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ABSTRACT

We investigated the phenological and physiological susceptibility of the endangered Karner blue butterfly (*Lycaeides melissa samuelis*) to *Bacillus thuringiensis* var. *kurstaki* (Bt), a product widely used for gypsy moth (*Lymantria dispar*) suppression in Michigan and other infested states. We monitored phenology of the bivoltine Karner blue in two regions of Michigan from 1993 to 1995 to determine if larval stages overlapped temporally with the period of Bt application for gypsy moth suppression. Karner blue larvae of the spring generation were found during the period that Bt was applied in nearby areas in 1993 only. However, spring-generation adults or newly laid eggs were observed up to 11 days before applications in 1994 and 1995. Since Karner blue eggs develop within one week, summer-generation larvae were most likely present during or shortly after 1994 and 1995 Bt application periods. These larvae would have been at risk, assuming Bt persistence of 4 to 6 days.

Physiological susceptibility of Karner blue larvae to Bt was determined in a laboratory bioassay. Larvae were reared on wild lupine (*Lupinus perennis*) foliage that was untreated, or sprayed with Bt formulations at rates of 30–37 or 90 BIU/ha. A similar bioassay with second instar gypsy moth larvae on similarly treated white oak (*Quercus alba*) foliage was conducted concurrently. Karner blue survival was 100%, 27% and 14% on control, low and high Bt treatments, respectively. Early and late Karner blue instars were equally susceptible to Bt. Survival of gypsy moth was 80%, 33% and 5% on control, low and high Bt treatments, respectively, and did not differ significantly from Karner blue survival. We conclude that Karner blue is both phenologically and physiologically susceptible to Bt used for gypsy moth suppression, although the larval generation at risk and extent of phenological overlap may vary from year to year.

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The Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov; Lepidoptera: Lycaenidae) was added to the United States federal endangered species list in December 1992 due to dramatic population declines throughout its range from Minnesota to New Hampshire (Schweitzer 1989, USFWS 1992). The species is extirpated in several states and Ontario (USFWS 1992, Haack 1993). Michigan, Wisconsin and New York have the largest populations, and the best opportunities for species conservation (Baker 1994).

In Michigan, Karner blue populations occur in the western portion of the Lower Peninsula (Baker 1994), primarily in oak savannas and pine-oak barrens (Schweitzer 1989). These dry, sparsely-wooded habitats support grasses and herbaceous plants, including wild lupine (*Lupinus perennis* L.), the only known larval host plant of Karner blue (Schweitzer 1989). Karner blue overwinters in the egg stage and completes two generations per year. Both larval generations feed on lupine, and spring and summer adults require nectar sources (Schweitzer 1989, Dirig 1994).

Gypsy moth (*Lymantria dispar* L.) (Lepidoptera: Lymantriidae) populations have recently spread into areas occupied by Karner blue in Michigan. Microbial insecticides containing *Bacillus thuringiensis* Berliner var. *kurstaki* (Bt) are widely used to suppress or eradicate gypsy moth populations in Michigan and other infested states. For example, 42,000 to 91,000 ha of wooded residential land or forested recreation areas were aerially treated with Bt annually from 1993 to 1995 in Michigan, through the Voluntary Cooperative Gypsy Moth Suppression Program, administered by federal and state agencies (USDA 1994a, 1994b, 1995). An additional but unknown amount of private land also was treated annually with Bt during gypsy moth outbreaks.

Bt, an entomopathogenic bacterium that occurs naturally in the soil and on leaf surfaces (DeLucca et al. 1981, Martin & Travers 1989), is widely used in North America to control outbreaks of forest-defoliating Lepidoptera (Beegle & Yamamoto 1992, Reardon et al. 1994, van Frankenhuyzen 1990). Bt produces proteinaceous crystals during sporulation (Dubois & Lewis 1981). Current formulations of Bt contain these crystals, comprised of *d*-endotoxins, and live spores, which act synergistically with crystals to cause insect mortality (Bauer 1995, Dubois & Lewis 1981, Gill et al. 1992, van Frankenhuyzen et al. 1991). Due to its selective toxicity, safety to vertebrates, and apparently short field persistence of 4 to 6 days on foliage (Beegle et al. 1981, Reardon et al. 1994, Wagner & Miller 1995), Bt presents little risk to nontarget organisms when compared to conventional insecticides (Dimond & Morris 1984, Luthy et al. 1982, Meadows 1993).

Extensive use of Bt, however, has led to growing concern about potential impacts on nontarget Lepidoptera (Brower 1986, Laird 1973, Miller 1990, 1992), especially for declining species such as the Karner blue. Laboratory bioassays have found that several native butterfly and moth species are physiologically susceptible to Bt (Peacock et al. 1993, Wagner & Miller 1995). In addition, recent evidence suggests that Bt may remain active against some lepidopteran species longer than generally thought following field application (Johnson et al. 1995).

Research results demonstrated variability both among and within lepidopteran species in susceptibilty to Bt. Wagner and Miller (1995) concluded that susceptibility could not be generalized from one family or species to another and must be considered on a species-by-species basis (Peacock et al. 1993). To date, no studies have examined the susceptibility of Karner blue or other lycaenids to Bt.

In Michigan and other recently infested states, public pressure to treat gypsy moth-infested woodlands is high, especially in residential and recre-

ational areas (USDA 1994a), and in nurseries, Christmas tree plantations, and other production areas affected by gypsy moth quarantines (D. McCullough, Michigan State University, and R. Priest, MDA, pers. comm.). Areas known to be inhabited by Karner blue, however, cannot be treated with Bt unless approved during a formal consultation process with the US Department of Interior (USDI) Fish & Wildlife Service (USFWS 1992, USDA 1994a). In addition, a 0.8 km spray buffer must be maintained around known Karner blue-occupied sites to protect them against aerial drift (Borak 1994).

These regulations have posed problems where Karner blue populations occur on or adjacent to private property. In addition, surveys to locate all Michigan populations of Karner blue are not complete. More than 100 new populations were discovered during surveys from 1993 to 1995, following listing of the Karner blue as an endangered species (J. Kelly, Huron-Manistee National Forests, pers. comm.). As gypsy moth populations expand into new areas, unknown Karner blue populations may inadvertently be treated with Bt. Information on susceptibility of Karner blue to Bt is needed to evaluate how Karner blue populations could be affected by gypsy moth management.

