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The Endangered Karner Blue Butterfly (Lepidoptera: Lycaenidae) in Michigan: Habitat Suitability, Potential Impacts of Gypsy Moth (Lepidoptera: Lymantriidae) Suppression, and Laboratory Rearing

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THE ENDANGERED KARNER BLUE BUTTERFLY (LEPIDOPTERA: LYCAENIDAE) IN MICHIGAN: HABITAT SUITABILITY, POTENTIAL IMPACTS OF GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) SUPPRESSION, AND LABORATORY REARING

By

Catherine Papp Herms

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ABSTRACT

THE ENDANGERED KARNER BLUE BUTTERFLY (LEPIDOPTERA: LYCAENIDAE) IN MICHIGAN: HABITAT SUITABILITY, POTENTIAL IMPACTS OF GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) SUPPRESSION, AND LABORATORY REARING

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The Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov) is an endangered species found in oak savanna and pine barren habitats of the northeastern and central United States. Populations have declined drastically or become extirpated as a result of habitat destruction. In 1993 and 1994, studies were conducted on the Karner blue in Michigan to investigate habitat suitability, potential impacts of gypsy moth (*Lymantria dispar*) suppression and methods for laboratory rearing. Habitat studies revealed that Karner blue abundance was highly associated with densities and frequencies of wild lupine (*Lupinus perennis*), the sole larvae food source. Ant tending was observed for over 80 percent of Karner blue larvae that were found. Thirteen species of tending ants were identified for Michigan. In field phenology surveys, Karner blue larvae were found to be phenologically susceptible to gypsy moth suppression activities using *Bacillus thuringiensis* var. *kurstaki* (*Btk*). In a laboratory bioassay, mortality of Karner blue larvae was significant when larvae were fed foliage treated with two levels of *Btk*. Larvae were highly physiologically susceptible to *Btk*. In 1994, spring generation female butterflies were collected and housed in the laboratory to collect eggs. Larvae were successfully reared through to adulthood, and released back into collection sites.

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Federal permits for this study were obtained in 1993 (subpermit 93-23-I) (Appendix 2) and 1994 (subpermit 94-23-R) (Appendix 3) from the US Fish & Wildlife Service. Threatened / Endangered Species permits (Appendix 4) and Use permits (Appendix 5) were also obtained in 1993 and 1994 from the Michigan Department of Natural Resources and Allegan State Game Area, respectively.

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INTRODUCTION

Many species of invertebrates are declining as a result of habitat alteration and destruction. The federally endangered Karner Blue butterfly (*Lycaeides melissa samuelis* Nabokov; Lepidoptera: Lycaenidae), is a prime example. This butterfly species occupies the declining oak savanna and pine barren habitats of the northeast and central United States. These habitats support wild lupine (*Lupinus perennis* L.), the only known larval food plant of the Karner blue. The butterfly was added to the United States' federal endangered species list in December 1992 as a result of drastic population declines within the last 20 years. The species is currently extirpated in several states. The Karner blue is recognized as an indicator species of the disappearing oak savanna and pine barren communities. Current management programs are focused on conserving and restoring Karner blue populations based upon its habitat requirements, for long-term maintenance of the Karner blue and of the savanna and barrens communities as a whole. The potential for captive rearing is also being explored. Concern has been raised regarding the recent spread of gypsy moth (*Lymantria dispar* L.; Lepidoptera: Lymantriidae), an introduced forest pest, into Karner blue habitat and the potential threats from gypsy moth suppression using a bacterial insecticide, *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*).

The following chapters discuss investigations into various aspects of conservation of the Karner blue butterfly. The first chapter discusses methods used to rear Karner blue

in the laboratory from eggs to adulthood. Karner blue eggs were obtained from spring generation female butterflies that were collected in the field and housed in the laboratory. The goal of the second chapter was to determine the phenological and physiological susceptibility of Karner blue larvae to *Btk* as used for gypsy moth suppression in Michigan. The last chapter presents results from an investigation of habitat suitability of Karner blue in the oak savanna, focusing on larval and adult resources, and other aspects of the butterfly's environment. I hope that information from these studies will contribute to the conservation of this species.

CHAPTER 1

Laboratory Rearing of the Endangered Karner Blue Butterfly (Lepidoptera: Lycaenidae) in Michigan

Abstract

The Karner blue butterfly (*Lycaeides melissa samuelis*) is a federally listed endangered species in the United States, occupying oak savanna and pine barren habitats from eastern Minnesota to New Hampshire. In 1994, we successfully reared Karner blue larvae under controlled laboratory conditions for experimental purposes, and report on those rearing methods here. We collected 20 female Karner blue adults of the spring generation from two areas in Michigan, and housed them in cages in an environmental chamber at 24° - 26°C for 5 days. The female butterflies produced 154 eggs, of which 72 hatched in an average of 4.5 days, and 68 first instars survived. All larvae used as controls for a related research project, plus those not used in the research, successfully completed the 4 instars and survived to adulthood. Eggs, larvae and pupae were kept in a growth chamber at 24°C. Developmental time from egg to adult averaged 26 days; the average duration of each instar ranged from 3 to 4 days, and the average pupal duration was 8 days. In total, 33 laboratory-reared Karner blue adults were released into the maternal collection sites. Laboratory rearing may be a viable means of providing Karner blue individuals for reintroduction into areas where the species has already gone extinct, for supplementation of small populations, or for research with minimal risk to wild

populations. Ultimately, such methods may become an integral part in the recovery of this and other rare invertebrate species.

Introduction

Lycaeides melissa samuelis Nabokov (Lepidoptera: Lycaenidae), commonly referred to as the Karner blue butterfly, is a federally endangered species, and occurs in discontinuous populations along a narrow band from eastern Minnesota to New Hampshire (Shapiro 1969; USFWS 1992; Haack 1993). This species occupies oak savanna in the Midwest and pine barrens in eastern states, both of which are xeric, sparsely wooded, prairie-like communities (Schweitzer 1989). The butterfly's range corresponds generally with the northern limits of its only known larval hostplant, wild lupine (*Lupinus perennis* L.), which grows in the sandy soils of the savanna and barrens habitats (USFWS 1992; Dirig 1994). The Karner blue overwinters in the egg stage and has two generations per year. Larvae of both the spring and summer generations feed on wild lupine, and adults utilize a variety of nectar sources (Schweitzer 1989; Haack 1993; Dirig 1994; Swengel 1995).

The Karner blue was added to the United States federal endangered species list in December 1992 in response to dramatic rangewide reductions in butterfly abundance and distribution (USFWS 1992). Karner blue numbers have declined an estimated 99 percent over the last 100 years, with 90 percent of that decline occurring within the past decade (Schweitzer 1989). Population declines are attributed to habitat loss and fragmentation resulting from anthropogenic activities such as agriculture, residential and commercial development, off-road vehicle use and fire suppression (Packer 1987; USFWS 1992;

Haack 1993; Dirig 1994). Currently, the species occurs in localized areas in Minnesota, Wisconsin, Indiana, Michigan, New York and New Hampshire, and is extirpated in Massachusetts, Pennsylvania, Ohio, Ontario and most likely Illinois (USFWS 1992; Haack 1993; Baker 1994; Grigore and Windus 1994; Packer 1994). Michigan, New York and Wisconsin harbor the greatest numbers of Karner blue populations (Bleser 1992; Haack 1993; Baker 1994).

Conservation of Karner blue is mandated by the Endangered Species Act of 1973, which provides federal protection for the butterfly and its designated critical habitat, and requires the development and implementation of management plans for species recovery (USFWS 1992). Specific recovery measures to-date include ongoing research to elucidate Karner blue ecology and critical habitat needs, habitat restoration and management, and investigation into the potential for Karner blue propagation and reintroduction (USFWS 1992; Baker 1994). Researchers have yet to define all the components of critical habitat, limiting the abilities of managers to restore or improve habitat (Andow et al. 1994). The potential for propagation of Karner blue through captive rearing is gaining increasing attention, especially in states such as Minnesota and New Hampshire, where only a few, small Karner blue populations are known to occur (Schweitzer 1994). These populations could become extirpated before necessary information regarding Karner blue ecology is acquired, or before the habitat has time to respond to management activities (Packer 1994).

Investigations into techniques for captive rearing have been conducted as part of the conservation of other declining butterfly species in the family Lycaenidae (New 1993). Captive rearing may provide a means to supplement low butterfly populations,

reestablish recently extirpated populations (New 1993), or provide individuals for research, with minimal risk to existing butterfly populations (Lane and Welch 1994). However, only recently have attempts been made to identify methods for collection and captive rearing of the Karner blue (Savignano 1992; VanLuven 1993, 1994; Lane and Welch 1994). We describe the methods and success of our efforts to rear Karner blue from spring generation butterflies under controlled laboratory conditions in 1994. Larvae acquired from this study were used in a related study to evaluate the susceptibility of Karner blue to *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*), a microbial insecticide specific to Lepidoptera, commonly used for gypsy moth (*Lymantria dispar* L.; Lepidoptera: Lymantriidae) suppression in Michigan (Chapter 2).

Methods & Materials

Lupine foliage: Wild lupine foliage used for Karner blue rearing activities were obtained from a small field in Ingham County, Michigan, which supports lupine and other remnant prairie plant species but no Karner blue. The lupine was harvested by cutting stems, placing them in a large, water-filled container, and then recutting the ends of the stems under water. In the laboratory, the container with lupine was refrigerated at 5°C until needed. A plastic bag was placed over the top of the foliage to reduce desiccation. New lupine stems were harvested and the old stems discarded every 4 - 5 days. Leaves with previous insect feeding or other damage were not used for rearing Karner blue larvae.

Field collection of Karner blue adults: We collected a total of 20 female Karner blue adults during the spring flight in June 1994 from five collection sites in the Lower

Peninsula of Michigan. Three sites were located in the Allegan State Game Area (Allegan County) in the southwest, and two sites were in the Huron-Manistee National Forest (Montcalm and Newaygo Counties) farther north. Sites were chosen in cooperation with officials from the Michigan Natural Features Inventory, the Michigan Field Office of The Nature Conservancy, the Allegan State Game Area, and the Huron-Manistee National Forest, and were approved by the US Fish & Wildlife Service. Ten females were collected from the Game Area and 10 from the National Forest. We collected only in sites that had 1993 summer generation adult counts of more than 200 butterflies (Michigan Natural Features Inventory, unpublished data; Huron-Manistee National Forest, unpublished data), with no more than five females collected from any one site to minimize possible impacts on local populations.

