Genetically Engineered Plants, Endangered Species, and Risk: A Temporal and Spatial Exposure Assessment for Karner Blue Butterfly Larvae and Bt Maize Pollen

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Genetically engineered maize (*Zea mays*) containing insecticidal endotoxin proteins from *Bacillus thuringiensis* (Bt) δ-endotoxin proteins has been adopted widely in the Midwestern United States. The proteins are toxic to several lepidopteran species and because a variety of maize tissues, including pollen, may express the endotoxins, the probability of exposure to nontarget species, including endangered species, needs to be understood. The objective of this study was to assess the potential temporal and spatial exposure of endangered Karner blue butterfly larvae (*Lycaeides melissa samuelis*) to Bt maize pollen in Wisconsin using probabilistic exposure techniques and geographic information systems analysis. Based on degree-day modeling of butterfly phenology and maize pollen shed, there is some potential for temporal exposure of larvae to maize pollen. However, in the majority of years and locations, maize pollen shed most likely will occur after the majority of larval feeding on wild lupine (*Lupinus perennis*). The spatial analysis indicates that some Karner blue butterfly populations occur in close proximity to maize fields, but in the vast majority of cases the butterfly's host plant and maize fields are separated by more than 500 m. A small number of potential or existing Karner blue butterfly sites are located near maize fields, including sites in two of the four counties where temporal overlap is most likely. The exposure assessment indicates that these two counties should receive the highest priority to determine if Karner blue butterfly larvae are actually at risk and then, if needed, to reduce or prevent exposure.

KEY WORDS: Biosafety; biotechnology risk; Karner blue butterfly; *Lycaeides melissa samuelis*; risk assessment

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1. INTRODUCTION

The Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov) is a U.S. endangered subspecies that has been the target of numerous scientific investigations and millions of dollars in conservation funds. It is restricted to distinct habitats in the eastern United States, with Wisconsin supporting the largest and most widespread populations. In Wisconsin, the species is documented in 22 counties (Wisconsin Dept. Natural Resources, 1999). The species has two complete generations per year and overwinters in the egg stage. Normally, first-generation larvae hatch in April and

adults emerge in late May to early June. Second-generation larvae feed into July and adults emerge in late July to August. The larvae feed exclusively on wild lupine (*Lupinus perennis* L.) in sandy pine and oak barrens, lake shore dunes, abandoned agricultural fields, mowed utility areas and rights-of-ways, and managed forest lands (Swengel, 1995).

Genetically engineered maize (*Zea mays* L.) containing insecticidal δ-endotoxin proteins from *Bacillus thuringiensis* (Bt) Berliner has been adopted widely in the Midwestern United States. The Cry1 Bt endotoxins are toxic to several lepidopteran species (Glare & O'Callaghan, 2000) and because a variety of maize tissues, including pollen, may express the endotoxins, the risk of exposure to nontarget species, including endangered species, needs to be understood. Risks to two lepidopteran species, the monarch butterfly (*Danaus plexippus*) and the eastern black swallowtail (*Papilio polyxenes*), have been assessed using quantitative techniques (Sears *et al.*, 2001; Zangerl *et al.*, 2001; Wolt *et al.*, 2003; Dively *et al.*, 2004).

One of the goals of ecological risk assessment is to characterize the magnitude and probabilities of adverse effects to ecological receptors, such as nontarget species, resulting from the presence of environmental stressors (U.S. EPA, 1999; U.S. OSTP, 1999). Because knowledge of effects, exposure, and risk is inexact, considerable attention has been directed toward the use of probabilistic risk assessment techniques that statistically quantify ecological risks as well as the associated uncertainty and variability in the subsequent risk conclusions (SETAC, 1994; U.S. EPA, 1999).

Ecological risk assessment is a valuable framework from which to measure, communicate, and make decisions about the environmental impacts of agricultural biotechnology (Wolt & Peterson, 2000; Wolt *et al.*, 2003). Wolt and Peterson (2000) and NRC (2000) argued that exposure and risk assessment paradigms for genetically engineered plants do not differ from those for other technological risks; therefore, quantitative risk assessment, especially quantitative exposure assessment (Cullen & Frey, 1999; Vose, 1996, 2000), should be an important component of crop biotechnology risk assessments.

The objective of this study was to assess the potential temporal and spatial exposure of Karner blue butterfly larvae to Bt maize pollen. We focused on exposure with the assumption that Karner blue butterfly would be sensitive to Bt maize pollen. We made the assumption that because the Karner blue butterfly is

an endangered subspecies, there is no specific information on its susceptibility to Bt toxins from commercialized Bt maize events (see below for more information). We used probabilistic exposure techniques and geographic information systems (GIS) analysis to assess the potential for temporal and spatial overlap between larval presence and maize pollen shed. This general approach has proven utility for considerations of Bt pollen exposure to monarch butterfly (Hellmich *et al.*, 2001; Sears *et al.*, 2001), but here we apply the methodology at a scale consistent with the restricted and isolated habit of extant Karner blue butterfly populations.

2. MATERIALS AND METHODS

2.1. Problem Formulation

Bt subspecies have different insecticidal activities. Typically, only certain insect species within a given taxonomic order are susceptible to a given insecticidal Bt endotoxin protein (Glare & O'Callaghan, 2000). Additionally, susceptibility of insect species within a taxonomic order can vary substantially to a single endotoxin protein (Wolt *et al.*, 2003). Genetically engineered maize events that are currently commercialized express a variety of endotoxins. The purpose of incorporating Bt endotoxins into maize is to protect the plants from pests. The Cry1Ab and Cry1F plant-expressed Bt endotoxins are active against lepidopteran species; therefore, an assessment of the risks to nontarget lepidopteran species, including endangered species, inhabiting or near maize production systems is needed (Wolt *et al.*, 2003).