We investigated the phenological and physiological susceptibility of Karner blue to Bt, in relation to gypsy moth suppression activities in Michigan. Our first objective was to monitor development of Karner blue in the field to determine if larval stages overlapped temporally with Bt spray periods. Our second objective was to evaluate the physiological susceptibility of Karner blue larvae to Bt in a laboratory bioassay.

METHODS

Karner blue phenology and gypsy moth suppression. We monitored spring phenological development of Karner blue and gypsy moth populations in two regions of Michigan from 1993 to 1995 to determine if Karner blue larval stages coincided temporally with the timing of aerial Bt applications for gypsy moth suppression. Bt applications in the Voluntary Cooperative Gypsy Moth Suppression Program are timed to occur when the majority of gypsy moth larvae are late first and early second instars, and when oak foliage is 40–50% expanded (Dubois 1991, USDA 1985).

Five Karner blue-occupied sites in Allegan State Game Area (ASGA) (Allegan Co.) and one site on the Huron-Manistee National Forest (HMNF) (Oceana Co.) (Fig. 1) were monitored. We surveyed spring-generation Karner blue larvae and adults once a week from late April through late May in 1993 and 1994, and from early May through early June in 1995. In 1995, surveys for summer-generation eggs and larvae were also conducted.

Approximately 500 to 1000 wild lupine stems along randomly located transects in each site were examined for Karner blue larvae. Larval length was recorded and the location flagged so that plants with larvae could be relocated. Larvae were classified as either early (first and second) or late (third and fourth) instars based on length. During subsequent surveys, we rechecked previous larval locations and searched new lupine stems. Surveys for eggs in 1995 were conducted in a similar manner by visually inspecting 500 to 1000 lupine stems per site. Karner blue adults were surveyed during 30 to 60 minute walks that traversed each site. Time allocated to adult surveys was based on the size of sites (Herms 1996).

We monitored development of gypsy moth larvae in one population located ca 16 km east of the ASGA study sites, and in one population which overlapped with our Karner blue study site in the HMNF. Gypsy moth egg masses and the foliage of 20 to 30 understory host trees were inspected for

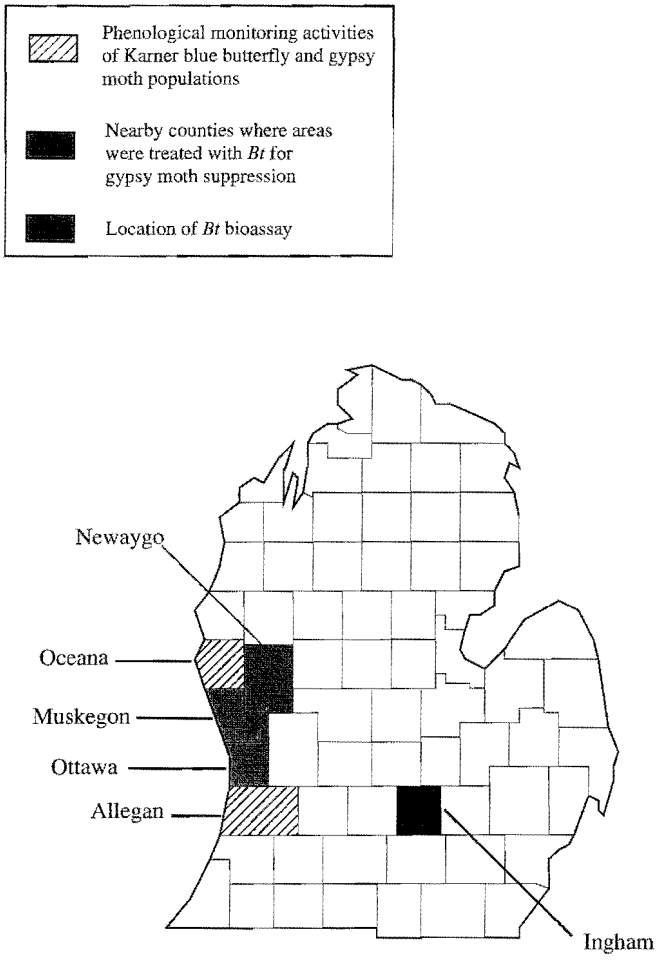

FIGURE 1. Michigan counties where Karner blue butterfly study sites were located (Allegan, Oceana), where Bt was applied at least once from 1993 to 1995 for gypsy moth suppression (Muskegon, Newaygo, Oceana, Ottawa), and where Bt laboratory bioassays were conducted (Ingham).

larvae once a week from egg hatch through early June. We recorded the instar of up to 100 gypsy moth larvae observed during each survey.

We evaluated the potential overlap of Karner blue larval stages with gypsy moth suppression in two ways. Information on gypsy moth larval development was used to predict the timing of a hypothetical Bt application (i.e., when the majority of gypsy moth larvae were late first instars and early seconds) in the ASGA and the HMNF sites. We also compared Karner blue

phenology with dates of actual Bt sprays applied through the Voluntary Cooperative Gypsy Moth Suppression Program in areas near the two Karner blue study sites (Ottawa, Muskegon, Newaygo and Oceana Counties) (Fig. 1).

Bt bioassays. Survival of Karner blue larvae exposed for 7 days to wild lupine leaves treated with Foray 48B (Abbott Laboratories, North Chicago, IL), a commercial Bt formulation commonly used in Michigan for gypsy moth suppression (USDA 1994a, 1995), was determined. A concurrent bioassay with second instar gypsy moth larvae on treated white oak (*Quercus alba* L.) leaves was conducted as a check for the Foray 48B dosages. Bioassays with each species consisted of three treatments: control (untreated foliage), a low Bt dose equivalent to 30–37 Billion International Units (BIU)/hectare (12–15 BIU/acre) field rate, and a high Bt dose equivalent to 90 BIU/hectare (36 BIU/acre) field rate. Typical Bt application rates for gypsy moth suppression range from 40–90 BIU/hectare (16–36 BIU/acre) (Dubois et al. 1993, Reardon et al. 1994). Application rates used in the 1994 and 1995 Michigan Voluntary Cooperative Gypsy Moth Suppression Program ranged from 40–60 BIU/hectare (16–24 BIU/acre) (USDA 1994a, 1995).

Karner blue larvae were reared in the laboratory from eggs of spring-generation female butterflies (Herms et al. 1996). Twenty female butterflies from sites in Montcalm and Newaygo Counties (Fig. 1) were collected in early June 1994, and housed in the laboratory for 5 days to obtain eggs (Herms et al. 1996). A total of 61 larvae hatched, but 2 died soon after emerging, leaving a total of 59 larvae available for the bioassay.