We collected the Karner blue females 2 weeks after the first spring generation adults were observed, approximately halfway into the spring flight period (Table 1). Since butterflies began flying approximately 5 days sooner in the more southerly sites of Allegan State Game Area than in the Huron-Manistee National Forest, Karner blue females were collected on 1 June 1994 in the Game Area and on 9 June 1994 in the National Forest. We attempted to select females with moderate wing wear, rather than extremely fresh-looking females or those with worn wings, assuming that females with moderate wear would have already mated but still retain much of their egg complement. At the time of collection in the Game Area, the ratio of males to females in Karner blue populations near the collection sites ranged from 2:1 to 3:1 (no butterfly surveys were conducted in the collection sites) (Chapter 2).

Collections were initiated around 11 am and completed by 1 pm. On both days, the weather was sunny, with temperatures around 22°C. We caught each Karner blue female individually in a butterfly net, and transferred it to a glassine envelope by holding the wings. Envelopes with butterflies were then placed in individual plastic containers to prevent crushing, and kept in a slightly chilled cooler (approximately 20°C) in the shade (Saul-Gershenz et al. 1995). A layer of newspaper was used to prevent direct contact of the containers with ice packs at the bottom of the cooler. Transportation time from each collection site to our laboratory at Michigan State University was ca. 2 hours.

Housing of butterflies: In the laboratory, butterflies were transferred to aluminum frame cages (61 x 61 x 61 cm) with 32 mesh Lumite screen (BioQuip Products, Gardena, CA). We opened each envelope inside the cage and allowed the female to walk out onto lupine foliage (described below). Butterflies were caged together by site. Cages were kept on fluorescent-lighted shelves in a walk-in environmental chamber maintained at 24 - 26°C, with an 18:6 hr light:dark photoperiod, and relative humidity of 57 - 68 percent.

We provisioned each cage with a water source, partial shading, nectar source, and ovipositional site. The water source was a wet sponge cut to tightly fit the bottom of a petri dish (100 x 15 mm). One sponge was provided per cage, and was moistened daily. Any standing water or condensation was wiped up immediately, to prevent butterflies from becoming trapped or drowning (Lane and Welch 1994). We provided partial shading by placing layers of paper towels over one corner of the top of the cage.

The nectar source was a 5 percent honey:95 percent water solution presented as per Lane and Welch (1994). The solution was placed in a sterile 150-ml flask, and then sealed with parafilm. Cotton dental wicking (Accu Bite Dental Supply Inc., East

Lansing, MI) was pushed partially into the flask through the parafilm, leaving 3 - 5 cm of wicking protruding, to provide a suitable place for butterflies to perch and feed. We provided two nectar flasks in each cage, and replaced them every 2 days.

The ovipositional site consisted of a wild lupine stem, 20 - 30 cm tall, with flowers and leaves, in a water-filled 250-ml flask with a parafilm seal. We placed two flasks with lupine in each cage, and replaced them every 2 days with fresh lupine.

We housed the females for 5 days in the cages, and then returned all survivors to their original collection sites. Female butterflies were transported in a ca. 20°C cooler, in glassine envelopes and plastic containers as above, to the appropriate site. At the sites, we released each female by opening the envelope near a lupine plant, and allowing the butterfly to walk onto a leaf.

Egg collection and care: We removed the lupine stems from the cages and inspected them for Karner blue eggs once per day. Eggs were carefully dislodged from the plant using a small blade (Lane and Welch 1994), and placed individually into 30-ml plastic cups (Jet Plastica Industries, Hatfield, PA). When lupine stems were replaced, the old stems were kept with the flasks in the environmental chamber, and examined periodically for any eggs or developing larvae that had been initially overlooked.

Plastic cups containing individual eggs were placed in large, lidded plastic boxes (19 x 10 x 8 cm; Tri-State Plastics, Dixon, KY) lined with moist paper towels, and kept in a fluorescent-lighted growth chamber maintained at 24°C, with an 18:6 hr light:dark photoperiod and ambient relative humidity. Relative humidity inside each box with moist paper towels was ca. 80 - 85 percent, as measured with a Bionaire instrument

(model BT-254F, accuracy ± 5 percent; Bionaire Environmental Air Products, Blauvelt, NY). We checked the eggs once per day for hatch. Two days after the eggs were collected, we added a small piece of lupine foliage to each cup in anticipation of hatch. The paper towels in each box were rewetted once at most, but only if there was no condensation on the sides of the box or in the cups. No additional moisture was added to the boxes once the lupine foliage was added to the cups, and the box lids were propped for short periods when necessary to allow excess moisture to dissipate.

Larval rearing: We kept larvae in the same growth chamber as the eggs, and checked them daily for molting, mortality, food supply and condition of container. Molting was noted via presence of exuvia. Larval length was measured at the beginning of each instar using a dissecting microscope fitted with an ocular micrometer.

First and second instars were reared individually in 30-ml plastic cups, which were kept in the growth chamber in lidded plastic boxes as the eggs. Larvae were transferred while on the lupine foliage to fresh cups every 2 days. If necessary, a #000 paintbrush was first used to place each larva on the lupine foliage. We supplied fresh pieces of lupine every 2 days for first instars, and daily for second instars. Old foliage was removed the following day after larvae had moved to the new leaves.

Third and fourth instars were reared individually in petri dishes (100 x 15 mm), which were kept in the growth chamber on trays. We provided an entire lupine leaf to each larva by placing the leaf stem in a water-filled 0.5 dram (2-ml) glass vial stoppered with a cotton plug. In this way, the vials and leaves could be placed in the petri dishes horizontally without water leakage, thus preventing larvae from drowning. Lupine leaves

were replaced when more than half of the leaf was eaten, usually every 1 - 2 days. Third instars were transferred to new petri dishes every 2 days, and fourth instars were transferred to new dishes daily. When replacing old lupine or transferring larvae to new dishes, we cut the leaflets that had the larvae, and then moved the larvae while on the leaflets.

After daily use, paintbrushes, forceps and scissors were sterilized by first soaking in a bleach:water solution (1:4), then washing with soapy water and rinsing in distilled water, and finally autoclaving. To avoid potential disease transmission between individuals, we also cleaned utensils after use with each larva by dipping utensils in the bleach solution, and then rinsing thoroughly with water.

Pupae: We kept pupae in the same growth chamber as the eggs and larvae. Pupae were placed individually in small, lidded plastic boxes (14 x 7 x 4 cm; Tri-State Plastics, Dixon, KY) to allow room for adult emergence. When pupae were attached to a lupine leaf, we cut away excess foliage from around the pupal case to avoid leaf molding. When pupae were attached to the petri dish, we sterilized the dish surface around the pupa with 70 percent ethyl alcohol, and placed the open dish in the box.

Adult butterflies: After emergence, each Karner blue adult with its container was removed from the growth chamber, and kept in a refrigerator at 5°C for 1 or 2 days prior to field release. On the day of release, we transported adults in their boxes in a ca. 20°C cooler to the maternal collection sites. The boxes were then removed from the cooler, and opened in a shady area to allow each butterfly to acclimate and fly away.

Statistical analysis: Developmental times for male and female Karner blue were compared by ANOVA using SYSTAT (Wilkinson 1990). All statistical analyses were conducted at $p < 0.05$ level of significance.

Results

Collection and housing of female butterflies: All 20 Karner blue adult females were collected and transported without mortality from the collection sites to Michigan State University. The butterflies appeared to adjust quickly to the cages, and began using the nectar and water sources within the first few hours. Females from Allegan State Game Area and Huron-Manistee National Forest began laying eggs 2 and 3 days after collection, respectively.

Ten of the 20 Karner blue females were still alive after 5 days (five each from Allegan State Game Area and Huron-Manistee National Forest), and were returned to the original collection sites. We observed male Karner blue butterflies of the spring generation in the sites when the females were released, so presumably all females could have mated. The ten females that did not survive died after 4 - 5 days in captivity of apparently natural causes. These specimens were donated to the Center for Insect Diversity Studies, Department of Entomology, Michigan State University, East Lansing, Michigan.

Egg collection and hatch: We collected a total of 154 eggs from the caged butterflies, of which, 61 percent were from Allegan State Game Area females, and 39 percent were from Huron-Manistee National Forest females (Table 2). Once females began laying eggs, we collected from 0 - 23 eggs per cage per day. Eggs were most often

found on the leaves, petioles and stems of the lupine, and occasionally on flowers. We did not find eggs on the sides of the cages or flasks. Nine eggs laid by the Huron-Manistee National Forest females were overlooked, and were later discovered as second and third instars on the old lupine stems in the environmental chamber. Since females were caged in groups, the exact number of eggs from each female could not be distinguished. Based upon cage averages, the average overall number of eggs per female ranged from 1 - 16.

Overall egg hatch was 47 percent; however, egg hatch varied by region and site (i.e. cage) (Table 2). Forty-three percent of eggs from Allegan State Game Area, and 53 percent of eggs from the Huron-Manistee National Forest hatched (Table 2). Of the 72 first instars obtained, two died (one was deformed so that it could not feed properly and one became diseased), and two escaped (and presumably died), leaving 68 first instars.

A total of 82 Karner blue eggs (53 percent) did not hatch. Of these eggs, we observed six cases where two eggs were stuck together (each was counted as 1 egg, not 2), two eggs which were oddly shaped as compared to the others, and an unidentified species of mite on five of the unhatched eggs. Mold developed on 47 eggs, even though no excessive moisture was apparent. Twenty of those eggs became moldy 5 - 6 days after they were collected, and the other 27 eggs developed mold in 8 - 11 days.

Development of larvae, pupae, adults: We used 59 of the 68 Karner blue larvae in a related study (Chapter 2) to determine the susceptibility of Karner blue to *Btk* used for gypsy moth suppression. The other nine Karner blue that were found as larvae on the old lupine were not used in the *Btk* study, and were reared under normal conditions. Of the larvae used in the *Btk* study, 15 were reared under normal conditions for controls, and the

other 44 larvae were placed at varying instars on *Btk* treatments. Information reported here regarding larval and pupal development (Table 3, 4) was taken from the 15 control larvae, and the 44 treatment larvae up to their placement on the treatments.