For example, toxicological hazard has been demonstrated for monarch butterfly larvae consuming common milkweed (*Asclepias syriaca*) leaves containing surface-deposited pollen from maize expressing Bt protein (Losey *et al.*, 1999; Jesse & Obrycki, 2000). Subsequent studies (Hellmich *et al.*, 2001; Oberhauser *et al.*, 2001; Pleasants *et al.*, 2001; Stanley-Horn *et al.*, 2001) were used by Sears *et al.* (2001) to quantitatively evaluate risks of short-duration exposures to monarch butterfly using probabilistic exposure and risk assessment principles. More recently, a similar approach has been applied to the consideration of risk for long-term exposure to monarch butterfly populations (Dively *et al.*, 2004).

To understand the potential risks to the Karner blue butterfly, both hazard (ability of the stressor to affect ecological receptors) and exposure (the interaction of the stressor and ecological receptors) should be

considered. However, because the Karner blue butterfly is an endangered subspecies, there is no specific information on its susceptibility to Bt toxins from currently commercialized Bt maize events. Herms *et al.* (1997) demonstrated similar sensitivity of Karner blue butterfly larvae and gypsy moth larvae (*Lymantria dispar*) to a sprayable Bt formulation containing Cry1 proteins. However, this information is of limited value for conclusions about the toxicity of Bt maize pollen because of the specific Cry proteins expressed in currently marketed Bt maize.

The focus of concern for an endangered species is not effect, but exposure, because public policy adheres in this instance to a rights-based (zero risk) criterion where "independent of benefits and costs, and of how big the risks are, [the goal is to] eliminate, or do not allow the introduction of, the risk" (Morgan & Henrion, 1990). Thus, in the case of Karner blue butterfly and in the absence of specific toxicity (hazard) information, it is crucial to assess the temporal and spatial exposure of the potentially susceptible stage of the species to Bt maize. Because the larvae are monophagous on lupine, we assumed in this assessment that exposure of larvae to Bt endotoxins resulted from Bt maize pollen deposited on the surface of lupine leaves. The exposure assessment reported here was based on input assumptions in the absence of specific effects and exposure data.

2.2. Temporal Exposure Assessment

We used ClimProb (Meyer *et al.*, 1996), a climate software package that assigns probabilities to the likelihood of occurrence of specific climatic events based on the long-term climatological history of a specific weather station. ClimProb assigns probabilities to events such as the first fall freeze occurring before or after a certain date, the number of degree days being accumulated within a specified time period, the number of days that will have maximum temperatures above a user-specified threshold temperature, and the accumulation of "X" mm of precipitation within a specified time period.

The software generates both a chronological history of the climatic event for the time window specified and a ranked-order listing from which probabilities are assigned. ClimProb data sets, derived from the U.S. National Weather Service and Cooperative Observer Network (typically rural settings), consist of the date, maximum and minimum temperature, and precipitation.

The technique presented here uses long-term temperature data to estimate the dates when a degree-day accumulation has been reached. This technique has been used for insect pests of soybean (Herbert *et al.*, 1988), alfalfa (Peterson & Meyer, 1995), and maize (Meyer & Peterson, 1998). In this study, degree days were accumulated using the linear method:

$$\text{CDD} = \sum_d [(\text{Tmax}_d + \text{Tmin}_d) \div 2] - T_{\text{base}}, \quad (1)$$

where CDD is the Celsius degree days accumulated over "x" days; Tmax_d is the daily maximum temperature (°C) at day "x"; Tmin_d is the daily minimum temperature (°C) at day "x"; and T_{base} is the base temperature (°C).

We did not use a developmental threshold on the maximum temperature as it typically is not used when determining insect development. Most insects do not experience temperatures at the developmental maximum in temperate regions either because the ambient temperature does not reach the developmental maximum or because the insects move from maximum temperatures to lower temperatures (i.e., behavioral thermoregulation) (Higley *et al.*, 1986). We used a developmental minimum threshold of 12°C based on data from Swengel (1995). The same temperature (12°C) also was used for the base temperature.

2.3. Karner Blue Butterfly Phenological Events

We based the degree-day values for specific phenological events on life-stage development and temperature data from Herms *et al.* (1996) and from first- and second-generation butterfly counts and observations from Swengel (1995) and Swengel and Swengel (1996, 1999). The 1990–1998 observations of butterfly flight from Swengel (1995) and Swengel and Swengel (1996, 1999) were from the following nine counties in Wisconsin: Clark, Eau Claire, Green Lake, Jackson, Juneau, Monroe, Portage, Waushara, and Wood. We used weather data from 11 stations in 8 of those 9 counties (adequate weather data from Jackson County could not be found) to determine degree-day accumulations necessary for pertinent phenological events (Table I).

Records of Karner blue butterfly flights from Swengel (1995) and Swengel and Swengel (1996, 1999) were used with developmental minimum temperature data from Swengel (1995) to determine the degree-day accumulations necessary for first flight, peak flight, and end flight (date of last adult observed) for the first and second generations. To assess the potential temporal overlap between larvae and maize pollen shed, we needed to predict the end of feeding for the second generation because this

Table I. Locations Used for Degree-Day Model Development

County	Weather Station	Latitude	Longitude	Elevation (m)
Clark	Neillsville	44°32′	90°38′	316
Eau Claire	Eau Claire	44°52′	91°29′	268
Green Lake	Dalton	43°39′	89°21′	262
Juneau	Mauston	43°47′	90°04′	264
	Necedah	44°02′	90°05′	282
Monroe	Viroqua[a]	43°32′	90°52′	354
Portage	Rosholt	44°45′	89°14′	354
	Stevens Point	44°31′	89°35′	329
Waushara	Hancock	44°07′	89°32′	328
Wood	Marshfield	44°38′	90°08′	381
	Wisconsin Rapids	44°23′	89°48′	317

[a]Not in Monroe County, but located within 16 km of the county border.

life-history event represents the presence of potentially susceptible larvae during maize pollen shed. This was done by determining the degree-day accumulations required for second-generation peak pupation (which indicated the peak end of larval feeding). Degree-day accumulations required for peak pupation are an indication of the end of larval feeding because at peak pupation, 50% of the individuals most likely would be in late larval stages and still feeding.