Gypsy moth larvae were obtained from USDA APHIS (Animal and Plant Health Inspection Service) Methods Development Center insect rearing facilities, Otis Air National Guard Base, Massachusetts. Larvae were shipped as first instars on artificial diet several days prior to the bioassay, kept at 24°C and checked daily. Gypsy moth larvae used in the bioassay were second instars that had molted within the previous 24 hours.

Wild lupine foliage obtained from an isolated field in Ingham Co. (Fig. 1) was used for Karner blue rearing and the bioassay (Herms et al. 1996). White oak leaves for the gypsy moth bioassay were obtained from a rural site in Ingham Co.. Lupine and oak foliage for the bioassay were harvested one day before Bt treatments were applied. Foliage for control treatments was kept at 5°C in containers with moist towelling. Foliage for the Bt treatments was placed in water pics, secured in a chilled cooler and flown to Hamden, Connecticut. The following morning, the lupine and oak foliage was brought to room temperature. Low and high Bt treatments were applied using a cylindrical spray tower, 2.5 m in diameter and ca. 4 m high (Hubbard & Lewis 1973), located at the USDA Northeastern Forest Experiment Station in Hamden, Connecticut. The spray tower was designed to simulate aerial Bt application, and was equipped with a Mini-Beecomist nozzle calibrated to generate drops between 75–125 mm volume median diameter (VMD) (Hubbard & Lewis 1973), the drop size range generally used in gypsy moth suppression programs (Reardon et al. 1994). Kromekote spray cards (Mead Corporation, Dayton, OH) were placed next to the leaves and later analyzed to confirm actual spray deposition rates. Bt-treated foliage was returned to Michigan by 6 pm that day.

Bioassays were set up 7 to 8 h after foliar Bt application. Of the 59 available Karner blue larvae, 22 were early instars and 37 were late instars. Fifteen late instars were randomly chosen for controls. Twenty-two larvae (11 early and 11 late instars) were randomly assigned to each Bt treatment. We used only late instars as controls because of the limited number of larvae available for the test. Each larva was placed in a clean petri dish (100 × 15

mm) with one lupine leaf (untreated, or low or high Bt), which had its petiole inserted into a water-filled 2 ml vial plugged with cotton.

For the gypsy moth bioassay, 40 second instar larvae were randomly assigned to each of the three treatments and placed in large, lidded plastic boxes (19 × 9 × 8 cm) (Tri-State Plastics, Dixon, KY), 10 larvae per box. Each box contained a bouquet of five oak leaves (untreated, or low or high Bt) in a water pic. Paper towels were used to line the bottom of the box.

All Karner blue and gypsy moth larvae were reared on treated or untreated foliage for 7 days in a growth chamber at 24°C. Larvae were checked daily for molting and mortality. To avoid buildup of secondary bacteria, sanitation practices included daily removal of frass from the leaves and petri dishes, replacing the paper towel lining in gypsy moth boxes every 2 days, and replacing petri dishes for Karner blue every 1 to 2 days. At the end of 7 days, all surviving larvae were placed in clean containers with fresh, untreated foliage. Karner blue pupae were weighed several times prior to adult emergence. Surviving Karner blue were reared to adulthood and released into their parental collection sites (Herms et al. 1996). The gypsy moth bioassay was terminated after 13 days.

Data analysis: Percentage survival of Karner blue and gypsy moth larvae on control and Bt treatments were analyzed together as a two-dimensional contingency table using SAS CATMOD, a nonparametric procedure for categorical data analysis (SAS Institute Inc., 1987). Two separate analyses were conducted, the first to test for effects of Bt, species and Bt × species interactions, and the second to test for linear effects of the incremental Bt rates (none, low and high). The nonparametric one-sided Smirnov test (Conover 1980) was used to evaluate differences in larval survival for all paired combinations of insect species and treatments. Differences in survival between early and late instar Karner blue were evaluated for each Bt rate as a nonparametric 2 × 2 contingency table using the chi-square test of independence (Conover 1980). To assess sublethal effects of Bt on pupal weight, mean pupal weights (measured 2 days after pupation) of female and male Karner blues reared on control foliage were compared with Karner blue reared on Bt-treated foliage by ANOVA using SYSTAT (Wilkinson 1990). All statistical analyses were conducted at $p<0.05$ level of significance.

RESULTS

Karner blue phenology and gypsy moth suppression. We monitored gypsy moth and Karner blue phenology at our study sites in ASGA and the HMNF to estimate what stage of Karner blue would be present during the optimal period for Bt application, had gypsy moth suppression occurred in these sites. We also related our observations of Karner blue development in the ASGA and HMNF sites to timing of actual Bt sprays that occurred in areas of adjacent counties that participated in the Voluntary Cooperative Gypsy Moth Suppression Program.

In 1993, we found spring-generation Karner blue larvae present at AGSA during the period when Bt application would have hypothetically occurred (Table 1). In 1994 and 1995, spring-generation Karner blue adults were observed at ASGA during the predicted spray period. In 1994, these adults had already been flying for approximately 5 days before Bt application would have been appropriate (Table 1). In the HMNF site, we observed spring-generation Karner blue adults during the predicted spray period each year. In 1994, the first adults were seen six days before the window for Bt application (Table 1).

Table 1. Phenological development of spring and summer-generation Karner blue and gypsy moth in Allegan State Game Area (Allegan Co.) and Huron-Manistee National Forests (Oceana Co.) in Michigan in 1993 to 1995. Life stages of Karner blue observed at the time of hypothetical Bt application, predicted from gypsy moth development, are in bold. Surveys for second generation eggs and larvae were conducted in 1995 only.