Total developmental time of Karner blue from egg collection to adulthood at 24°C averaged 26 days overall; however, developmental time for females differed significantly from males by 2 days on average ($F = 11.47$, $df = 1$; $p < 0.005$) (Table 3). Karner blue eggs hatched on average 4 days after egg collection, with several eggs hatching after only 2 days (Table 3). One egg hatched after only 1 day; however, this egg was probably overlooked during egg collection and left on the lupine foliage for a day. No eggs hatched more than 6 days after collection. Total larval duration (first - fourth instar) averaged 13 days overall; larval duration was ca. 1.5 days longer for females than males on average, but was not significantly different ($F = 4.41$, $df = 1$; $p < 0.056$) (Table 3). The duration of individual instars averaged 3 - 4 days (Table 3). At the prepupal stage, which lasted ca. 1 day (Table 3), Karner blue larvae stopped feeding and became stationary, attaching themselves to the petri dish or to a lupine leaf with a few silk threads. The pupal stage averaged 8 days (Table 3) for both Karner blue males ($n = 7$, SE = 0.2) and females ($n = 8$, SE = 0.2). Pupae darkened significantly 1 day before adult emergence.

Larval body length was difficult to measure accurately because larvae were often moving, appearing more elongate than when stationary. Based on the average initial lengths for each instar, larvae grew 1 mm from first to second instar, 2.7 mm from second to third, and 3.3 mm from third to fourth (Table 4).

The nine larvae not used in the *Btk* study and the 15 control larvae, plus nine of the 44 treatment larvae that survived the *Btk* bioassay, developed successfully to adulthood, producing 33 Karner blue adults for release. Nineteen adults (9 males, 10 females) were released into Allegan sites; 14 adults (6 males, 8 females) were released into Huron-Manistee sites. We observed summer generation Karner blue adults from the wild populations in the sites at the time of release (Table 1).

Discussion

Laboratory, or captive, rearing and subsequent reintroduction have been successful components in the conservation of several butterfly species in the family Lycaenidae, such as the atala hairstreak (*Eumaeus atala* Poey; New 1993) in Florida, and the large blue (*Maculinea arion* L.; Clarke 1977; New 1993) and large copper (*Lycaena dispar* Obth.) in England (Duffey 1977; Pyle et al. 1981). Our results confirm those of recent Karner blue studies (Savignano 1992; VanLuven 1993, 1994; Lane and Welch 1994) that eggs can be collected from females in the laboratory, and can be reared successfully from larva to adult.

In the present study, we obtained 154 eggs, and subsequently 72 first instars, from 20 spring generation Karner blue females. Survival of larvae, pupae and adults reared under normal conditions was high; only four first instars died. Developmental time from egg to adult averaged 26 days at 24°C. The controlled environments of the walk-in environmental chamber and growth chamber used to maintain butterflies and other lifestages ensured that individuals would not experience detrimental temperature extremes. Although most Karner blue larvae were used in related research (Chapter 2),

the 24 larvae reared under normal conditions, plus nine experimental larvae, survived to adulthood (a total of 33), and were released into maternal collection sites. We observed summer generation Karner blue adults from wild populations at the time of release, a fortuitous result. The rate at which Karner blue developed in the laboratory at 24°C was similar enough to that of field individuals to allow for overlap. Ultimately, synchronous development of lab and field populations would be a desired outcome for a reintroduction program.

In Wisconsin, Lane and Welch (1994) reported the highest oviposition and hatching rates of any rearing study to date. They obtained 876 eggs from 40 spring generation Karner blue females after a 2-day housing period, and 88 percent of the eggs hatched. Two hundred larvae were use in a laboratory experiment, and 149 survived to adulthood. The remaining 570 larvae were placed out in the field, with 5 percent survival. Lane and Welch (1994) concluded that captive rearing produced large numbers of larvae with minimal or no impact to local populations, and that survival of larvae to adulthood was higher in the laboratory than in the field.

Summer generation Karner blue females have been used successfully for captive rearing activities in New Hampshire, although overwintering of the eggs and providing lupine for newly hatched larvae in the spring posed some challenges (VanLuven 1993, 1994). In 1992, VanLuven (1993, 1994) obtained 117 eggs from 11 summer generation females that were housed for 3 - 5 days. These eggs were placed outdoors in jars to overwinter, and 110 hatched the following spring. Of those, 88 developed successfully to adulthood.

In this study, we observed lower oviposition rates (Karner blue eggs per female) and hatching success than in previous studies (Savignano 1992; VanLuven 1993; Lane and Welch 1994). These results may have been due to random, uncontrollable variables, such as field conditions experienced by the females prior to collection, that impacted egg production and viability. Savignano (1992) reported year-to-year variability in egg hatch among rearing experiments, ranging from 60 - 90 percent hatch. Lederhouse and Scriber (1987) obtained low oviposition rates and/or egg viability for 10 - 20 percent of field-collected female tiger swallowtail butterflies (*Papilio glaucus* L.; Lepidoptera: Papilionidae) in each of several trials; they attributed these results to random mating failure. However, oviposition and hatching rates in this study may also have been affected by experimental variables such as age (based on wing wear) of collected females, handling of females (collection, transport), size and type of ovipositional cage, and environmental conditions (temperature, relative humidity, light) used to maintain females and eggs in the laboratory. Of these four variables, female age and environmental laboratory conditions are the most probable ones to explain our results.

Like VanLuven (1993, 1994), we attempted to collect females with moderate wing wear, assuming that these females would have mated (Friedrich 1986) but still retain many eggs. In contrast, Lane and Welch (1994) captured fresh females, many of which were observed ovipositing in the field and were presumed to be gravid. It is possible that the moderately worn females collected in our study had already laid a large proportion of their eggs in the field (Friedrich 1986), which would explain the low numbers of eggs obtained. Age of the Karner blue females may also have impacted egg viability. Lederhouse and Scriber (1987) reported significant declines over time in egg

viability of female tiger swallowtail butterflies. Although unlikely, some of the Karner blue females we collected may not have been gravid, as proposed by VanLuven (1994) to explain low egg numbers in his 1993 study; any eggs laid by these females would have contributed to the low hatching success we recorded.

Our adult butterfly collection and transportation methods differed somewhat from other studies. After netting the Karner blue adults, we transferred individuals to glassine envelopes to confine their movement, and kept them in a ca. 20°C cooler for transport (Saul-Gershenz et al. 1995). We handled the females only by the wings. In other studies, butterflies were not directly handled, and had some freedom of movement during transport (VanLuven 1993, 1994; Lane and Welch 1994). Lane and Welch (1994) also provisioned butterflies with water and nectar sources. Transport time from the field to the laboratory was considerably longer in our study than in the other studies. Keeping the butterflies immobile and cool ensured that they would not experience temperature extremes (Saul-Gershenz et al. 1995), reduced their need for resources during transportation, and did not appear to stress or damage them.

Small butterflies, such as lycaenids, can be induced to oviposit in small containers that restrict movement (Friedrich 1986). VanLuven (1993, 1994) used 240-ml glass jars to house summer generation females for oviposition, with varying success. For this study, we chose to use larger mesh cages, with access provided by a cloth sleeve, to facilitate the provisioning of resources such as lupine stems for oviposition and honeywater, and to minimize the risk of butterflies escaping. Lane and Welch (1994) used a similar type of mesh cage to ours; however, their cage was half the size (30 x 30 x 30cm), which caused the lupine stems to touch the top of the cage. Females were often

observed walking on the cage top and coming into contact with the lupine (C. Lane, University of Minnesota, pers. comm.). A smaller cage may be more successful to induce oviposition of Karner blue females by increasing the likelihood of contact between butterflies and ovipositional sites.

Environmental laboratory conditions, such as temperature, relative humidity and light, used to maintain females and eggs in this study may have affected oviposition rate and egg hatch (Singh and Ashby 1985). Our rearing methods mimicked field conditions less than other studies because of our use of an environmental walk-in chamber to house caged butterflies and growth chambers to house the other butterfly lifestages.

Temperature is an important variable for determining insect activity and development (Goodenough and Parnell 1985; Singh and Ashby 1985; Saul-Gershenz et al. 1995). We housed female butterflies at 24 - 26°C, temperatures slightly lower than daytime temperatures in the field. VanLuven (1993) observed that female Karner blue butterflies of the summer generation were relatively inactive when housed in the laboratory at temperatures below 27°C. However, guidelines for butterfly rearing have suggested 25°C as an acceptable temperature for oviposition (Friedrich 1986). Lane and Welch (1994) kept caged females at ambient room temperature, which averaged 28°C, but fluctuated widely from 23° to 31°C during the day. Temperatures higher than what were used in this study, or fluctuating temperatures, may be important to facilitate egg production or stimulate oviposition with Karner blue. The same may be true for egg development. We maintained eggs at 24°C, whereas Lane and Welch (1994) kept eggs in ambient room temperature, which averaged 24°C, but ranged daily from 20° - 28°C.

The appropriate level of relative humidity for insect development varies with different lifestages (Saul-Gershenz et al. 1995). Relative humidity can impact egg development (Goodenough and Parnell 1985) by either causing desiccation when humidity is too low or molding when humidity is too high (Singh and Ashby 1985; Friedrich 1986). In our study, molding appeared to have reduced egg hatch; approximately half of the unhatched eggs developed mold, some within 6 days and others within 11 days of collection. After collection, eggs were kept in plastic boxes in a growth chamber with ambient relative humidity. We attempted to control the humidity in the boxes in two ways: adding wetted paper towels (prior to the addition of foliage) to increase humidity, or propping the lids of the boxes to reduce condensation. Lane and Welch (1994) similarly reported molding as a significant factor in preliminary rearing attempts with Karner blue. Surface disinfection of eggs would presumably reduce this problem (Singh and Ashby 1985). The remaining unhatched eggs in our study neither developed mold, nor appeared desiccated.

The quality of light, both wavelength and intensity, and photoperiod, can impact insect physiology, biochemistry and behavior, including oviposition behavior (Singh and Ashby 1985; Saul-Gershenz et al. 1995). In our study, lighting experienced by caged Karner blue females was provided entirely by fluorescent bulbs, with an 18:6 hr light:dark photoperiod. In the studies by Lane and Welch (1994) and VanLuven (1993, 1994), caged butterflies experienced some indirect natural lighting. However, in the study by Lane and Welch (1994), most lighting came from fluorescent bulbs, with a 16:8 hr light:dark photoperiod. VanLuven (1993, 1994) supplemented the natural light with an incandescent lamp during cloudy days.

We did not encounter any problems rearing larvae to adulthood in the laboratory. Karner blue larvae developed successfully without the provision of tending ant species; however, this may be a requirement for other ant-tended lycaenid species (New 1993). Only one larva died from an apparent disease. We emphasized sanitation throughout the rearing process (Singh and Ashby 1985; Saul-Gershenz et al. 1995), especially during larval rearing. Protocols included housing larvae in individual containers which were changed often, keeping larval containers free of frass and moisture build-up, supplying clean foliage regularly, and using sterilized tools.