Life-stage-specific events such as the second-generation end of feeding and pupation, for which there are no field observations, were determined by considering both developmental data of Herms *et al*. (1996) and records of adult flight (Swengel, 1995; Swengel & Swengel, 1996, 1999) (Table II). Developmental data determined by Herms *et al*. (1996)

Table II. Degree-Day Requirements for Specific Phenological Events of the Karner Blue Butterfly

Phenological Event	CDD ± SE[a]	Years[b]
First generation		
First flight	145 ± 8.1	8
Peak flight	246 ± 10.3	8
End flight	339 ± 29	6
Second generation		
First flight	515 ± 47.7	5
Peak flight	683 ± 24.1	9
End flight	965 ± 176	4
End of larval feeding	574[c]	

[a]Celsius degree-day accumulations from January 1, base temperature 12°C.
[b]Years of observational data (Swengel & Swengel, 1996, 1999).
[c]End of larval feeding not directly observed, but determined by subtracting 109 centigrade degree-days from second-generation peak flight.

were converted to degree days, assuming a 12°C minimum developmental threshold (Swengel, 1995). Consequently, the peak pupation of second-generation larvae (and therefore the end of larval feeding) was assumed to occur 109 CDD before the degree-day accumulation necessary for peak flight of the second generation (Table II). The value of 109 CDD is the degree-day requirement for completion of the pre-pupal and pupal stages as determined by Herms *et al*. (1996).

Using ClimProb, the degree days and concomitant calendar dates for several life-history events were calculated for 26 locations in 19 counties to determine areas of potential overlap between maize pollen shed and Karner blue butterfly larval presence. The counties and locations were: Barron (Cumberland), Burnett (Danbury, Grantsburg), Chippewa (Bloomer), Clark (Neillsville), Dunn (Menomoni), Eau Claire (Eau Claire), Green Lake (Dalton), Jackson (Blair), Juneau (Mauston), Kenosha (Kenosha, Lake Geneva), Oconto (Oconto), Outagamie (Appleton), Polk (St. Croix Falls, Amery), Portage (Rosholt, Stevens Point), Sauk (Baraboo, Wisconsin Dells), Shawano (Shawano), Waupaca (New London, Waupaca), Waushara (Hancock), and Wood (Marshfield, Wisconsin Rapids). We used the most recent 30-year climatic normal period (1971–2000) from each location to calculate accumulated degree days beginning January 1 of each year. These locations were chosen because all of the counties had records of Karner blue butterfly occurrence (Wisconsin Dept. Natural Resources, 1999). Four counties with known occurrences (Adams, Marquette, Menominee, and Monroe) were not used in this analysis because of either the lack of acceptable weather stations or inadequate weather data.

To validate the Karner blue butterfly degree-day model, predictions of peak butterfly flights were compared to two years of observations of first- and second-generation peak flights in Waupaca County, Wisconsin (1991–1992) (Bleser, 1994) and one year of observations of second-generation peak flight in Allegan County, Michigan (1989) (Lawrence, 1994). Model predictions were compared to both degree days and calendar dates from observations at the locations discussed above.

2.4. Spatial Exposure Assessment

Karner blue butterfly distributions in Wisconsin have been documented by numerous field studies, summarized by the National Heritage Program of the

Fig. 1. Distribution of wild lupine sites (•) documented by the Wisconsin Department of Natural Resources (Wisconsin DNR, pers. comm.) and maize fields (shaded gray) planted during the year 2003, as reported by the U.S. Department of Agriculture's National Agricultural Statistics Survey (USDA, 2004a). Geographic range of Karner blue butterflies in Wisconsin (lower left) is derived from the Wisconsin Dept. Natural Resources (1999).
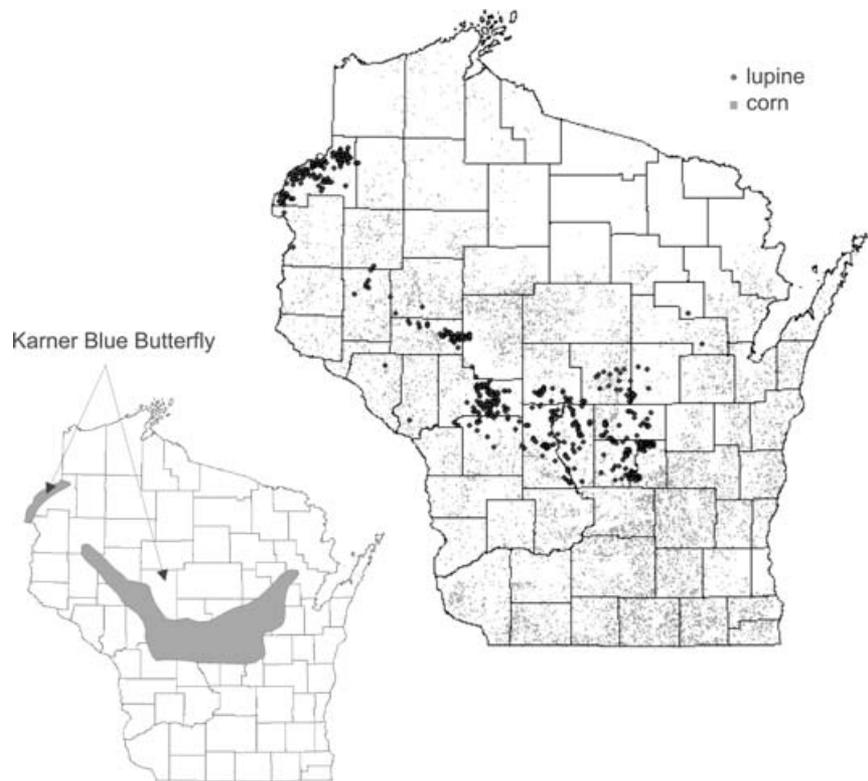
Wisconsin Department of Natural Resources (DNR) (Fig. 1). DNR biologists provided us with the most recent database on lupine distributions, identifying 1,026 localities where the plant has been recorded in Wisconsin. We used this database as a conservative estimate of potential Karner blue butterfly distribution even though the butterfly has not been documented at all of these localities. The distribution of maize fields was obtained from the GIS Cropland Data Layer for 2003, compiled by the USDA National Agricultural Statistics Survey (USDA, 2004b); at the time of this writing, data were not available in digital form for any other year. This digital data resource consists of geo-referenced, categorized land cover classifications derived from satellite imagery produced by the Enhanced Thematic Mapper (ETM+) on Landsat 7. Classification was developed by using two images collected on April 22, 2003 and September 13, 2003, respectively, with a resolution of 30 m × 30 m. Satellite data were ground-truthed with extensive field observations collected during the annual USDA June Agricultural Survey. Details about the cropland data can be found in the public domain at http://www.nass.usda.gov/research/Cropland/SARS1a.htm. Meta-data include the Analysis District coverage, sensors used, percent correct and