		Allegan State Game Area		Huron-Manistee National Forests	
Sampling Year	Date	Karner blue[1]	Gypsy moth	Karner blue[1]	Gypsy moth
1993	30 April	Early instars	Eggs	Early instars	Eggs
	6 May	Early instars	1st instar	Early instars	Not surveyed
	12 May	Early/late instars	1st/2nd instars	Early/late instars	1st instar
	18 May	**Late instars**	**1st/2nd instars**	Late instars	1st instar
	25 May	Adults	1st/2nd instars	**Adults**	**1st/2nd instars**
1994	28 April	Early instars	Eggs	Early instars	Eggs
	8 May	Early/late instars	1st instar	Early instars	Not surveyed
	14 May	Late instars	1st instar	Early/late instars	1st instar
	19 May	Late instars/adults	1st/2nd instars	Late instars	1st instar
	24 May	**Adults**	**1st/2nd instars**	Adults	1st instar
	30 May	Adults	2nd/3rd instars	**Adults**	**1st/2nd instars**
1995	3 May	Early instars	Eggs	Early instars	Eggs
	10 May	Early/late instars	Eggs	Early instars	Eggs
	15 May	Late instars	1st instar	Early/late instars	1st instar
	22 May	**Adults**	**1st/2nd instars**	Late instars	1st instar
	29 May	Adults/Eggs	1st/2nd instars	**Adults**	**1st/2nd instars**
	5 June	Adults/Eggs/Early instars	1st/2nd/3rd/4th instars	Adults/Eggs	2nd/3rd instars

[1]Karner blue larvae were approximated as early (first and second) or late (third and fourth) instars during field surveys.

Table 2. Timing of Bt applications for gypsy moth suppression applied in Michigan counties near Karner blue study sites in 1993 to 1995.

	Bt Application[1]	
County[2]	Year	Date
Muskegon	1994	27 May
	1995	30 May–2 June
Newaygo	1993	28 May
	1994	2–3 June
		15 June[3]
	1995	5 June
Oceana	1993	26 May
	1994	31 May–2 June
	1995	30-31 May
Ottawa	1993	17 May
	1994	23 May
	1995	25 May 25–2 June

[1]Aerial application of Bt in the Michigan Voluntary Cooperative Gypsy Moth Suppression Program administered by the Michigan Department of Agriculture.
[2]See Fig. 1 for location of counties.
[3]Date of second Bt application.

Several areas in Ottawa Co., north of the ASGA study sites (Fig. 1), were treated with Bt for gypsy moth suppression from 1993 to 1995 (Table 2). During the period of Bt application in Ottawa Co., we observed late instar Karner blue larvae of the spring-generation in the ASGA sites in 1993 (Table 1). No Karner blue larvae were seen in 1994 or 1995 during the Ottawa Co. sprays. However, spring-generation Karner blue adults were first observed 4 and 3–11 days before the 1994 and 1995 Ottawa Co. Bt applications, respectively (Table 1). In 1995, Karner blue eggs were first seen 4 days into the 8-day spray period, one week after adults were initially observed. Early instar larvae of the summer-generation were first observed 3 days after completion of the Ottawa Co. spray period, 2 weeks after adults were seen (Table 1).

Bt also was applied for gypsy moth suppression in areas of Oceana and Newaygo Counties from 1993 to 1995, and in Muskegon Co. in 1994 and 1995 (Table 2; Fig. 1). Timing of these sprays was related to Karner blue phenology at the HMNF site. From 1993 to 1995, no spring-generation Karner blue larvae were observed in the HMNF site during the spray periods in those counties. However, the first spring-generation Karner blue adults were observed 1–3 and 7–10 days before the 1993 and 1994 Bt applications, respectively, in Oceana and Newaygo Counties (Table 1). Some areas of Newaygo Co. that were heavily infested with gypsy moth were treated with a second Bt application in 1994. Karner blue adults began flying almost 3 weeks before this second Newaygo Co. application (Table 1), so early instar larvae were probably present. In 1995, we first observed spring-generation Karner blue adults in the HMNF site 1–4 days prior to Muskegon and Oceana Co. applications, and 7 days before Bt application in Newaygo Co.(Table 1). Eggs from spring-generation adults were first seen on the same day as the 1995 Newaygo Co. spray, and 3 and 5 days after the Muskegon and Oceana Co. spray periods, respectively (Table 1).

Bt bioassays. Results of categorical analysis indicated overall survival of larvae on leaves sprayed with Bt was significantly lower than larval sur-

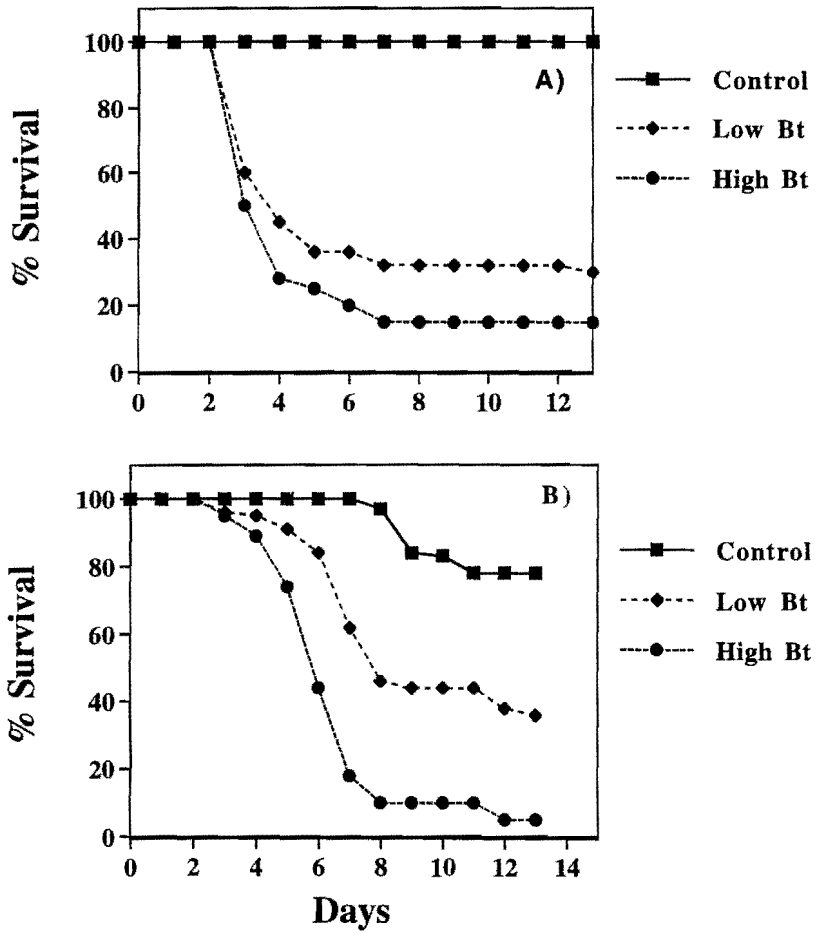

Figure 2. Larval survival of (A) Karner blue butterfly and (B) gypsy moth over 13 days on control (untreated) foliage, on foliage treated with a low-Bt dose (Low; 30–37 BIU/ha), or on foliage treated with a high-Bt dose (High; 90 BIU/ha). On Day 7, all surviving larvae were placed on untreated foliage.

vival on unsprayed leaves (chi-square = 259.1, $p<0.001$). However, there were no significant effects of insect species or Bt × species interactions (chi-square = 2.2 and 3.9, respectively; $p>0.05$), suggesting that Karner blue and gypsy moth did not differ in their overall response to Bt. There was a significant increase in mortality of each species at the higher Bt dose (chi-square = 362.3 for both species combined; chi-square = 459.1 and 111.4 for Karner blue and gypsy moth, respectively; $p<0.001$).

