = 0.05) of planted corn acres in Iowa (or 20% of planted *Bt* corn acres in 2000), an extreme upper bound estimate based on historical marketing data, the risk of impact (R) to monarch populations exposed to effects from event 176 pollen is:

$$R = P_e \times P_t = 0.0042 \times 0.9 = 0.0038, \quad [3]$$

or 0.4% of the population. The LOEC of pollen for all other events (Bt11 and Mon810 comprise the remaining 20% of total area planted; $a = 0.20$) equals or exceeds 0.7% of expected pollen densities, thus the proportion of the monarch population at risk of impact from effects of Cry proteins, other than event 176, in *Bt* cornfields in Iowa is:

$$R = P_e \times P_t = 0.0168 \times 0.007 = 0.00012, \quad [4]$$

or 0.012% of the population. The combined risk estimate for monarchs in Iowa is the sum of these two values, or 0.41%.

Following the same logic as above and assuming that (i) adoption rate of *Bt* corn reached its maximum limit of 80% (based on current refuge requirements) ($a = 0.80$) in Iowa, and

(ii) pollen from current and future *Bt* corn events will pose a hazard less than or equal to that established here for Bt11 and Mon810, the proportion of the monarch population in Iowa that would be at risk with market saturation is:

$$R = P_e \times P_t = 0.067 \times 0.007 = 0.00047, \quad [5]$$

Table 2. Parameter estimates for probability of exposure (P_e) of monarch larvae to *Bt* corn pollen within cornfields in states and provinces of the Corn Belt that constitute 50% of the eastern North American monarch population

State	Parameter estimates for risk of exposure in each state				P_e
	l^*	$o^†$	a	$m^‡$	
IA	0.560	0.15*	0.25	0.192	0.0040
IL	0.423	0.25	0.14	0.177	0.0026
IN	0.376	0.25	0.07	0.101	0.0007
KS	0.119	0.15	0.26	0.038	0.0002
KY	0.132	0.15	0.11	0.024	<0.0001
MI	0.211	0.40	0.08	0.035	0.0002
MN	0.287	0.40*	0.30	0.083	0.0028
MO	0.120	0.15	0.22	0.054	0.0002
NE	0.393	0.15	0.26	0.071	0.0011
NY	0.135	0.40	0.11	0.003	<0.0001
OH	0.263	0.25	0.06	0.090	0.0004
ON	0.300	0.62*	0.20	0.020	0.0007
PA	0.220	0.25	0.11	0.019	0.0005
SD	0.229	0.40	0.37	0.063	0.0021
WI	0.261	0.40	0.14	0.045	0.0007
WV	0.026	0.15	0.11	0.005	<0.0001
Average (totals)	0.253	0.28	0.17	(1.020)	(0.0161)

* l , proportion of monarchs from corn; o , overlap of pollen shed with susceptible larval stages; a , adoption rate of *Bt* corn.

[†]Values marked with * were derived from field observations in 2000 (see ref. 4).

[‡] m represents the proportion land area of each state or province that constitutes 50% of the breeding habitat of the eastern North American monarch population.

or 0.05% of the Iowa population. If, instead, only event 176 hybrids were grown to the maximum extent in Iowa, 6.1% of the monarch population would be at risk. By using this format and data from Tables 1 and 2, risk of exposure and toxicity from *Bt* corn can be applied to each of the states and provinces in which monarch breeding and corn production overlap.

Discussion

Previous reports (1, 8) indicating the hazard of *Bt* corn pollen to monarch butterfly are inadequate to assess risk, because assigning risk can be accomplished only when the likelihood of toxic response can be properly expressed and the likelihood of exposure is estimated through appropriate observations. We have used a comprehensive set of new data and a formalized approach to risk assessment that integrates aspects of exposure to characterize the risk posed to monarchs from *Bt* corn pollen. Characterization of acute toxic effects alone indicates that the potential for hazard to monarchs is currently restricted to event 176 hybrids, which express Cry1Ab protein in pollen at a level sufficient to show measurable effects. Event 176 hybrids have always had a minor presence in the corn market and current plantings, which comprise <2% of corn acres, are rapidly declining.