Karner blue larvae appeared to do well on cut foliage from wild lupine plants. Our initial intention was to rear larvae on wild lupine grown from seed in the greenhouse, and a preliminary attempt in 1993 to produce greenhouse lupine was successful. Unfortunately, in our 1994 study, the lupine seedlings became infested with western flower thrips (*Frankliniella occidentalis* Pergande; Thysanoptera: Thripidae), a common greenhouse pest, and no plants survived. Savignano (1992) successfully reared Karner blue larvae from eggs of spring generation butterflies on Russell Hybrid, a cultivated lupine hybrid that grows more quickly in the greenhouse and produces larger leaves than does wild lupine. Cultivation of lupine in the greenhouse may become a useful way of providing foliage for Karner blue rearing projects, especially when overwintered eggs are used and wild lupine may be difficult to obtain in the spring.

While we need more information on proper laboratory conditions for Karner blue oviposition and development, captive rearing appears to be a viable means of producing large numbers of Karner blue individuals with potentially little impact to source populations. These individuals can be used to supplement or reestablish populations, or

used in research. In considering the use of reared Karner blue for reintroduction, some questions still remain, such as which generation of Karner blue (spring or summer) should be used for the egg source, and which life stage should be released in the field (Lane and Welch 1994; Schweitzer 1994). Based upon previous recommendations for captive rearing programs, reintroductions should occur only within the historic range of the Karner blue, and reared individuals that are to be used for supplementation or re-establishment should be genetically similar to native individuals in or near the release site (Pyle 1976; New et al. 1995). While captive rearing does not replace the need for conservation of butterfly populations in the natural environment (New 1993; Robinson 1995), it appears to be a viable option in the overall conservation program of the Karner blue.

Table 1.1. Adult flight periods of 1994 spring and summer Karner blue generations in Allegan State Game Area (Allegan Co) and Huron-Manistee National Forest (Oceana Co) in Michigan.

Area	Flight period	First adult seen	Last adult seen
Allegan State Game Area	Spring	May 19	June 18
	Summer	June 27	August 12
Huron-Manistee National Forest	Spring	May 24	*not recorded*
	Summer	July 5	*not recorded*

Table 1.2. Total numbers of eggs obtained and hatched from caged female Karner blue butterflies collected from Allegan State Game Area (Allegan Co) and Huron-Manistee National Forest (Montcalm Co and Newaygo Co) in Michigan.

Karner blue collection area	Cage no.		No. Karner blue adult females	No. eggs	
				Laid	Hatched
Allegan State Game Area	1		4	64	29
	2		3	13	2
	3		3	17	9
		Subtotal	10	94	40
Huron-Manistee National Forest	4		5	54	28
	5		5	6	4
		Subtotal	10	60	32
		Total	**20**	**154**	**72**

Table 1.3. Mean duration (± SE) of Karner blue life stages captively reared at 24°C.

Life stage	Sample size[1]	Duration of life stages (days)	
		Mean ± SE	Range
Egg	62	4.1 ± 0.2	1 - 6[2]
1st instar	38	3.2 ± 0.2	2 - 6
2nd instar	36	3.1 ± 0.1	1 - 5
3rd instar	31	3.4 ± 0.1	2 - 5
4th instar	15	4.0 ± 0.2	3 - 6
Prepupa	15	1.2 ± 0.1	1 - 2
Pupa	15	7.9 ± 0.2	7 - 9
1st - 4th instar	15	13.1 ± 0.4	11 - 16
Males	7	12.4 ± 0.5 a	11 - 14
Females	8	13.8 ± 0.5 a	12 - 16
Egg - adult	15	26.0 ± 0.4	24 - 29
Males	7	25.0 ± 0.2 a	24 - 26
Females	8	26.9 ± 0.5 b	25 - 29

NOTE: For gender comparisons, means followed by the same letter are not significantly different by ANOVA at $p < 0.05$.

[1] Some larvae reared in this study were used in related research (Chapter 2). Data reported here represent development of 'treatment' Karner blue larvae before they were assigned to treatments, and 'control' larvae in the related research.

[2] Only one egg hatched 1 day after collection; however, it was probably overlooked during egg collection and left on the lupine foliage for 1 day.

Table 1.4. Average body length (± SE) of captively reared Karner blue larvae at the onset of each instar.

Instar	Sample size[1]	Body length (mm)	
		Mean ± SE	Range
1st	25	1.5 ± 0.04	1.1 - 1.9
2nd	18	2.5 ± 0.12	1.9 - 3.5
3rd	31	5.2 ± 0.20	3.3 - 6.8
4th	28	8.5 ± 0.25	6.2 - 12.5

[1] Some larvae reared in this study were used in related research (Chapter 2). Data reported here represent development of 'treatment' Karner blue larvae before they were assigned to treatments, and 'control' larvae in the related research.

CHAPTER 2

Susceptibility of the Endangered Karner Blue Butterfly (Lepidoptera: Lycaenidae) to *Bacillus thuringiensis* var. *kurstaki* Used for Gypsy Moth (Lepidoptera: Lymantriidae) Suppression in Michigan

Abstract

Management conflicts have arisen in Michigan due to the recent spread of gypsy moth (*Lymantria dispar*), an introduced forest pest, into oak savanna habitat occupied by the endangered Karner blue butterfly (*Lycaeides melissa samuelis*). Microbial insecticides formulated from *Bacillus thuringiensis* var. *kurstaki* (*Btk*), a naturally occurring soil bacterium, are commonly used for gypsy moth suppression; however, widespread use has raised concern regarding the impacts of *Btk* on nontarget Lepidoptera. In this study, we investigated the phenological and physiological susceptibility of Karner blue to *Btk* as used for gypsy moth suppression in Michigan. In the spring of 1993 - 1995, we monitored phenology of spring generation Karner blue populations in two regions of Lower Michigan to determine if larval stages overlapped temporally with the *Btk* spray period for gypsy moth in nearby areas. In 1993, some late instar Karner blue of the spring generation were found during *Btk* application in one region. In 1994 and 1995, no spring generation larvae overlapped the *Btk* spray periods in either region; however, spring generation Karner blue adults were observed up to 11 days prior to *Btk* application, and in 1995, newly laid eggs were observed at the time of or a few days before *Btk*

application. Since Karner blue eggs hatch quickly, summer generation early instars were most likely present during or shortly after *Btk* application in 1994 and 1995, and assuming that *Btk* persists in the field for 4 - 6 days post-spray, some larvae would have been at risk.

In a laboratory bioassay, captively-reared Karner blue larvae (first through fourth instars) were fed foliage of the host plant, wild lupine (*Lupinus perennis*), which were untreated or treated with the *Btk* formulation Foray 48B, at rates of ca. 30 - 37 BIU/hectare (12 - 15 BIU/acre) and 90 BIU/hectare (36 BIU/acre). A similar bioassay with second instar gypsy moth larvae on white oak foliage (*Quercus alba*) was conducted concurrently. Karner blue larval survival was 27 percent and 14 percent on low and high *Btk* treatments, respectively, and was significantly lower for all instars on both *Btk* treatments than for controls. Survival of gypsy moth larvae was 33 percent and 5 percent on low and high *Btk* treatments, respectively. Overall survival of Karner blue did not differ significantly from that of gypsy moth; however, Karner blue mortality was significantly higher than gypsy moth mortality in the first 3 - 6 days of the bioassay, suggesting that Karner blue may be more sensitive to *Btk* than gypsy moth. We conclude that Karner blue is highly susceptible physiologically to *Btk*, and is phenologically susceptible to gypsy moth suppression activities, though the extent of phenological overlap and the larval generation (spring vs. summer) at risk may vary from year to year. Information regarding the susceptibility of nontarget Lepidoptera to *Btk*, including physiological susceptibility and temporal overlap of larval stages with *Btk* application and the period of toxic persistence, must be considered in management plans for gypsy moth.

However, impacts of gypsy moth defoliation, in the absence of suppression, on nontargets must also be considered.

Introduction

The Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov; Lepidoptera: Lycaenidae) is a federally endangered species occurring in localized areas of the northeastern and midwestern United States. Recently, in Michigan, gypsy moth (*Lymantria dispar* L.; Lepidoptera: Lymantriidae), an introduced defoliator of hardwoods, has spread into oak savanna habitat, to which the Karner blue is restricted. *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*), a microbial insecticide, is widely sprayed in Michigan to suppress gypsy moth populations. However, concern regarding potential nontarget impacts of *Btk* on Karner blue has brought about management conflicts in areas where gypsy moth and Karner blue co-occur.

The Karner blue was added to the United States' federal endangered species list in December 1992 due to dramatic population declines throughout its range (Schweitzer 1989; USFWS 1992). Historically, Karner blue populations occurred in a narrow band from Minnesota to New Hampshire. However, the species is currently extirpated in Ohio, Pennsylvania, Massachusetts and Ontario (USFWS 1992; Haack 1993). Habitat of the Karner blue consists primarily of oak savannas in the Midwest and pine barrens in the Northeast (Schweitzer 1989). These dry, sandy, sparsely wooded habitats support many grasses and herbaceous plants including wild lupine (*Lupinus perennis* L.), the only known host plant of Karner blue larvae (Schweitzer 1989). The butterfly completes two generations per year; both larval generations feed on lupine, and spring and summer

adults require nectar sources (Schweitzer 1989; Dirig 1994). Rangewide decline of Karner blue is attributed to loss of suitable habitat due largely to human activities, such as agriculture, residential and commercial development and fire suppression (Packer 1987; USFWS 1992; Haack 1993; Dirig 1994; Lane 1994). As with all federally listed species, the Endangered Species Act of 1973 mandates that conservation measures be provided for the Karner blue to ensure its survival (USFWS 1992).

Gypsy moth was first recorded in eastern Michigan in 1954 (O'Dell 1955). Despite control efforts, populations have continued to spread west throughout the state, causing severe defoliation of oak-dominated woodlands (Gage et al. 1990; Witter and Stoyenoff 1992). Current efforts to suppress gypsy moth populations in wooded residential areas and high-value recreation sites in Michigan are administered jointly by the Michigan Department of Agriculture and the United States Department of Agriculture (USDA) Forest Service through the Michigan Voluntary Cooperative Gypsy Moth Suppression Program (USDA 1994a). This is a large program, which recently has involved aerial application of *Btk* to more than 91,200 hectares in Michigan in 1993, 56,720 hectares in 1994, and 42,800 hectares in 1995 (USDA 1994a, 1994b; USDA 1995).