kappa coefficients, regression analysis by Analysis District, the sampling frame scheme, and the original cover type signatures. Classification accuracy is generally between 85% and 95% correct for agricultural-related land cover categories (USDA, 2004b). Although the majority of maize fields in Wisconsin typically are rotated during alternate years with another crop (such as soybean), we assumed that a distribution estimate for 2003 would be representative of the location and total acreage of maize with respect to lupine each year.

We created GIS buffers of 100 and 500 m around each lupine locality and calculated the percent area occupied by maize fields. These distances are very conservative based on published data on maize pollen dispersal and butterfly mortality. Wraight *et al.* (2000) found an 8× decrease in mean Bt pollen deposition between 0.5 and 7 m from the edge of maize fields; levels of pollen at the 7-m distance were below those shown to cause significant mortality in black swallowtails and other butterfly species (Stanley-Horn *et al.*, 2001; Hellmich *et al.*, 2001). The larger distances used in our study allow for variation in the geographic extent of lupine populations relative to the edges of maize fields. For this analysis, we also use the conservative assumption that all of the fields are planted

with Bt varieties, although this is unlikely to be the case. In 2004, only 22% of Wisconsin maize acres were planted with Bt maize (USDA, 2004a).

2.5. Maize Pollen Shed

The emergence of ear silks is an indicator of maize pollen shed because the two events are highly synchronized (Hanway, 1971). Therefore, we developed a model to predict dates of maize pollen shed based on silking. Model development required dates for three stages of maize growth: 50% emergence, 50% silking, and 50% maturity. These data are published weekly on a Crop Reporting District (CRD) level (Wisconsin Agricultural Statistics Service, 2003). The range of the Karner blue butterfly (Fig. 1) is essentially limited to three CRDs—northwest, west central, and central. Data for these three growth stages were only available beginning with the 1998 growing season. Therefore, we used data from the 1998 to 2000 growing seasons to develop the model and data from the 2001 to 2002 growing seasons to validate the model.

Model development was based on work by Neild and Seeley (1977), in which we observed that the date of silking, when determined as a function of the percentage of degree days required to reach maturity, was very consistently independent of maize hybrid. Thus, we used the ClimProb software to accumulate degree days between the 50% emergence date and the 50% silking date, and between the 50% emergence date and the 50% maturity date (10°C was used for the base temperature and the minimum temperature threshold, 30°C used for the maximum temperature threshold). Gilmore and Rogers (1958) found that the correlation between maize growth and accumulated growing degree days was optimized using thresholds of 10°C for the minimum base temperature and 30°C for the maximum base temperature. The use of the 10°C and 30°C thresholds is a common standard in accumulating growing degree days. Based on these accumulated degree days, we calculated the percentage of each growing season (1998–2000) required to reach the 50% silking date for 5 locations in the northwest CRD, 3 locations in the west central CRD, and 13 locations in the central CRD (Table III).

To validate the maize pollen shed model (predicted date of 50% silking), we determined the dates of 50% emergence and 50% maturity for 2001–2002 (Table III). We then accumulated degree days from emergence to maturity and multiplied that degree-day total by the average percentage of each growing sea-

son required to reach the silking date as determined in the model development stage. ClimProb was then used to match that (emergence to silking) degree-day total to the day-of-year on which 50% silking had occurred. This procedure was followed for each of the 21 locations in the northwest, west central, and central Wisconsin CRDs. Differences in day-of-year for predicted versus observed pollen shed were subjected to Pearson correlation analysis (PROC CORR, SAS v. 9.0).

3. RESULTS

Degree-day accumulations were calculated for each of the major Karner blue butterfly phenological events (Table II). Estimates of degree-day accumulations necessary for predicting end of flight for the first and second generations were more variable than first flight or peak flight. This was expected given that observations of first and peak flight involved greater numbers of adults relative to end flight (Swengel & Swengel, 1996, 1999). End flight typically represented the date of the last adult observed, which could be highly variable, especially for the second generation (Swengel & Swengel, 1996, 1999).

For most locations, modeling suggested that 50% of the first-generation flight is completed by the end of the first week in June (Table IV). However, 10% of the time (1 year in 10), peak flight most likely occurs through the third week in June. Adults of the second generation typically fly by the end of July. Ten percent of the time, the flight will occur through the second week in August (Table IV).

For most locations, modeling suggested that 50% of second-generation larval feeding would be completed by mid July (Table V). However, 10% of the time, larval feeding may occur in late July to early August.

For most locations, our models suggested that 50% of the time, maize pollen shed (indicated by initiation of silking) occurs between July 21 and 23 across most locations (Table V). Ninety percent of the time, silking initiation occurs before approximately July 27.