Other events either express negligible Cry1Ab protein in corn pollen (Mon810 and Bt11) or express Cry protein of significantly less toxicity to monarch (Dbt418, Cbh351, and Tc1507 expressing Cry1Ac, Cry9c, and Cry1F proteins, respectively). These corn hybrids have little or no effect on monarch populations, although sublethal effects due to chronic exposure to *Bt* pollen over the entire larval growth of monarchs has not been accounted for in these studies. Should chronic effects be documented, the impact on monarch populations will remain low or negligible, because overall exposure of monarch larvae to *Bt* pollen is low.

Monarch populations share their habitat with corn ecosystems to a degree previously undocumented (4). Despite this conclusion, the portion of the monarch population that is potentially exposed to toxic levels of *Bt* corn pollen is negligible and declining as planting of event 176 hybrids is phased out through 2003. The exposure portion (P_e) of the risk (R) equation described above is low, and the toxicity portion (P_t) of this equation for the dominant corn hybrids is negligible, therefore the impact of *Bt* corn on monarch populations should remain low.

Evidence supporting this risk conclusion has been collected over a wide geographic area and under a variety of conditions in both laboratory and field settings (3–6). Findings from studies done in multiple locations were consistent, even though methods differed from one study to another. This approach to risk characterization is consistent with accepted risk assessment procedures and shares many similarities with previous assessments over a wide range of situations describing potential risk associated with a described hazard. It is imperative that future conclusions concerning the environmental or nontarget impacts of transgenic crops be based on appropriate methods of investigation and sound risk-assessment procedures.

We thank Jeffrey Wolt, Keith Solomon, and Anthony Shelton for their input and critical comments and suggestions during the development of this paper. This research was supported by a pooled grant provided by USDA–ARS and the Agricultural Biotechnology Stewardship Technical Committee (ABSTC), and by funding from the Canadian Food Inspection Agency (CFIA), Environment Canada, and the Ontario Ministry of Agriculture, Food and Rural Affairs, the Maryland Agricultural Experiment Station, and the Leopold Center for Sustainable Agriculture, Ames, IA. Members of ABSTC are Aventis CropScience USA LP, Dow AgroSciences LLC, E. I. du Pont de Nemours and Company, Monsanto Company, and Syngenta Seeds, Inc.