Bacillus thuringiensis var. *kurstaki* is an entomopathogenic bacteria that occurs naturally in the soil (DeLucca et al. 1981; Dulmage and Aizawa 1982; Martin and Travers 1989), and is selectively toxic to larvae of some lepidopteran species (Dubois and Lewis 1981). The *Bacillus thuringiensis* group of bacteria produce proteinaceous crystalline inclusions, or crystals, during spore formation (Cherwonogrodzky 1980; Dubois and Lewis 1981; Gill et al. 1992). The crystals of *Btk* are a matrix within which

glycoproteins, known as δ-endotoxins or insecticidal crystal proteins (ICP) (Gill et al. 1992; Bauer 1995), are contained. The insecticidal activity of *Btk* is largely attributed to the solubilization of the crystal in the gut of the insect and activation of the δ-endotoxins (van Frankenhuyzen et al. 1991). Gut perforations occur and the spores invade the haemolymph and cause septicemia; death occurs from ICP toxicity and is enhanced by septicemia (Bauer 1995; Dubois and Dean 1995). Most, if not all, commercial preparations of *Btk* contain both crystals and spores (Lüthy et al. 1982; Bauer 1995).

Bacillus thuringiensis var. *kurstaki* is widely used as a microbial pesticide for control of forest defoliating Lepidoptera in North America (van Frankenhuyzen 1990; Beegle and Yamamoto 1992; Reardon et al. 1994). Due to its selective toxicity, safety to vertebrates, and apparently short field persistence of 4 - 6 days on foliage (Beegle et al. 1981; Reardon et al. 1994; Wagner and Miller 1995), *Btk* is thought to present little risk to nontarget organisms compared to alternative insecticides (Morris et al. 1975; Lüthy et al. 1982; Dimond and Morris 1984; Meadows 1993; Bauer 1995). However, as a result of *Btk*'s extensive use, there is growing concern regarding the potential impacts on nontarget Lepidoptera (Laird 1973; Brower 1986; Miller 1990), especially for declining species such as the Karner blue. In addition, recent evidence suggests that *Btk* may remain toxic to some lepidopteran species for much longer than generally thought following field application (Johnson et al. 1995).

Management conflicts have arisen in areas of Michigan where gypsy moth and Karner blue populations overlap. Public pressure to treat gypsy moth-infested woodlands is on the rise, especially in residential or recreational areas (USDA 1994a), and in

nurseries, Christmas tree plantations, and other plant industry production areas (D. McCullough, Michigan State University, and R. Priest, Michigan Department of Agriculture, pers. comm.). However, according to US federal regulations, areas inhabited by Karner blue cannot be treated with *Btk* (USDA 1994a), except through formal consultation with the US Fish & Wildlife Service (USFWS 1992), because of potential negative impacts. In addition, a 0.8 km spray buffer must be maintained around known Karner blue-occupied sites to protect them against drift (Borak 1994).

A limited number of field and laboratory studies to date have addressed the issue of susceptibility of nontarget Lepidoptera to *Btk.* In field studies in Oregon and West Virginia where only a single application of *Btk* was used for western spruce budworm (*Choristoneura occidentalis* Freeman; Lepidoptera: Tortricidae) and gypsy moth, respectively, larval abundance and species richness of Lepidoptera were reduced for at least two years after treatment (Miller 1992; Sample et al. 1993). Decreases in species richness and larval abundance of oak-feeding lepidopterans were also observed for up to two years following repeated *Btk* applications over one season for gypsy moth eradication in Oregon (Miller 1990). *Btk* toxicity has been determined for the cinnabar moth (*Tyria jacobaeae* L.; Lepidoptera: Arctiidae) (James et al. 1993), a biocontrol agent of tansy ragwort (*Senecio jacobaea* L.), and for two swallowtail butterfly species (*Papilio glaucus* L. and *P. canadensis* Rothschild and Jordan; Lepidoptera: Papilionidae) and the promethea moth (*Callosamia promethea* Drury; Lepidoptera: Saturniidae) (Johnson et al. 1995) in field trials. Laboratory bioassays have demonstrated *Btk* susceptibility for several other native species of butterflies and moths (Peacock et al. 1993; Wagner and Miller 1995). Though negative effects of *Btk* have been demonstrated for a broad range

of nontarget lepidopteran species, *Btk* susceptibility cannot be generalized from one family or species to another (Wagner and Miller 1995), 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 lycaenid species to *Btk*.

Surveys to locate all Michigan populations of Karner blue have not been completed. Many new populations were discovered in 1993 - 1995, following listing of the Karner blue as an endangered species (J. Kelly, Huron-Manistee National Forest, pers. comm.). As gypsy moth populations expand into new areas, it is possible that unknown Karner blue populations will be inadvertently treated with *Btk*. Information on phenological and physiological susceptibility of Karner blue to *Btk* is required to ensure that populations are not negatively affected by gypsy moth management programs.

In this study, we investigated the susceptibility of the Karner blue butterfly to *Btk*, as used for gypsy moth suppression activities in Michigan. Our first objective was to monitor development of Karner blue in the field to determine if larval instars or other life stages overlap temporally with the *Btk* spray period. Our second objective was to evaluate the physiological susceptibility of Karner blue larvae to *Btk* in a laboratory bioassay.

Methods & Materials

Phenology of Karner blue with respect to gypsy moth suppression

We monitored the phenological development of Karner blue and gypsy moth populations in two regions of Lower Michigan in the springs of 1993 - 1995 to determine if Karner blue larval stages would coincide temporally with the timing of aerial *Btk*

spraying for gypsy moth suppression. *Btk* application in the Michigan Voluntary Cooperative Gypsy Moth Suppression Program is timed to occur when the majority of gypsy moth larvae are late first instars and early second instars, and when oak foliage is 40 - 50 percent expanded (USDA 1985; Dubois 1991).

Five Karner blue-occupied sites in Allegan State Game Area (Allegan County) in southwestern Michigan, and one site located farther north on the Huron-Manistee National Forest (Oceana County) (Figure 1) were chosen for monitoring activities. We surveyed the sites for 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 (Table 1). In 1995, surveys for eggs and larvae of summer generation Karner blue were also conducted.

For each larval survey, approximately 500 - 1000 randomly chosen wild lupine stems were examined for window-feeding damage indicative of Karner blue larvae (Dirig 1994). Lupine stems with feeding damage were inspected for larvae. When Karner blue larvae were found, larval length was recorded, and the plant's location was flagged so that plants could be relocated. Larval length was used to classify larvae as either early (first and second) or late (third and fourth) instars. During subsequent surveys, we rechecked all previous larval locations and searched new lupine stems for additional larvae. Surveys for eggs in 1995 were conducted in a similar manner by visually inspecting 500 - 1000 randomly chosen lupine plants. To survey for the presence of Karner blue adults, we randomly walked through each site for ca. 30 - 60 minutes.

We monitored gypsy moth larval development in one population located approximately 16 km east of the Karner blue study sites in Allegan State Game Area, and

in one population which occurred in our Karner blue study site in the Huron-Manistee National forest. Foliage of 20 - 30 understory host trees with or near gypsy moth egg masses were inspected for gypsy moth larvae once a week from egg hatch through early June. We recorded the larval stage of up to 100 larvae found.

We evaluated the potential overlap of Karner blue larval stages with gypsy moth suppression activities in two ways. We used the information gathered on gypsy moth larval development to predict the timing of a hypothetical *Btk* application in each of the two Karner blue areas. We also compared Karner blue phenology with dates of actual *Btk* application in spray areas near the Karner blue study sites in Allegan and the Huron-Manistee (Ottawa County, and Muskegon, Newaygo and Oceana Counties, respectively) (Figure 1).

Btk susceptibility bioassays

Bioassay treatments: We measured the susceptibility of Karner blue larvae fed wild lupine leaves treated with Foray 48B (Abbott Laboratories, North Chicago, IL), a commercial *Btk* formulation commonly used in Michigan for gypsy moth suppression (USDA 1994a, 1995). A concurrent bioassay with second instar gypsy moth larvae on *Btk*-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 *Btk* dose equivalent to 30 - 37 Billion International Units (BIU)/hectare (12 - 15 BIU/acre) field rate, and a high *Btk* dose equivalent to 90 BIU/hectare (36 BIU/acre) field rate. Typical rates of *Btk* application for gypsy moth range from 40 - 90 BIU/hectare (16 - 36 BIU/acre) (Dubois et al. 1993; Reardon et al.

1994). Application rates used in the 1994 Michigan Voluntary Cooperative Gypsy Moth Suppression Project ranged from 40 - 60 BIU/hectare (16 - 24 BIU/acre) (USDA 1994a, 1995).

Experimental insects and foliage: Karner blue larvae were reared in the laboratory from eggs of spring generation female butterflies as described in Chapter 1. Twenty female butterflies were collected from two areas in Michigan during the first 2 weeks of June 1994, and housed in the laboratory for five days to obtain eggs. Collection sites of the butterflies were located in Allegan State Game Area (Allegan Co.) and Huron-Manistee National Forest (Montcalm and Newaygo Counties) (Chapter 1). Overall, 59 larvae were 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, and were checked daily for second instars. All second instars used for the bioassay were no more than 24 hours old.

Wild lupine foliage, obtained from an isolated lupine population in a small field in Ingham County, Michigan (Chapter 1), was used for general rearing and for the *Btk* bioassay of the Karner blue larvae. White oak leaves used for the gypsy moth bioassay were obtained from a semi-residential site located in Ingham County, Michigan. Lupine and oak foliage used in the bioassay were harvested 1 day prior to application of *Btk* treatments.

Btk Application: Low and high *Btk* doses were applied to lupine and oak foliage using a cylindrical spray tower, 2.5 m in diameter and ca. 4 m high (Hubbard and Lewis

1973), located at the USDA Northeastern Forest Experiment Station in Hamden, Connecticut. The spray tower was designed to simulate aerial *Btk* application, and was equipped with a Mini-Beecomist nozzle calibrated to generate *Btk* drops between 75 - 125 *u*m volume median diameter (VMD) (Hubbard and Lewis 1973), the drop size range generally used in gypsy moth suppression spray programs (Reardon et al. 1994).

One day before foliage treatment, freshly harvested wild lupine and white oak leaves were placed as bouquets of five leaves in water picks. Excess lupine and oak foliage was harvested for the control treatments and kept at 5°C in water-filled containers. The bouquets of foliage were secured in a chilled cooler and flown that evening to Hamden, Connecticut. The following morning, the oak and lupine bouquets were brought to room temperature and sprayed at the doses described above. Kromekote spray cards (Mead Corporation, Dayton, OH) were also placed next to the leaves and later analyzed to confirm actual spray deposition rates. *Btk* treated foliage was returned to Michigan by 6 pm the same day.