Karner blue survival: All Karner blue larvae reared on untreated

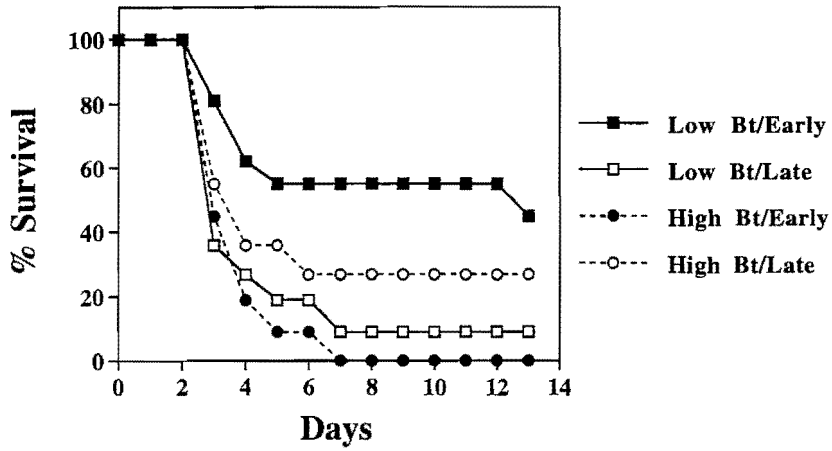

Figure 3. Survival over 13 days of early (first and second) and late (third and fourth) instar Karner blue reared on lupine foliage treated with a low Bt dose (Low; 30–37 BIU/ha) or a high-Bt dose (High; 90 BIU/ha). On day 7, all surviving larvae were placed on untreated lupine foliage.

lupine leaves survived to adulthood (Fig. 2A). Survival of Karner blue larvae in both Bt treatments dropped steeply from Day 3 to 7 (Fig. 2A). By Day 7, 68% of larvae on low-Bt foliage and 86% of larvae on high-Bt foliage had died (Fig. 2A). After larvae were placed on clean foliage, one additional larva on low-Bt foliage died (Fig. 2A). Six larvae reared on low-Bt foliage and 3 larvae reared on high-Bt foliage survived to adulthood. In total, 24 out of 59 Karner blue larvae were released as adults (13 females, 11 males).

The Smirnov test indicated significant differences in overall survival between the control and each of the two Bt treatments ($p<0.001$), confirming results of categorical analysis. However, mortality did not differ significantly between the low and high-Bt rates at any time during the bioassay ($p>0.05$).

On Day 3 of the bioassay, survival of early instar Karner blue larvae on low-Bt foliage was significantly higher than late instar survival on low-Bt foliage (chi-square = 4.70; $p<0.05$). Differences between early and late instar survival also were significant during the Day 7–12 period after larvae were removed from the low-Bt foliage (chi-square = 5.24; $p<0.025$) (Fig. 3). Overall survival on the low-Bt foliage, however, did not differ significantly between early and late instars (chi-square = 3.67; $p>0.05$).

On the high-Bt treatment, there were no differences in survival between early instar and late instar Karner blue larvae at any point of the bioassay (Fig. 3). Overall survival of early instar larvae was significantly higher on the low-Bt foliage than on high-Bt foliage (chi-square = 6.47; $p<0.025$). Survival of late instars did not differ significantly between low and high-Bt treatments (chi-square = 1.22; $p>0.5$).

Pupal weights of Karner blue used in the bioassay were quantified to assess possible sublethal effects of Bt. The data suggest a Bt concentration-dependent decrease in Karner blue pupal weight (Fig. 4). However, the only significant difference was between male pupal weights for the control versus

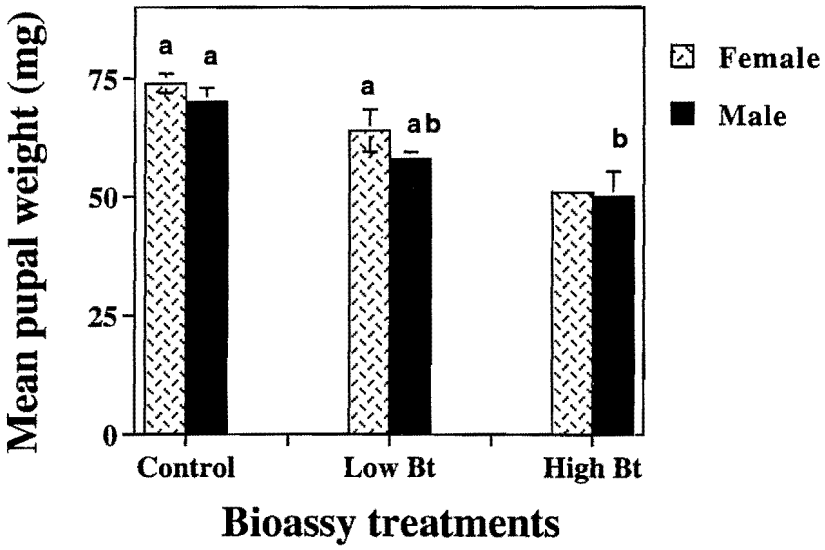

Figure 4. Mean pupal weight (+ 1 SE) two days after pupation for female and male Karner blue larvae used in the Bt bioassay. There were 8, 4, and 1 female survivors and 7, 2, and 2 male survivors on control, low-Bt (Low; 30–37 BIU/ha) and high-Bt (High; 90 BIU/ha) treatments, respectively. Letters above bars indicate significant differences among Bt treatments ($p<0.05$); male and female data were tested separately. Female pupal weight for the high-Bt treatment was not included in ANOVA.

high-Bt treatment ($F = 6.84$; $df = 1$, $r < 0.05$). No other within-gender comparisons of mean pupal weight were significant ($p>0.05$), probably due to the small sample sizes. Female pupal weight for the high-Bt treatment was not included in ANOVA because only a single female survived.