1. Losey, J. E., Rayor, L. S. & Carter, M. E. (1999) *Nature (London)* **399**, 214.
2. U.S. Environmental Protection Agency (1995) *Publ. No. EPA731-F-95-004* (U.S. Govt. Printing Office, Washington, DC).
3. Hellmich, R. L., Siegfried, B. D., Sears, M. K., Stanley-Horn, D. E., Daniels, M. J., Mattila, H. R., Spencer, T., Bidne, K. G. & Lewis, L. (2001) *Proc. Natl. Acad. Sci. USA*, in press.
4. Oberhauser, K. S., Prysby, M., Mattila, H. R., Stanley-Horn, D. E., Sears, M. K., Dively, G., Olson, E., Pleasants, J. M., Lam, W.-K. F. & Hellmich, R. L. (2001) *Proc. Natl. Acad. Sci. USA*, in press.
5. Pleasants, J. M., Hellmich, R. L., Dively, G., Sears, M. K., Stanley-Horn, D. E., Mattila, H. R., Foster, J. E., Clark, P. L. & Jones, G. D. (2001) *Proc. Natl. Acad. Sci. USA*, in press.
6. Stanley-Horn, D. E., Dively, G. P., Hellmich, R. L., Mattila, H. R., Sears, M. K., Rose, R., Jesse, L. C. H., Losey, J. F., Obrycki, J. J. & Lewis, L. (2001) *Proc. Natl. Acad. Sci. USA*, in press.
7. Hellmich, R. L. & Siegfried, B. D. (2001) in *Genetically Modified Organisms in Agriculture—Economics and Politics*, ed. Nelson, G. C. (Academic, London), pp. 283–289.
8. Jesse, L. C. H. & Obrycki, J. J. (2000) *Oecologia* **125**, 241–248.
9. Wraight, C. L., Zangerl, A. R., Carroll, M. J. & Berenbaum, M. R. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 7700–7703. (First Published June 6, 2000; 10.1073/pnas.130202097)
10. Giddings, J. M., Hall, L. W., Jr. & Solomon, K. R. (2000) *Risk Anal.* **2**, 545–572.
11. Giesy, J. P., Solomon, K. R., Coats, J. R., Dixon, K. R., Giddings, J. M. & Kenega, E. E. (1999) *Rev. Environ. Contam. Toxicol.* **160**, 1–129.
12. Solomon, K. R., Baker, D. B., Richards, P., Dixon, K. R., Klaine, S. J., La Point, T. W., Kendall, R. J., Weisskopf, C. P., Giddings, J. M., Giesy, J. P., et al. (1996) *Environ. Toxicol. Chem.* **15**, 31–76.
13. Solomon, K. R., Giesy, J. P., Kendall, R. J., Best, L. B., Coats, J. R., Dixon, K. R., Hooper, M. J., Kenega, E. E. & McMurphy, S. T. (2001) *Environ. Toxicol. Chem.* **7**, 497–632.
14. Geisy, J. P., Dobson, S. & Solomon, K. R. (2000) *Rev. Environ. Contam. Toxicol.* **167**, 35–120.
15. Klaine, S. J., Cobb, G. P., Dickerson, R. L., Dixon, K. R., Kendall, R. J., Smith E. E. & Solomon, K. R. (1996) *Environ. Toxicol. Chem.* **15**, 21–30.
16. Hall, L. W., Jr., Giddings, J. M., Solomon, K. R. & Balcomb, R. (1999) *Crit. Rev. Toxicol.* **29**, 367–437.
17. Kendall, R. J., Lacher, T., Jr., Bunck, E. C., Daniel, F. B., Driver, C., Glue, G. E., Leighton, F., Stansley, W., Watanabe, P. G. & Whitworth, M. (1996) *Environ. Toxicol. Chem.* **15**, 4–20.
18. Kreig, A. & Langerbruch, G. A. (1981) in *Microbial Control of Pests and Plant Diseases*, ed. Burges, H. D. (Academic, New York), pp. 837–896.
19. Peacock, J. W., Schweitzer, D. F., Dale, F., Carter, J. L. & Dubois, N. R. (1998) *Environ. Entomol.* **27**, 450–457.
20. Miller, J. C. (1990) *Am. Entomol.* **36**, 135–139.
21. Johnson, K. S., Scriber, J. M., Nitao, J. K. & Smitley, D. R. (1995) *Environ. Entomol.* **24**, 288–297.
22. Hall, S. P., Sullivan, J. B. & Schweitzer, D. F. (1999) *USDA Bull. No. FHTET-98-16* (USDA, Washington, DC).
23. Malcolm, S. B., Cockrell, B. J. & Brower, L. P. (1993) in *Biology and Conservation of the Monarch Butterfly*, eds. Malcolm S. B. & Brower L. P. (Natural History Museum of Los Angeles County, Los Angeles, CA), pp. 253–267.
24. National Research Council (1983) *Risk Assessment in the Federal Government: Managing the Process* (Natl. Acad. Press, Washington, DC).
25. Koziel, M. G., Beland, G. L., Bowman, C., Carozzi, N. B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., et al. (1993) *Biotechnology* **11**, 194–200.
26. Christensen, A. H., Sharrock, R. A. & Quail, P. H. (1992) *Plant Mol. Biol.* **18**, 675–689.
27. Raynor, G. S., Ogden, E. C. & Hayes, J. V. (1972) *Agron. J.* **64**, 420–427.
28. Wassenaar, L. I. & Hobson, K. A. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 15436–15439.
29. USDA–National Agricultural Statistics Service (2000) *Census of Agriculture (USDA–NASS, Washington, DC)*, Vol. 1, Part 57.
30. USDA, Natural Resources and Conservation Service, & Iowa State University, Statistics Laboratory (1997) *Summary Report, National Resources Inventory (USDA, Washington, DC)*.