Initiation and monitoring of bioassays: The bioassays were set up ca. 7 - 8 hours after foliar application of *Btk*. Treatment leaves were labeled without reference to the dosage to maintain a "blind" experiment. Due to differences in collection dates of female butterflies, 22 of the 59 Karner blue larvae were early instars (all from Huron-Manistee National Forest females), and 37 were late instars (36 from Allegan State Game Area females, and 1 from a Huron-Manistee female). Fifteen late instar Karner blue larvae were randomly chosen for controls. Twenty-two larvae (11 early and 11 late instars) were randomly assigned to each *Btk* treatment. We felt it was necessary to use only late instars as controls because of the limited number of larvae available for the test. Prior to

the bioassay, only two of 61 larvae died (one was deformed upon hatch, one died of unknown causes). All available early instars were used in the bioassay to evaluate possible stage-specific differences in *Btk* susceptibility. Each larva was placed in a clean petri dish (100 x 15 mm) with one lupine leaf (untreated, or low or high *Btk* treatments), which had been transferred to a water-filled 2-ml vial plugged with cotton.

For the gypsy moth bioassay, 40 1-day old second instars were randomly assigned to each of the three treatments and placed in large, lidded plastic boxes (19 x 9 x 8 cm) (Tri-State Plastics, Dixon, KY), 10 larvae per box, for a total of four replicates per treatment. Each box contained a bouquet of five white oak leaves (untreated, or low or high *Btk* treatments) in a water pick. Paper towels were used to line the bottom of the box.

Karner blue and gypsy moth larvae were maintained on the same treated or untreated leaves for up to 7 days, and were checked daily for molting and mortality. All larvae were kept in a growth chamber at 24°C. Larvae were considered dead if they did not respond to physical stimulus. Petri dishes and plastic boxes were kept free of frass to avoid buildup of secondary bacteria. Sanitation practices included daily removal of frass from the leaves, replacing the paper towel lining in the gypsy moth boxes every 2 days, and replacing petri dishes for Karner blue every 1 - 2 days.

At the end of 7 days, surviving Karner blue and gypsy moth larvae were placed in clean containers (petri dishes and plastic boxes, respectively) with fresh, untreated foliage. Karner blue pupae were weighed several times prior to adult emergence to assess potential sublethal effects of *Btk* on pupal weight. The gypsy moth bioassay was terminated after 13 days (Figure 2). Surviving Karner blue were reared to adulthood

following protocol described in Chapter 1 and subsequently released into the parental collection sites.

Statistical analysis: Percent survival of Karner blue and gypsy moth larvae on control and *Btk* 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 with this procedure, the first to test for effects of *Btk*, species and *Btk* x species, and the second to test for linear effects of the incremental doses of *Btk* (no, low and high *Btk*). 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, at selected times throughout the bioassay. Differences in survival between early and late instar Karner blue were evaluated for each *Btk* dose as a nonparametric 2 x 2 contingency table using the chi-square test of independence (Conover 1980). To assess sublethal effects, mean pupal weights (measured 2 days after pupation) of female and male control Karner blue were compared with those of female and male survivors, respectively, of the *Btk* treatments by ANOVA using SYSTAT (Wilkinson 1990). All statistical analyses were conducted at $p < 0.05$ level of significance.

Results

Phenology of Karner blue with respect to gypsy moth suppression

Predicted *Btk* application: Based upon the phenology of gypsy moth larvae, i.e. when the majority of larvae were late first instars and early seconds, we predicted that hypothetical *Btk* applications for gypsy moth management near Allegan State Game Area

would have occurred during the week of 18 May in 1993, 24 May in 1994 and 22 May in 1995 (Table 1). Spring generation Karner blue larvae were found during the predicted *Btk* application only in 1993, when late instars were observed. In 1994 and 1995, spring generation Karner blue adults were observed during the predicted spray times, and in 1994, adults had already been flying for approximately 5 days (Table 1).

For the Huron-Manistee National Forest, we predicted that hypothetical *Btk* applications for gypsy moth management would have occurred during the week of 25 May in 1993, 30 May in 1994 and 29 May in 1995 (Table 1). During all of these periods, we observed only spring generation Karner blue adults, and in 1994, the first adults were seen 6 days prior to the predicted spray date (Table 1).

Actual *Btk* application: Areas in Ottawa County, north of Allegan State Game Area, were sprayed with *Btk* from 1993 to 1995 for gypsy moth suppression (Table 2; Figure 1). In 1993, we observed late instar spring generation Karner blue in Allegan State Game Area during the Ottawa County spray period (Table 1). In 1994 and 1995, no larvae were found during the spray periods; however, we first observed spring generation Karner blue adults 4 days prior to the 1994 spray period, and 3 - 11 days prior to 1995 spray applications (Table 1). In 1995, Karner blue eggs were first seen 4 days into the 8-day spray period, 1 week after adults were observed, and the first observation of a summer generation early instar Karner blue larva was made 3 days after the end of the Ottawa County spray period, 2 weeks after the first adults were seen (Table 1).

Areas in Muskegon, Newaygo and Oceana Counties, near our Karner blue site in the Huron-Manistee National Forest, were also treated with *Btk* for gypsy moth suppression. *Btk* applications occurred in Oceana and Newaygo Counties 1993 - 1995,

and in Muskegon County 1994 and 1995 (Table 2; Figure 1). For the years considered, no spring generation larvae were observed during the spray periods in those counties. In 1993, the first spring generation Karner blue adults were observed 1 - 3 days prior to *Btk* application in Oceana and Newaygo Counties (Table 1). In 1994, adults began flying in the Huron-Manistee site 7 - 10 days before *Btk* treatments were completed in Newaygo and Oceana Counties, and close to 3 weeks before the second *Btk* application in Newaygo County (Table 1). In 1995, we first observed spring generation Karner blue adults 1 - 4 days prior to *Btk* application in Muskegon and Oceana Counties, and 7 days prior to treatment in Newaygo County (Table 1). Karner blue eggs from spring generation adults were first seen on 5 June, the date of *Btk* application in Newaygo County, and 3 and 5 days after the Muskegon and Oceana County spray periods, respectively (Table 1).

Btk bioassays

Overall survival: Categorical analysis indicated that overall survival of larvae on leaves sprayed with *Btk* was significantly reduced (chi-square = 259.1, $p < 0.001$), but there were no significant effects of insect species or *Btk* x species interactions (chi-square = 2.2 and 3.9, respectively), suggesting that Karner blue and gypsy moth did not differ in their overall response to *Btk*. Linear analysis showed a significant tendency for increased mortality of each species with increased *Btk* 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 (n=15) on untreated leaves survived to adulthood (Figure 2A). With both *Btk* treatments, Karner blue larval mortality began

on Day 3, with a subsequent steep drop in survival (Figure 2A). By Day 7, 32 percent of larvae on the low *Btk*, and 14 percent of larvae on the high *Btk* larvae had survived (Figure 2A). After removing larvae from the treatments to clean foliage, one additional low *Btk* larva died (larva was unable to complete pupation), decreasing larval survival on the low *Btk* dose to 27 percent (Figure 2A). The remaining six larvae exposed to low *Btk* and three exposed to high *Btk* survived to adulthood. In total, 24 out of 59 Karner blue larvae used in this study were released as adults (13 females, 11 males).

The Smirnov test indicated significant differences in larval survival between the control and each of the two *Btk* doses ($p < 0.001$) as suggested by categorical analysis. However, mortality did not differ significantly between the low and high doses at any time during the bioassay ($p > 0.05$).

On the low *Btk* dose, survival of early instar Karner blue was significantly higher than survival of late instars on Day 3 (chi-square = 4.70; $p < 0.05$) and Days 7 - 12 (chi-square = 5.24; $p < 0.025$) of the bioassay (Figure 3); however, differences in overall survival were not significant (chi-square = 3.67; $p < 0.1$). On the high *Btk* dose, survival of early instar Karner blue was not significantly lower than survival of late instars at Day 13 (chi-square = 3.47; $p < 0.1$) or at any time during the bioassay ($p > 0.05$) (Figure 3). Overall survival of early instars was significantly higher on the low versus high *Btk* treatment (chi-square = 6.47; $p < 0.025$), but survival of late instars on the two treatments did not differ significantly (chi-square = 1.22; $p < 0.5$).

Gypsy moth survival: All gypsy moth larvae on untreated control foliage survived to Day 8. Some mortality occurred after Day 8, and 80 percent of the larvae survived to Day 13 (Figure 2B). For the two *Btk* treatments, some mortality occurred on

Day 3, but we did not observe a steep drop in survival until Day 6 (Figure 2B). By Day 13, 33 percent of low *Btk* and 5 percent of high *Btk* larvae had survived (Figure 2B).

As with the Karner blue, results from the Smirnov analysis indicated that survival of gypsy moth larvae on each *Btk* treatment differed significantly from the control ($p < 0.001$), but differences between the low and high *Btk* dose were not significant ($p > 0.05$).

Karner blue vs. gypsy moth survival: Although overall survival of Karner blue did not differ significantly from survival of gypsy moth on any of the *Btk* treatments, the steep decrease in survival observed for Karner blue on Day 3 suggests that Karner blue larvae were affected more quickly by *Btk* than gypsy moth (Figure 2). Smirnov analysis indicated that gypsy moth larvae had significantly higher survival than Karner blue on Days 4 - 6 ($p < 0.01$) for the low *Btk* treatments, and on Days 3 - 5 ($p < 0.05$) for the high *Btk* treatments (Figure 2).

Sublethal effects on Karner blue: There appeared to be a *Btk* concentration-dependent decrease in mean pupal weight of female and male Karner blue on control and *Btk* treatments (Figure 4). However, the only statistically significant difference occurred between male pupal weights for the control versus high *Btk* treatment ($F = 6.84$; $df = 1$, $p < 0.05$); all other within-gender comparisons of mean pupal weight were not significant ($p > 0.05$), possibly due to the small sample sizes. Female pupal weight for the high *Btk* treatment could not be included in an ANOVA because there was only a single sample (Figure 4).

Discussion

Conflicts between management of forest pests such as gypsy moth, that involve *Btk* and nontarget endangered Lepidoptera are likely to increase. Management problems regarding the use of *Btk* similar to those in Michigan exist in Wisconsin, where Karner blue have been found in jack pine (*Pinus banksiana* Lambert) stands infested with jack pine budworm (*Choristoneura pinus* Freeman; Lepidoptera: Tortricidae) (Baker 1994). In general, susceptibility of nontarget Lepidoptera to *Btk* will depend on three conditions, the presence of vulnerable larval stages around the time of *Btk* application, larval consumption of foliage treated with *Btk*, and toxicity and/or viability of *Btk* to larvae when ingested (Dubois and Lewis 1981; Venables 1990), and will be greatly influenced by the length of time that toxic effects of *Btk* persist post-spray (Johnson et al. 1995).