Gypsy moth survival: All gypsy moth larvae reared on untreated foliage survived to Day 8. Some mortality occurred after Day 8, although 80% of the larvae survived to Day 13 (Fig. 2B). In the Bt treatments, some larval mortality occurred on Day 3, but a steep drop in survival was not observed until Day 6 (Fig. 2B). At Day 13, larval survival was 33% and 5% on the low and high-Bt treatments, respectively (Fig. 2B). As with Karner blue, Smirnov analysis indicated that gypsy moth larval survival on both low and high-Bt treatment differed significantly from the control ($p<0.001$), but did not differ significantly from each other ($p>0.05$).

DISCUSSION

Conflicts between forest pest management involving Bt and conservation of nontarget endangered Lepidoptera are likely to increase. For example, is-

sues regarding the use of Bt recently arose in Wisconsin, where Karner blue occurs in jack pine (*Pinus banksiana* Lambert) stands infested with jack pine budworm (*Choristoneura pinus pinus* Freeman; Lepidoptera: Tortricidae) (Baker 1994). In general, susceptibility of nontarget Lepidoptera to Bt depends on the presence of vulnerable larval stages when Bt is sprayed (e.g., phenological susceptibility), toxicity and/or viability of Bt to larvae when ingested (e.g., physiological susceptibility) (Dubois & Lewis 1981, Venables 1990), larval consumption of Bt-treated foliage, and the length of time that Bt remains toxic after spraying (Johnson et al. 1995).

Bt application for gypsy moth suppression is generally timed to occur when highly susceptible first and second instars predominate, and when 40% to 50% canopy development has occurred (Dubois 1991). However, timing varies considerably from year to year due to factors such as weather, and rates of canopy and larval development (Dubois 1991, Reardon et al. 1994). For example, some suppression program managers may spray while most larvae are first instars, to ensure that Bt penetrates the overstory and reaches shrub vegetation where early season feeding may occur (R. Mech, MI Dept. of Natural Resources, pers. comm.). Typically, there is about a 2 week "window" for effective Bt application (Smitley & Davis 1993).

Our phenological data over a three-year period indicated that Bt application for gypsy moth suppression in Michigan is likely to coincide temporally with vulnerable stages of Karner blue. For example, in 1993, late instar Karner blue of the spring-generation were actively feeding during both the predicted and actual Bt spray periods in southwestern Michigan. In 1994 and 1995, spring-generation Karner blue adults were present in the ASGA sites 3 to 11 days prior to Bt applications in nearby areas. Adults were present in the HMNF site as much as 7 to 10 days prior to nearby Bt applications (ca. 3 weeks prior to a second Bt application in one county in 1994).

Our 1995 observations indicated that spring-generation adults can begin laying eggs within one week after the first butterflies emerge. Egg hatch is estimated to occur within one week in the field (Dirig 1994, Schweitzer 1989). Herms et al. (1996) found that Karner blue eggs laid in the laboratory took between 2 to 6 days to hatch at 24°C. Based on this information, we predicted that summer-generation larvae could begin hatching approximately 10 to 11 days after the first spring adults emerge. Thus, Karner blue first instars could have begun to hatch during or a few days after Bt application in 1994 and 1995, and would have been at risk, assuming Bt persistence of 4 to 6 days. In 1995, we searched the ASGA sites for summer-generation first instar Karner blue, which are small (ca. 1.5 mm), well-camouflaged and difficult to locate when newly hatched (Herms 1996, Swengel 1995). We first observed an early instar larva 14 days after spring-generation adults were initially observed (Table 1), and only 3 days after completion of the Bt spray period in a nearby area (Table 2).

Toxicity of Bt is generally thought to breakdown within 4 to 6 days of field application due to environmental factors such as sunlight, temperature, vapor pressure deficit, and rain (Beegle et al. 1981, Ignoffo et al. 1974, Leong et al. 1980, Pinnock et al. 1974, Reardon et al. 1994). However, some studies suggest that Bt may remain toxic for longer periods of time in the field than previously thought (Beckwith and Stelzer 1987, Johnson et al. 1995, Leong et al. 1980). Mortality rates may also be affected by interactions between Bt and other bacteria present as opportunists (Dubois & Dean 1995). Longer persistence of Bt toxins or spores increases the chance that early instars of summer-generation Karner blue could ingest lethal Bt fractions. Field bioassays would be the most conclusive way of determining persistence of Bt toxicity for Karner blue (Leong et al. 1980).

Results from our laboratory bioassays indicated that Bt-treated foliage was toxic to both early and late instar Karner blue larvae and that overall survival of Karner blue and gypsy moth larvae on treated leaves was similar. Most Lepidoptera, including gypsy moth, are more susceptible during early instars than later instars (Peacock & Schweitzer 1992, Reardon et al. 1994, Wagner & Miller 1995), although some exceptions have been reported (James et al. 1993, Peacock et al. 1993, Wagner & Miller 1995).

In our study, early (I and II) and late (III and IV) instar Karner blue appeared equally susceptible to Bt. This result may reflect a shift in feeding behavior as Karner blue larvae develop. Early instar larvae chew a small entry hole in the epidermal layer of the lupine leaf and feed by skeletonizing within the leaf (Haack 1993). Therefore, exposure to Bt by neonates occurs while chewing the entry hole on only one leaf surface, minimizing ingestion of a physiologically lethal dose. Late instar larvae feed freely on the entire leaf, risking consumption of more Bt. Given that all Karner blue larvae were negatively affected by Bt, we assume that the late instar spring-generation larvae observed in 1993 and early instar larvae of the summer-generation that were likely present in 1994 and 1995 would have been at risk if Bt had been applied for gypsy moth suppression.

Although there was a trend for reduced pupal weight and possibly lower fecundity (Honek 1993) of Karner blue reared on Bt-treated foliage, mean pupal weights differed significantly between control and high-Bt treatments only for male Karner blue. Since few females and males survived the Bt treatments to provide comparison, these data should be interpreted cautiously although similar effects were observed in studies with spruce budworm (*Choristoneura fumiferana* (Clemens)) (Bauer and Nordin 1989). Sublethal effects of Bt have been previously considered for beneficial insect predators and parasitoids (Croft 1990), but possible sublethal or multi-generational impacts of Bt on nontarget Lepidoptera need further investigation.

It should be noted that our bioassay was conducted using lupine and oak leaves, rather than intact plants. We assumed that this did not substantially affect our results, which seems reasonable since larval mortality on unsprayed leaves was minimal. Ideally, however, physiological susceptibility should be investigated in the field or at least on intact plants, to avoid any interactions between Bt and host plant quality.