Based on dates for the end of larval feeding at the 50th percentile probability of occurrence and the initiation of silking at the 50th percentile probability of occurrence, only 5 of 26 locations (19%) (4 counties) exhibited an overlap between larval feeding and pollen shed (Table V; Fig. 2). For four of those five locations (80%), the overlap was only 1 to 4 days. The end of larval feeding was determined by modeling peak pupation. Therefore, half of the population

Table III. Comparisons of Modeled and Observed Maize Silking Dates (DOY) by Location and Year

Crop Reporting District	Location	Year	Observed Silking Date (DOY)	Modeled Silking Date (DOY)	Modeled–Observed
NW	Bloomer	2001	218	213	−5
NW	Bloomer	2002	215	212	−3
NW	Cumberland	2001	218	212	−6
NW	Cumberland	2002	215	213	−2
NW	Amery	2001	218	214	−4
NW	Amery	2002	215	213	−2
NW	Danbury	2001	218	213	−5
NW	Danbury	2002	215	212	−3
NW	Grantsburg	2001	218	214	−4
NW	Grantsburg	2002	215	213	−2
NE	Oconto	2001	215	214	−1
NE	Oconto	2002	215	213	−2
WC	Menomonie	2001	213	212	−1
WC	Menomonie	2002	212	210	−2
WC	Eau Claire	2001	213	211	−2
WC	Eau Claire	2002	212	210	−2
WC	Blair	2001	213	210	−3
WC	Blair	2002	212	208	−4
C (NE)	Shawano[a]	2001	213	214	1
C (NE)	Shawano[a]	2002	212	212	0
C (NC)	Owen[b]	2001	213	215	2
C (NC)	Owen[b]	2002	212	215	3
C (NC)	Neillsville[b]	2001	213	214	1
C (NC)	Neillsville[b]	2002	212	213	1
C	Marshfield	2001	213	214	1
C	Marshfield	2002	212	211	−1
C	Wisconsin Rapids	2001	213	213	0
C	Wisconsin Rapids	2002	212	212	0
C	Stevens Point	2001	213	213	0
C	Stevens Point	2002	212	212	0
C	Rosholt	2001	213	213	0
C	Rosholt	2002	212	212	0
C	Waupaca	2001	213	213	0
C	Waupaca	2002	212	212	0
C	New London	2001	213	213	0
C	New London	2002	212	213	1
C	Necedah	2001	213	214	1
C	Necedah	2002	212	211	−1
C	Hancock	2001	213	213	0
C	Hancock	2002	212	212	0
C	Mauston	2001	213	213	0
C	Mauston	2002	212	212	0
C	Dalton	2001	213	214	1
C	Dalton	2002	212	212	0
SW	Virocqua	2001	212	209	−3
SW	Virocqua	2002	208	205	−3
SW	Baraboo	2001	212	207	−5
SW	Baraboo	2002	208	206	−2
SW	Wisconsin Dells	2001	212	209	−3
SW	Wisconsin Dells	2002	208	205	−3

[a]Site that is actually in the northeast Crop Reporting District (CRD), but, based on proximity, is more closely representative of the central CRD.
[b]Site that is actually in the north central CRD but, based on proximity, is more closely representative of the central CRD.

Table IV. Predicted Dates (Day-of-Year) and Probabilities of Occurrence for First- and Second-Generation Peak Flights of Karner Blue Butterflies by Location

County	Weather Station	First-Generation Peak Flight Probabilities of Occurrence			Second-Generation Peak Flight Probabilities of Occurrence		
		25%	50%	90%	25%	50%	90%
Barron	Cumberland	151	162	172	204	211	225
Burnett	Danbury	158	164	174	208	218	233
Burnett	Grantsburg	158	162	174	208	218	228
Chippewa	Bloomer	150	160	172	202	210	228
Clark	Neillsville	157	162	173	207	215	230
Dunn	Menomonie	148	156	168	196	203	224
Eau Claire	Eau Claire	151	161	170	200	208	219
Green Lake	Dalton	149	158	164	198	205	213
Jackson	Blair	152	161	172	205	211	224
Juneau	Mauston	151	157	171	201	206	223
Kenosha	Kenosha	168	175	186	215	224	238
Kenosha	Lake Geneva	149	156	165	193	200	206
Oconto	Oconto	162	169	178	215	224	237
Outagamie	Appleton	158	163	174	203	210	222
Polk	St. Croix Falls	150	158	170	198	206	220
Polk	Amery	160	166	176	209	216	229
Portage	Rosholt	157	163	176	211	216	235
Portage	Stevens Point	156	163	174	207	214	228
Sauk	Baraboo	152	160	169	203	210	222
Sauk	Wisconsin Dells	152	161	171	202	208	223
Shawano	Shawano	155	161	175	205	212	228
Waupaca	New London	155	159	175	201	208	228
Waupaca	Waupaca	155	160	174	202	208	225
Waushara	Hancock	152	158	168	200	206	222
Wood	Marshfield	155	162	171	204	214	222
Wood	Wisconsin Rapids	153	161	171	203	211	225

would be pupae and half would be late-stage larvae, which most likely would be less sensitive to the Bt toxin (Hellmich *et al.*, 2001; but see Herms *et al.*, 1997).

A more conservative, but most likely less accurate, estimation of potential overlap between larval feeding and pollen shed would be to evaluate the end of larval feeding and initiation of silking at the 90th percentile probability of occurrence. Using that endpoint, 24 of 26 locations (92%) exhibited an overlap between larval feeding and pollen shed.