Btk application for gypsy moth suppression is timed to occur when most gypsy moth larvae have hatched, and are predominantly highly susceptible first and second instars, and when 50 percent canopy development has occurred (Dubois 1991). Typically, there is a 2 week "window" for effective *Btk* application (Smitley and Davis 1993). 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).

Our phenological data over a three-year period indicated that *Btk* application for gypsy moth suppression in Michigan could impact Karner blue. For example, in 1993, late instar Karner blue of the spring generation were actively feeding during both the predicted and actual *Btk* spray periods in southwestern Michigan, and would likely have been at risk. In 1994 and 1995, we observed spring generation Karner blue adults, rather than larvae, during *Btk* application in nearby counties that had gypsy moth suppression

programs. However, early-instar larvae of the summer generation would likely have been at risk.

In 1994 and 1995, Karner blue adults of the spring generation were present in Allegan State Game Area 3 - 11 days prior to nearby *Btk* applications, and were present in the Huron-Manistee National Forest as much as 7 - 10 days prior to nearby *Btk* applications (ca. 3 weeks prior to a second *Btk* application in one county in 1994). Spring generation Karner blue can begin laying eggs within one week of the first emerged adults, as confirmed by our 1995 observations. Egg hatch is estimated to occur within 1 week in the field (Schweitzer 1989; Dirig 1994), and in Chapter 1, I found that Karner blue eggs laid in the laboratory took between 2 - 6 days to hatch at 24°C (average of 4 days). Based on this information, we predict that summer generation larvae could begin hatching approximately 9 - 10 days after the first spring adults emerge. Thus, assuming *Btk* persistence of 4 - 6 days, Karner blue first instars could have begun to hatch during the time of or a few days after *Btk* application in 1994 and 1995, and would have been at risk. In 1995, we conducted searches for early summer generation Karner blue larvae in Allegan State Game Area; first-instar Karner blue are small (ca. 1.5 mm), well-camouflaged and difficult to locate when newly hatched (Chapter 3). We found the first early instar 14 days after spring generation adults were first observed, which was only 3 days after the end of the *Btk* spray period in a nearby area.

Persistence of *Btk* crystals and spores in the field is a necessary consideration for evaluating the phenological susceptibility of Karner blue. *Btk* is generally thought to breakdown within 4 - 6 days of field application due to environmental factors such as sunlight, temperature, vapor pressure deficit, and rain (Ignoffo et al. 1974; Pinnock et al.

1974; Leong et al. 1980; Beegle et al. 1981; Reardon et al. 1994), and spore viability is impacted much more than crystal activity by UV light (Lüthy et al. 1982). However, recent studies have found *Btk* to remain toxic for longer periods of time in the field. Beckwith and Stelzer (1987) reported significant *Btk* mortality for western spruce budworm 10 days after application. Johnson et al. (1995) found that *Btk* was toxic to first instars of *P. glaucus* for at least 30 days in the field after application, potentially due to low levels of viable spores remaining of the leaf surface for long periods of time (Leong et al. 1980). Further research has revealed that increased sensitivity, several hundred- to several thousand-fold, to *Btk* doses occurs in four *Papilio* spp. as compared to gypsy moth sensitivity (Johnson et al. 1995). Thus, persistence may be determined, in part, by a particular species' sensitivity to *Btk*. In considering our Karner blue phenology data from 1994 and 1995, the longer the toxic persistence of *Btk*, the greater the number of early instars possibly impacted. Field bioassays would be the most conclusive way of determining persistence of *Btk* toxicity for Karner blue (Leong et al. 1980).

Toxicity of *Btk* to Lepidoptera depends upon the physiological makeup of each species. After ingestion by lepidopteran larvae, *Btk* crystals become toxic if conditions within the larval gut solubilize crystals into specific δ-endotoxins, which then bind to receptors on the gut wall (Reardon et al. 1994). The binding of δ-endotoxins causes gut wall cells to swell and lyse, creating perforations in the gut lining, leading to mortality by bacterial septicemia (Gill et al. 1992; Bauer 1995). Factors in the gut that determine *Btk*'s insecticidal activity include the presence of *Btk* spores, appropriate gut pH, digestive enzymes, receptors on the gut wall, and other factors that facilitate active pore

formation (Cherwonogrodzky 1980; van Frankenhuyzen et al. 1991; Bauer 1995). Though the exact role of spores in the synergism of crystal toxicity is not known, their presence in *Btk* formulations can have a significant influence on toxicity for some lepidopteran species (Moar et al. 1990; Johnson et al. 1995). Other bacteria present as opportunists could also significantly affect the observed mortality (Dubois and Dean 1995).

We found that *Btk* was toxic to Karner blue when larvae were fed treated lupine foliage. Karner blue larvae did not differ from gypsy moth larvae in their overall percent survival. However, Karner blue mortality was significantly higher than gypsy moth mortality in the first 3 - 6 days of the bioassay, suggesting that Karner blue may be more sensitive to *Btk* than gypsy moth.

Early (first and second) and late (third and fourth) instar Karner blue were equally susceptible. Generally for Lepidoptera, including gypsy moth, early instars are much more susceptible than later instars to *Btk* (Peacock and Schweitzer 1992; Reardon et al. 1994; Wagner and Miller 1995). However, many exceptions have been reported (Wagner and Miller 1995). *Btk* caused high mortality for late (fourth and fifth) instars of the cinnabar moth while early instars appeared to be impervious (James et al. 1993). Peacock and Schweitzer (1992) and Peacock et al. (1993) found substantial variation in early- versus late-instar susceptibility to *Btk* for related species within the families Geometridae and Noctuidae. As with Karner blue, early and late (fourth) instars of two species of swallowtails and the promethea moth were reported to be susceptible to *Btk* (Johnson et al. 1995). Since all instars of Karner blue were negatively affected by the *Btk* treatments, the late instar larvae observed in the field during the 1993 gypsy moth suppression

activities would have been at risk, along with the earlier instars which were most likely present during or soon after *Btk* application in 1994 and 1995.

Although there was a trend for reduced pupal weight, and possibly lower fecundity (Honek 1993), when Karner blue were reared on *Btk*-treated foliage, mean pupal weights differed significantly only between control and high *Btk* treatments for male Karner blue. Since very few females and males survived the *Btk* treatments to provide comparison, these data should be interpreted cautiously. However, potentially sublethal effects of *Btk* have only been previously considered for beneficial insect predators and parasitoids (Croft 1990). Possible sublethal or multi-generational impacts of *Btk* on nontarget Lepidoptera need further investigation.

Data on the individual roles of each *Btk* δ-endotoxin and *Btk* spores in Karner blue mortality could be useful in the future production of a *Btk* formulation which would impact gypsy moth, but have no effect on Karner blue. Van Frankenhuyzen et al. (1991) found that, of the three CryIA toxins in *Btk*, CryIA(c) toxin caused little gypsy moth mortality compared to CryIA(a) and CryIA(b). Dubois and Dean (1995) also showed that CryIA(a) was more toxic to gypsy moth than CryIA(c).

We conclude that Karner blue is highly physiologically susceptible to *Btk*, and is phenologically susceptible to the timing of *Btk* application for gypsy moth suppression, although the extent of phenological overlap and the larval generation (spring vs. summer) at risk may vary from year to year. The actual amount of risk posed by gypsy moth suppression to the survival of a particular Karner blue population must take into consideration the length of time that *Btk* remains toxic and/or viable to Karner blue larvae

after field application, as well as the size and level of isolation of each population. Small or isolated Karner blue populations would face more of a risk than populations which have large numbers of individuals or are in close proximity to other Karner blue areas to allow for recolonization (Schweitzer 1994).

Information regarding the susceptibility of nontarget Lepidoptera to *Btk*, including physiological susceptibility and the temporal overlap of larval stages with the application of *Btk* or the period of its toxic persistence (which appears to be species-specific; Johnson et al. 1995), must be considered in management plans for gypsy moth. However, nontarget impacts of gypsy moth defoliation, in the absence of suppression, such as a potential increase in parasitoids and predators, altered microclimate or a decrease in the availability or quality of host plants (Liebhold and Elkinton 1989; Sample et al. 1993; Johnson et al. 1995; Wagner and Miller 1995) must also be considered. The potential for development of modified *Btk* products that have higher specificity for gypsy moth, so as to reduce the physiological impact on select nontarget lepidopteran species, should be explored.

Karner blue and gypsy moth in Allegan State Game Area (Allegan Co) and Huron-Manistee

Table 2.1. Phenological development of first and second generation Karner blue and gypsy moth in Allegan State Game Area (Allegan Co) and Huron-Manistee National Forest (Oceana Co) in Michigan, 1993 - 1995. Life stages of Karner blue that were observed at the time of hypothetical *Btk* application, predicted from gypsy moth development, are in bold. Surveys for second generation eggs and larvae were conducted only in 1995.

		Allegan State Game Area			Huron-Manistee National Forest		
Year	Sampling date	Karner blue	Gypsy moth	Degree days (base 50° F)[1]	Karner blue	Gypsy moth	Degree days (base 50° F)[1]
1993	April 30	Early instars	Eggs	65	Early instars	Eggs	57
	May 6	Early instars	1st instar	120	Early instars	Not recorded	115
	May 12	Early/late instars	1st/2nd instars	226	Early/late instars	1st instar	224
	May 18	**Late instars**	**1st/2nd instars**	245	Late instars	1st instar	238
	May 25	Adults	1st/2nd instars	290	**Adults**	**1st/2nd instars**	275
1994	April 28	Early instars	Eggs	114	Early instars	Eggs	92
	May 8	Early/late instars	1st instar	130	Early instars	Not recorded	115
	May 14	Late instars	1st instar	159	Early/late instars	1st instar	152
	May 19	Late instars Adults	1st/2nd instars	182	Late instars	1st instar	182
	May 24	**Adults**	**1st/2nd instars**	238	Adults	1st instar	250
	May 30	Adults	2nd/3rd instars	312	**Adults**	**1st/2nd instars**	312

Table 2.1 (cont'd)

1995	May 3	Early instars	Eggs	91	Early instars	Eggs	72
	May 10	Early/late instars	Eggs	125	Early instars	Eggs	98
	May 15	Late instars	1st instar	168	Early/late instars	1st instar	145
	May 22	**Adults**	**1st/2nd instars**	198	Late instars	1st instar	175
	May 29	Adults Eggs	1st/2nd instars	270	**Adults**	**1st/2nd instars**	270
	June 5	Adults Eggs Early instars	1st/2nd/3rd/4th instars	370	Adults Eggs	2nd/3rd instars	390

[1] Degree days based upon degree day accumulation since March 1st, published in the Michigan State University Landscape Crop Advisory Team Alert Newsletter. Degree days calculated using the Baskerville-Emin method (Baskerville & Emin 1969).