Results of our field and laboratory studies lead us to conclude that Karner blue is both physiologically and phenologically susceptible to Bt used for gypsy moth suppression. The extent of phenological overlap and the larval generation (spring vs. summer) at risk, however, may vary from year to year. Evaluating potential risks of gypsy moth suppression on the survival of Karner blue populations requires consideration of several variables including the size and level of isolation of populations, and the length of time that Bt remains active against Karner blue larvae after field application. Small or isolated Karner blue populations would face greater risk than populations with large numbers of individuals or those in close proximity to other populations to allow for recolonization (Schweitzer 1994).

Information regarding the susceptibility of nontarget Lepidoptera to Bt, including physiological susceptibility, the temporal overlap of larval stages with Bt application, and the duration of Bt's toxic persistence, must be considered in management plans for gypsy moth. In the absence of suppression, however, severe gypsy moth defoliation could affect natural enemy abundance, microclimate, or host plant availability or quality (Johnson et al. 1995, Liebhold & Elkinton 1989, Sample et al. 1993, Wagner & Miller 1995). Development of Bt-based products with higher specificity for gypsy moth

(Dubois & Dean 1995, van Frankenhuyzen et al. 1991), would reduce the impact of gypsy moth control on nontarget lepidopteran species.

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LITERATURE CITED

Baker, R. J. 1994. The Karner blue butterfly: 1993 and beyond. pp. 163–169. In: D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84-1994, Minn. Agric. Exp. Sta., Univ. of Minnesota, St. Paul.

Bauer, L. S. 1995. Resistance: a threat to the insecticidal crystal proteins of *Bacillus thuringiensis*. Florida Entomol. 78(3):1–30.

Bauer, L.S. and G.L. Nordin. 1989. Response of spruce budworm (Lepidoptera: Tortricidae) infected with *Nosema fumiferanae* (microsporida) to *Bacillus thuringiensis* treatments. Environ. Entomol. 18:816–821.

Beckwith, R. C. and M. J. Stelzer. 1987. Persistence of *Bacillus thuringiensis* in two formulations applied by helicopter against the western spruce budworm (Lepidoptera: Tortricidae) in north central Oregon. J. Econ. Entomol. 80: 204–207.

Beegle, C. C. and T. Yamamoto. 1992. Invitation paper (C.P. Alexander Fund): History of *Bacillus thuringiensis* Berliner research and development. Can. Entomol. 124:587–616.

Beegle, C. C., H. T. Dulmage, D. A. Wolfenbarger and E. Martinez. 1981. Persistence of *Bacillus thuringiensis* Berliner insecticidal activity on cotton foliage. Environ. Entomol. 10:400–401.

Brower, L. P. 1986. Commentary: The potential impact of Dipel spraying on the monarch butterfly overwintering phenomenon. Atala 14(1):17–19.

Conover, W. J. 1980. Practical nonparametric statistics. 2nd ed. John Wiley & Sons, New York.

Croft, B. A. 1990. Microbial pesticides. pp. 269–303. In: Arthropod biological control agents and pesticides. John Wiley & Sons, New York.

DeLucca II, A. J., J. G. Simonson and A. D. Larson. 1981. *Bacillus thuringiensis* distribution in soils of the United States. Can. J. Microbiol. 27865–870.

Dimond, J. B. and O. N. Morris. 1984. Microbial and other biological control. pp. 103–114. *In:* D. M. Schmitt, D. G. Grimble and J. L. Searcy (eds.). Spruce Budworm Handbook: Managing the Spruce Budworm in Eastern North America. USDA Forest Service, Cooperative State Res. Ser. Agric. Hndbk. No. 620.

Dirig, R. 1994. Historical notes on wild lupine and the Karner blue butterfly at the Albany Pine Bush, New York. pp. 23–36. *In:* D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84-1994, Minnesota Agricultural Experiment Station, Univ. of Minnesota, St. Paul.

Dubois, N. R. 1991. Insect and foliage development. pp. 108–111. *In:* Aerial spraying for gypsy moth control: A handbook of technology. USDA Forest Service, Northeastern Area Northeastern Forest Experiment Station and Northeast Forest Aerial Application Technology Group, NA-TP-20. 167 pp.

Dubois, N. R. and D. H. Dean. 1995. Synergism between CryIA insecticidal crystal proteins and spores of *Bacillus thuringiensis*, other bacterial spores, and vegetative cells against *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae. Environ. Entomol. 24(6):1741–1747.

Dubois, N. R. and F. B. Lewis. 1981. What is *Bacillus thuringiensis*. J. Arboric. 7:233–240.

Dubois, N. R., R. C. Reardon and K. Mierzejewski. 1993. Field efficacy and deposit analysis of *Bacillus thuringiensis*, Foray 48B, against gypsy moth (Lepidoptera: Lymantriidae). J. Econ. Entomol. 86(1):26–33.

Gill, S. S., E. A. Cowles, and P. V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Annu. Rev. Entomol. 37:615–636.

Haack, R. A. 1993. The endangered Karner blue butterfly (Lepidoptera:Lycaenidae): biology, management considerations, and data gaps. pp. 83–100. *In:* A. R. Gillespie, G. R. Parker, P. E. Phillip and G. Rink (eds.). Proc., 9th Central Hardwood Forest Conference. Gen. Tech. Rep. NC-161. St. Paul, MN: USDA Forest Service, Nor. Cent. For. Exp. Sta., St. Paul, MN.

Herms, C. P. 1996. The endangered Karner blue butterfly (Lepidoptera: Lycaenidae) in Michigan: habitat suitability, potential impacts of gypsy moth (Lepidoptera: Lymantriidae) suppression, and laboratory rearing. M.S. Thesis. Michigan State University, East Lansing. 180 pp.

Herms, C.P., D.G. McCullough, D.L. Miller, L.S. Bauer and R.A. Haack. 1996. Laboratory rearing of *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae), an endangered butterfly in Michigan. Great Lakes Entomol. 29(2):63–75.

Honek, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos 66:483–492.

Hubbard, H. B., Jr. and F. B. Lewis. 1973. A spray tower for the application of microbial aerial sprays. USDA For. Serv. Res. Pap. NE-249. 8 pp.

Ignoffo, C. M., D. L. Hostetter and R. E. Pinnell. 1974. Stability of *Bacillus thuringiensis* and *Baculovirus heliothis* on soybean foliage. Environ. Entomol. 3:117–119.