3.1. Validation of Karner Blue Butterfly Phenological Events

Swengel and Swengel (1999) pooled butterfly flight data from as many as 10 Wisconsin counties from 1990 through 1998. We used weather data from 11 stations in 8 of those counties to determine degree-day accumulations for various phenological events. Validation is difficult because of the lack of systematic sampling of butterfly populations over several years and the relatively low numbers of insects observed. Predictions of peak butterfly flights from our model were compared to two years of observations of first- and second-generation peak flight in Wisconsin (1991–1992) (Bleser, 1994) and one year of observations in Michigan (1989) (Lawrence, 1994). From our model, we predicted that the peak of second-generation flight would occur on days 210, 198, and 231 in 1989, 1991, and 1992, respectively. Actual peak flights occurred on days 209, 198, and 220 in 1989, 1991, and 1992, respectively. We predicted that the peak of first-generation flight would occur on days 151, 164, and 160 in 1989, 1991, and 1992, respectively. Actual peak flights occurred on days 159, 163, and 167 in 1989, 1991, and 1992, respectively. The average magnitude of the difference ($\pm$SE) in days between modeled and observed flight for both flights over both locations was 4.7 $\pm$ 1.9. Therefore, the degree-day model seems to be fairly accurate for predicting phenological events of the Karner blue butterfly, especially peak flight times.

Table V. Predicted Dates (Day-of-Year) and Probabilities of Occurrence for Second-Generation End of Feeding by Karner Blue Butterfly Larvae by Location and Maize Pollen Shed. Bold, Underlined Numbers Indicate Temporal Overlap Between Maize Pollen Shed and End of Larval Feeding

Crop Reporting District	County	Weather Station	End of Larval Feeding Probabilities of Occurrence			Initiation of Silking Probabilities of Occurrence	
			25%	50%	90%	50%	90%
NW	Barron	Cumberland	194	199	212	203	206
NW	Burnett	Danbury	201	**206**	219	**203**	206
NW	Burnett	Grantsburg	197	**206**	216	**203**	206
NW	Chippewa	Bloomer	192	198	213	203	206
NC	Clark	Neillsville	196	203	214	204	207
WC	Dunn	Menomoni	187	192	210	202	203
WC	Eau Claire	Eau Claire	191	196	207	202	203
C	Green Lake	Dalton	187	194	200	204	204
WC	Jackson	Blair	193	199	212	202	203
C	Juneau	Mauston	191	195	210	204	204
SE	Kenosha	Kenosha	205	**212**	228	**203**	207
SE	Kenosha	Lake Geneva	184	190	196	203	207
NE	Oconto	Oconto	203	**209**	225	**205**	209
EC	Outagamie	Appleton	195	200	210	202	209
NW	Polk	St. Croix Falls	189	196	207	203	206
NW	Polk	Amery	198	**204**	216	**203**	206
C	Portage	Rosholt	199	203	219	204	204
C	Portage	Stevens Point	196	201	214	204	204
SW	Sauk	Baraboo	193	196	208	201	203
SW	Sauk	Wisconsin Dells	193	197	209	201	203
NE	Shawano	Shawano	195	200	214	205	209
C	Waupaca	New London	193	198	214	204	204
C	Waupaca	Waupaca	192	197	212	204	204
C	Waushara	Hancock	190	196	207	204	204
C	Wood	Marshfield	195	202	208	204	204
C	Wood	Wisconsin Rapids	193	198	211	204	204

3.2. Validation of Maize Pollen Shed

For the two years for which validation data were available (2001–2002), the maize pollen shed model was most accurate in the central CRD. For the 13 locations in this CRD, the prediction of the 50% silking date was usually within two days of the actual event (Table III). The average difference ($\pm$SE) in days between modeled and observed silking was 1.6 ± 0.25 (Pearson correlation coefficient $= 0.304$; $p = 0.05$; $n = 41$). For the three locations in the west central CRD, modeled silking dates were one to four days early. The pollen shed model was least accurate for the five locations in the northwest CRD. Here the modeled silking dates were two to six days early. However, the fact that the predicted pollen shed may be early suggests that comparisons with the overlap from larval feeding most likely are conservatively cast in this assessment.

3.3. Spatial Analysis

Maize is grown widely across the range of the Karner blue butterfly in Wisconsin (Fig. 1), so at least some local butterfly populations may be near fields where Bt maize is grown. Analysis of maize fields planted during 2003 (USDA, 2004b) in relation to known locations of the butterfly's exclusive host plant (wild lupine) reveals that more than 80% of the lupine localities have few (<1% total area) or no maize fields within 500 m, whereas more than 90% have few or no maize fields within 100 m (Fig. 3). A small but (among counties) geographically consistent number of lupine sites, however, lie within 500 m of maize fields. Statewide, USDA cropland data indicate that 7.3% of documented lupine localities have 10% or more area of maize fields within 500 m and 5% have 10% or more maize fields within 100 m (Fig. 3).
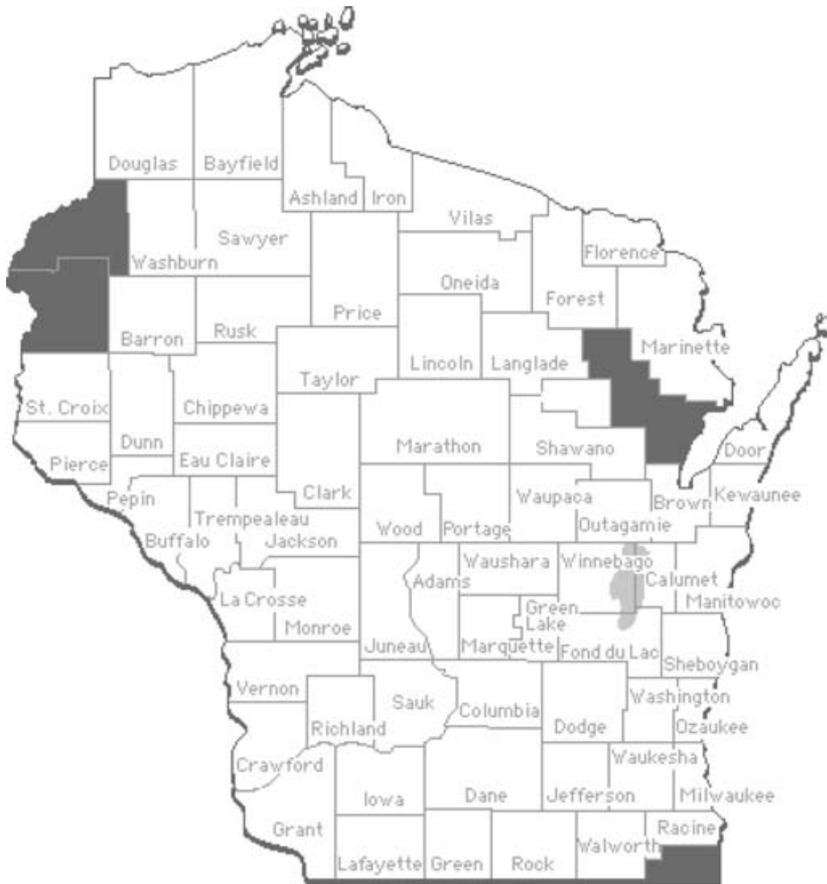
Fig. 2. Shaded counties (Burnett, Kenosha, Oconto, Polk) indicate potential temporal overlap between maize pollen shed and presence of Karner blue butterfly larvae.

Wild lupine has been documented in only two of the four counties where temporal overlap of Bt maize and Karner blue butterfly larvae is most likely (Burnett and Polk). Only two lupine sites are known from Polk County, one with no maize fields within 500 m and the other with 2.9% of the area within 100 m planted to maize and 2.6% of the area within 500 m planted to maize. Burnett County has many (206) documented lupine sites. Only 16 of these (7.7%) have any maize within 500 m, and 7 of these (3.4% of total)
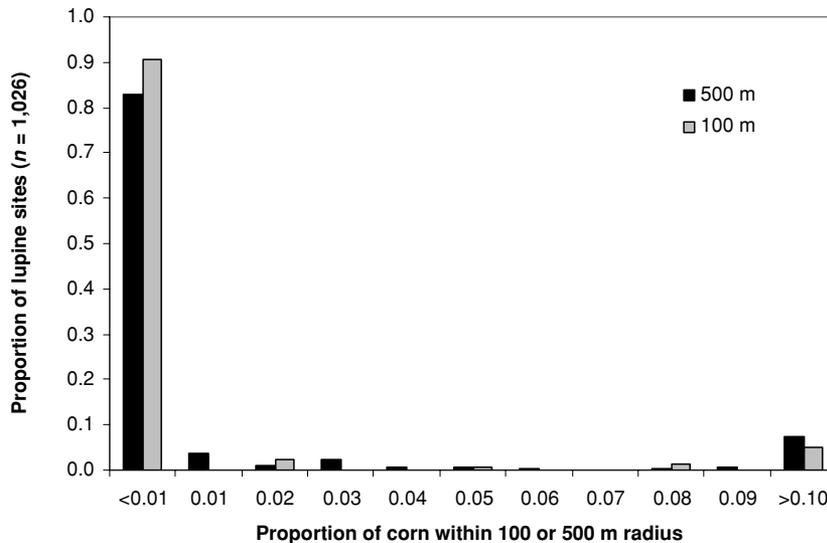
Fig. 3. Proximity of documented localities of wild lupine in Wisconsin (Wisconsin DNR, pers. comm.) to maize fields planted in 2003 (USDA, 2004b). Histograms represent the proportion of all lupine sites ($n = 1,026$) that occurred within the category shown on the horizontal axis. In most cases, only small proportions (<0.01) of the 100- or 500-m radius were occupied by maize fields. Proportion of crop area within 100- and 500-m buffers was calculated in ArcView GIS 3.3 (ESRI).

have more than 10% of the area within 500 m planted to maize. On a finer scale, 11 sites (5.3%) have at least some maize fields and 6 of these (2.9%) have more than 10% of the area within 100 m planted to maize.

An additional consideration of pollen exposure on lupine is the effect of precipitation. Maize pollen germinates quickly after being exposed to moisture and it is thought that the Bt endotoxin is not bioavailable after germination (Zangerl *et al.*, 2001). Using ClimProb, we determined historical probabilities of measurable precipitation for the five locations in which there was potential temporal overlap of Bt maize and Karner blue butterfly larvae. On the predicted 50% silking date, the average percent probability of precipitation ($\pm$SE) was 33.8 ± 1.9. The average percent probability of precipitation ($\pm$SE) for the period two days before and after the predicted 50% silking date was 84.4 ± 1.2. Therefore, this suggests that if Bt maize pollen reaches lupine leaves, it will not be persistent.

4. DISCUSSION

Based on degree-day modeling, there is some potential for temporal exposure of Karner blue butterfly larvae to maize pollen. However, in the majority of years and locations in Wisconsin, maize pollen shed will occur after the majority of larval feeding on lupine. If a more conservative percentile of occurrence is used as a decision aid (e.g., 90% vs. 50%), then there is greater potential for temporal overlap between maize pollen shed and larval feeding. However, the 50th percentile of occurrence most likely is sufficiently conservative for two reasons. First, greater cumulative probabilities of occurrence add additional variability. Second, at the phenological event we have termed "end of feeding" half of the second-generation larvae will already have pupated and the remainder of larvae will be late-stage. Older lepidopteran larvae are less susceptible to Bt endotoxins (Hellmich *et al.*, 2001; but see Herms *et al.*, 1996).

Similarly, the spatial analysis indicates that some Karner blue butterfly populations occur in close proximity to maize fields, but in a large majority of cases the butterfly's larval host plant and maize fields are separated by more than 500 m. As reviewed by Aylor *et al.* (2003), maize pollen is wind dispersed and because of its relatively large size it rapidly settles by gravitation. Perhaps, 5% of pollen dispersed by the wind is carried beyond 60 m (Raynor *et al.*, 1972). Pleasants *et al.* (2001) showed this limited off-source transport to be the case for off-field deposition of maize pollen

on milkweed leaves. The 95th percentile pollen densities at 1 m from the maize field edge (200 grains cm^{-2}) fell to 25 grains cm^{-2} at 4–5 m from the field edge. Milkweed intercepted about 20% of windblown pollen to which it was exposed.