James, R. R., J. C. Miller and B. Lighthart. 1993. *Bacillus thuringiensis* var. *kurstaki* affects a beneficial insect, the cinnabar moth (Lepidoptera: Arctiidae). J. Econ. Entomol. 86:334–339.

Johnson, K. S., J. M. Scriber, J. K. Nitao and D. R. Smitley. 1995. Toxicity of *Bacillus thuringiensis* var. *kurstaki* to three nontarget Lepidoptera in field studies. Environ. Entomol. 24:288–297.

Laird, M. 1973. Environmental impact of insect control by microorganisms. Ann. New York Acad. Sci. 217:218–226.

Leong, K. L. H., R. J. Cano and A. M. Kubinski. 1980. Factors affecting *Bacillus thuringiensis* total field persistence. Environ. Entomol. 9:593–599.

Liebhold, A. M. and J. S. Elkinton. 1989. Elevated parasitism in artificially augmented populations of *Lymantria dispar* (Lepidoptera: Lymantriidae). Environ. Entomol. 18(6):986–995.

Luthy, P., J. Cordier and H. Fischer. 1982. *Bacillus thuringiensis* as a bacterial insecticide: basic considerations and application. pp. 35–75. *In:* E. Kurstak (ed.). Microbial and Viral Pesticides. Marcel Dekker, Inc., New York.

Martin, P. A. and R. S. Travers. 1989. Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 55: 437–2442.

Meadows, M. P. 1993. *Bacillus thuringiensis* in the environment: ecology and risk assessment. pp. 193–220. *In:* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs (eds.). *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. John Wiley & Sons, New York.

Miller, J. C. 1990. Field assessment of the effects of a microbial pest control agent on nontarget Lepidoptera. Amer. Entomol. 36: 35–139.

Miller, J. C. 1992. Effects of a microbial insecticide, *Bacillus thuringiensis* kurstaki, on nontarget Lepidoptera in a spruce budworm-infested forest. J. Res. Lepidoptera 29:267–276.

Peacock, J. W. and D. F. Schweitzer. 1992. Toxicity of Btk to larvae of non-target Lepidoptera in laboratory bioassay. p. 35. In: K. W. Gottschalk and M. J. Twery (eds.). Proc. USDA Interagency Gypsy Moth Research Forum 1992. USDA For. Serv., NE For. Exp. Sta., Gen. Tech. Rep. NE-170.

Peacock, J. W., D. L. Wagner and D. F. Schweitzer. 1993. Impacts of Bt on non-target Lepidoptera. Newsletter of the Michigan Entomol. Soc. 38(4):1, 4–5.

Pinnock, D. E., R. J. Brand, K. L. Jackson and J. E. Milstead. 1974. The field persistence of *Bacillus thuringiensis* spores on *Cercis occidentalis* leaves. J. Invertebr. Pathol. 23:341–346.

Reardon, R., N. Dubois and W. McLane. 1994. *Bacillus thuringiensis* for managing gypsy moth: a review. USDA For. Serv., National Center of Forest Health Management, Morgantown, WV, FHM-NC-01–94. 32 pp.

Sample, B. E., L. Butler, C. Zivkovich and R. C. Whitmore. 1993. Evaluation of *Bacillus thuringiensis* and defoliation effects on native Lepidoptera. USDA For. Serv. NE Area , Forest Health Protection, Appalachian IPM, NA-TP-10–93. 12 pp.

SAS Institute Inc. 1987. SAS/STATTM guide for personal computers, Version 6 edition. Cary, NC: SAS Institute Inc. 1028 pp.

Schweitzer, D. F. 1989. Fact sheet for the Karner blue butterfly with special reference to New York. Report to The Nature Conservancy, Albany, New York. 7 pp.

Schweitzer, D. F. 1994. Recovery goals and methods for Karner blue butterfly populations. pp. 185–193. *In:* D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84-1994, Minn. Agric.l Exp. Sta., Univ. of Minnesota, St. Paul.

Smitley, D. R. and T. W. Davis. 1993. Aerial application of *Bacillus thuringiensis* for suppression of gypsy moth (Lepidoptera: Lymantriidae) in *Populus-Quercus* forests. J. Econ. Entomol. 86:1178–1184.

Swengel, A.B. 1995. Observations of spring larvae of *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae) in central Wisconsin. Great Lakes Entomol. 28:155–170.

United States Department of Agriculture. 1985. Gypsy moth suppression and eradication projects: Final environmental impact statement as supplemented, 1985. USDA For. Serv. and Animal & Plant Health Inspection Service, Washington, D.C.. 100 pp. plus appendices.

_____. 1994a. Decision notice and environmental assessment: cooperative gypsy moth suppression project— Michigan 1994. USDA For. Serv., Forest Health Protection, St. Paul, Minnesota. 44 pp. plus appendices.

_____. 1994b. Acres treated for gypsy moth in 1994. Gypsy Moth News, September 1994. USDA , Morgantown, West Virginia.

_____. 1995. Decision notice and enviromental assessment: cooperative gypsy moth

suppression project— Michigan 1995. USDA For. Serv. Forest Health Protection, St. Paul, Minnesota. 38 pp. plus appendices.
United States Fish & Wildlife Service. 1992. Endangered and threatened wildlife and plants: Determination of endangered status for the Karner blue butterfly. Federal Register (December 14). 57(240): 59236–59244.
van Frankenhuyzen, K. 1990. Development and current status of *Bacillus thuringiensis* for control of defoliating forest insects. For. Chron. 66:498–507.
van Frankenhuyzen, K., J. L. Gringorten, R. E. Milne, D. Gauthier, M. Pusztai, R. Brousseau and L. Masson. 1991. Specificity of activated CryIA proteins from *Bacillus thuringiensis* subsp. *kurstaki* HD-1 for defoliating forest Lepidoptera. Appl. Environ. Microbiol. 57:1650–1655.
Venables, B. A. B. 1990. Preliminary assessment of the susceptibilities of nontarget lepidopteran species to *Bacillus thuringiensis* (B.t.) and Dimilin used for gypsy moth suppression. Report to USDI National Park Service, National Capital Region, Center for Urban Ecology. 51 pp.
Wagner, D. and J. C. Miller. 1995. Must butterflies die for the gypsy moths' sins? Amer. Butterflies 3(3):19–23.
Wilkinson, L. 1990. SYSTAT: the system for statistics. Evanston, Illinois: SYSTAT Inc. 677 pp.