4.1. Uncertainties, Sensitivities, and Refinements

Our degree-day modeling potentially is limited by lack of data on Karner blue butterfly larval phenology, especially date-specific observations of second-generation larval feeding and pupation. Additionally, there has been no systematic study of degree-day requirements for the species. However, stage-specific development times determined by Herms *et al.* (1996) in a laboratory, which we converted to degree days, were similar to field observations (Swengel & Swengel, 1999). The minimum developmental temperature of 12°C used in our model is based on field observations of larval immobility at cool temperatures (Swengel, 1995). Therefore, laboratory determinations of the minimum temperature threshold may be different. However, a review of the literature indicated that minimum developmental temperatures for most lepidopterans are between 9°C and 12°C, suggesting that our use of 12°C is sufficiently conservative given that lower threshold temperatures would cause life-history events of the Karner blue butterfly to occur earlier, further separating the end of larval feeding and maize pollen shed. We also evaluated, using ClimProb, a range of minimum developmental thresholds from 8°C to 12°C and their effect on resulting degree-day accumulations. As expected, minimum developmental thresholds less than 12°C caused degree-day accumulations to occur earlier, supporting our assumption that the 12°C threshold is conservative for this analysis.

We also evaluated the effect of variability of degree-day requirements on predicted dates of second-generation peak flight. Using the standard error value of $\pm$24.1 CDD around the mean of 683 CDD, the 50th probability of occurrence ($\pm$SE) was only $+2.6 \pm 0.15$ and -2.3 ± 0.11 days different than the mean value. Therefore, the predicted dates of life stages are relatively insensitive to variability in degree-day estimates.

Historical modeling of maize pollen shed is limited by lack of data on site-specific maize planting date and plant emergence. Ideally, simulations for each site would be initiated on planting date or plant emergence for accurate estimations of silking date.

We also examined the variability in corn growth using two different methods. We limited the analysis to 10 locations that are either in the actual documented range or very near the edge of the actual documented range of the Karner blue butterfly in Wisconsin, and examined the five years (1998–2002) that we used for our study. For the first analysis, we examined the number of CDD accumulated from the observed emergence data to the observed silking date and compared that to the number of CDD accumulated during the respective timeframe for the 30-year normal period (1971–2000). For example, for Shawano, 617 CDD were accumulated from May 15, 1998 through July 23, 1998. For the 30-year normal period 1971–2000, between those same dates, a mean of 579 ($\pm$71 SE) CDDs was accumulated. The accumulated CDD fell within one SD of the 30-year normal period 78% of the time.

For the second analysis, we examined the date on which observed silking actually occurred and compared that to the 30-year average silking date based on the actual number of accumulated CDD from emergence to silking. For example, there were 617 CDD that actually accumulated from May 15, 1998 through July 23, 1998 in Shawano. Therefore, with a starting date of May 15, we accumulated 617 CDD for each year 1971–2000 (30-year normal period), to determine an average silking date. For the period from May 15, 1998 through July 23, 1998, the average silking date was day-of-year 209 (July 28) with an SD of 6.8 days. As with the first analysis, the observed silking date fell within one SD of the 30-year normal period 78% of the time.

Our analysis of spatial distributions is conservative in several ways. First, we examined all lupine localities, even though only some of these sites support populations of the Karner blue butterfly. The geographic accuracy of lupine localities provided by field observers is not known, so using GIS buffers of 100 and 500 m helps ensure that the actual location is represented in our analysis. If the localities are accurate, then the 100- and 500-m distances far exceed the distance over which lethal quantities of pollen are likely to be deposited (Pleasants *et al.*, 2001; Sears *et al.*, 2001; Zangerl *et al.*, 2001; Wolt *et al.*, 2003). We also made no distinction between fields planted with different types of maize, assuming that all of the fields might potentially be planted with Bt varieties.

Oberhauser *et al.* (2001) argued that Bt pollen might be a serious threat to populations of monarch butterflies because one of the butterfly's major host plants, common milkweed, is a widespread weed that exists within or adjacent to maize fields. Conversely, wild lupine favors dry soils in open woodlands (Wisconsin Dept. Natural Resources, 1999) and does not frequently occur, if it ever occurs, in maize fields. Zangerl *et al.* (2001) demonstrated negative effects of Bt maize pollen on larvae of monarch butterflies and black swallowtail butterflies outside maize fields, but the deposition of pollen was very low beyond 5 m from the edge of the field. Their results are representative of field maize pollen concentrations found by Pleasants *et al.* (2001); therefore, we can conclude that only negligible amounts of Bt pollen will be expected at distances of 100 m or more from the field edge. In short, given the vagaries of pollen dispersal (Ackerman, 2000), coupled with the small number of localities where Karner blue butterfly populations and maize are temporally and spatially juxtaposed, we can conclude that the probability of butterfly exposure most likely is very small and very restricted.

5. CONCLUSIONS

The exposure assessment methodology presented here is a valuable tool for assessing risk of nontarget organisms to potential stressors, such as genetically engineered plants. The approach is particularly relevant for cases in which toxicological information is difficult to obtain and exposure information is incomplete and uncertain. The approach allows decision-makers to focus on times and locations with the greatest probability of exposure (and subsequently risk) to ecological receptors. In the present case, the temporal exposure assessment focused attention on four Wisconsin counties, out of 22 counties where the endangered Karner blue butterflies are known to occur. The spatial assessment revealed that a small number of potential or existing Karner blue butterfly sites are located near maize fields, including sites in two of the four counties where temporal overlap is most likely. These two counties (Polk and Burnett) should receive the highest priority to determine if Karner blue butterfly larvae are actually at risk and then, if needed, to reduce or prevent exposure, which is critical in the case of endangered species.

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