Table 2.2. Actual timing of *Btk* applications for gypsy moth suppression in Michigan counties near Karner blue study sites, 1993 - 1995.

		Btk Application[1]	
County	Year	Date	Degree days (base 50° F)[3]
Muskegon	1994	May 27	250
	1995	May 30 - June 2	280 - 312
Newaygo	1993	May 28	300
	1994	June 2 - 3	340 - 360
		June 15[2]	525
	1995	June 5	358
Oceana	1993	May 26	284
	1994	May 31 - June 2	320 - 340
	1995	May 30 - 31	275 - 282
Ottawa	1993	May 17	280
	1994	May 23	320
	1995	May 25 - June 2	272 - 370

[1] Aerial application of *Btk* as conducted in the Michigan Voluntary Cooperative Gypsy Moth Suppression Program which is administered by the Michigan Department of Agriculture.

[2] Date of second *Btk* application.

[3] Degree days (base 50° F) based upon degree day accumulation since March 1st, published in the Michigan State University Landscape Crop Advisory Team Alert Newsletter. Degree days calculated using the Baskerville-Emin method (Baskerville and Emin 1969).

Figure 2.1. Michigan counties where Karner blue butterfly study sites were located (Allegan, Oceana), where *Btk* was applied at least once in 1993 - 1995 for gypsy moth suppression (Muskegon, Newaygo, Oceana, Ottawa) and where the *Btk* laboratory bioassay was conducted (Ingham).

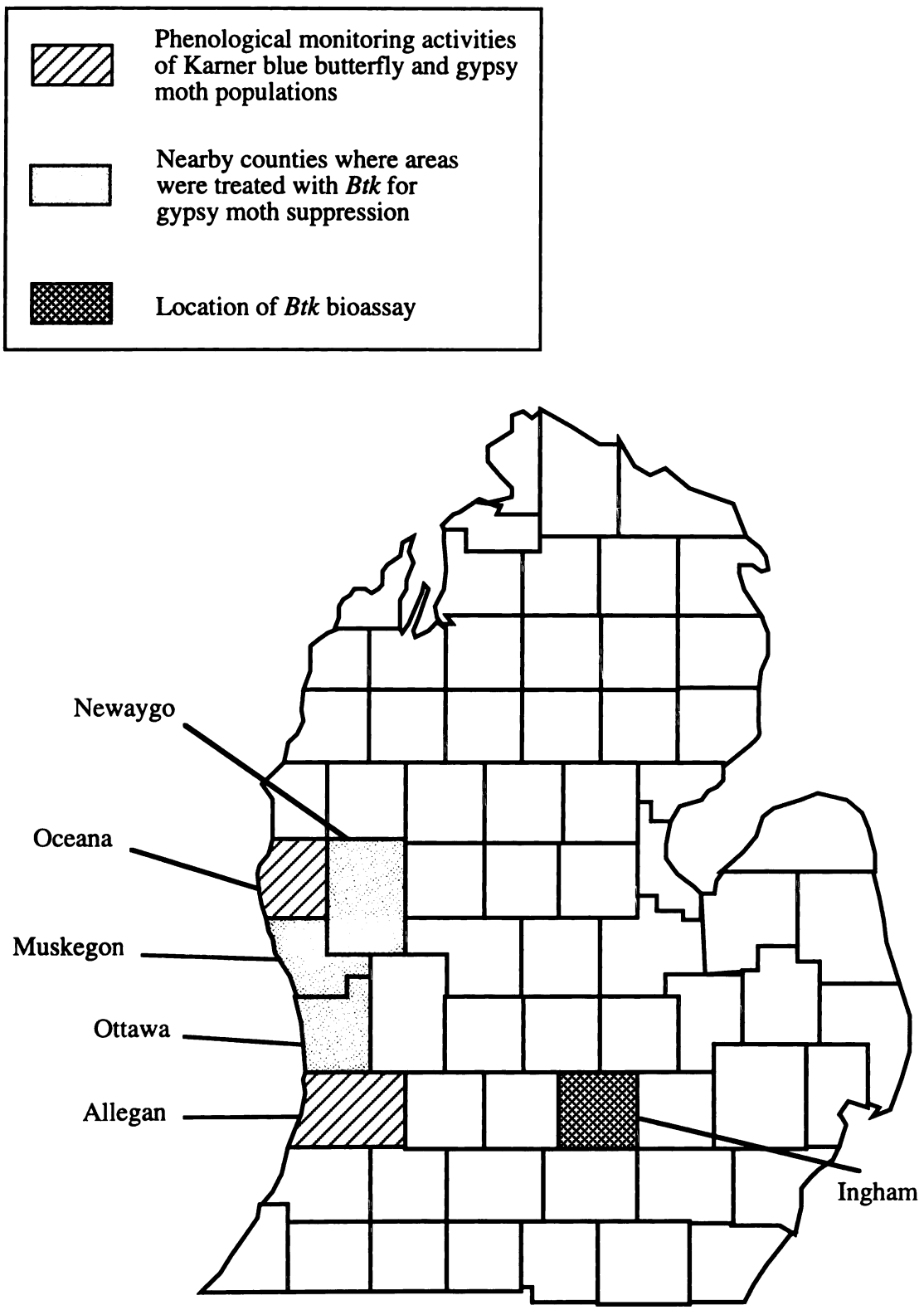
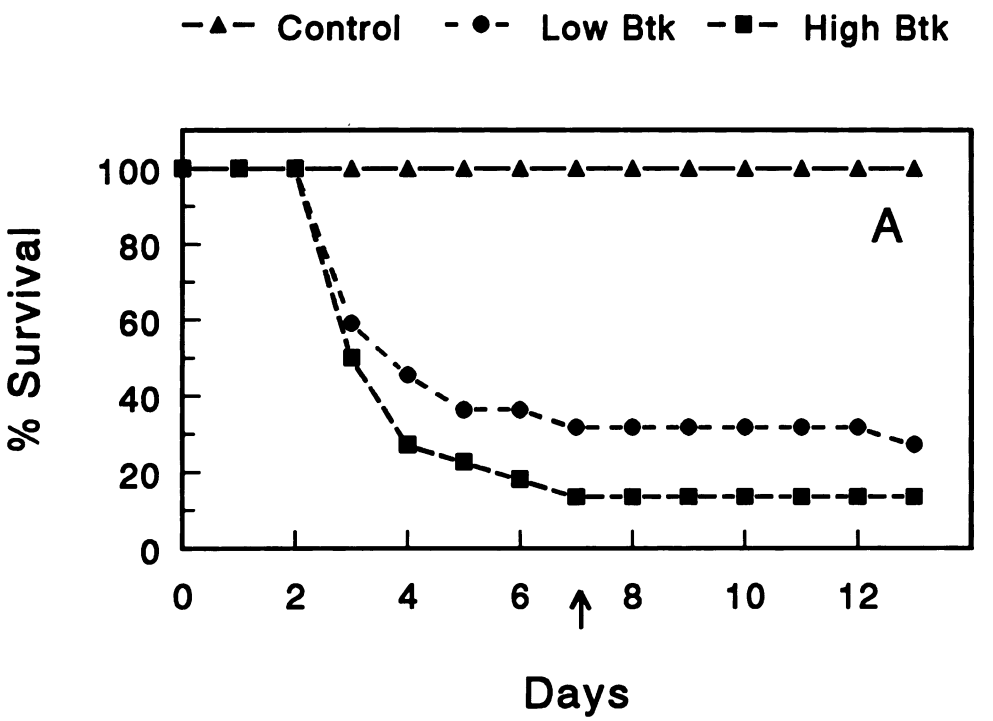
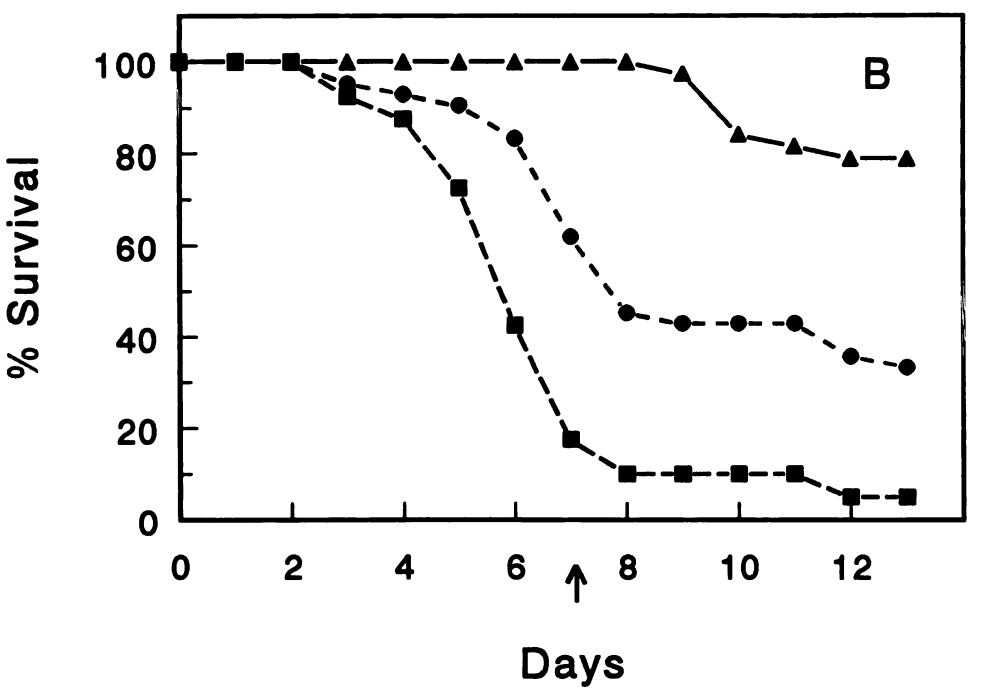

Phenological monitoring activities of Karner blue butterfly and gypsy moth populations
Nearby counties where areas were treated with *Btk* for gypsy moth suppression
Location of *Btk* bioassay
Newaygo
Oceana
Muskegon
Ottawa
Allegan
Ingham

Figure 2.2. Larval survival of (A) Karner blue butterfly and (B) gypsy moth over 13 days on control (untreated) foliage, on foliage treated with *Btk* (*Bacillus thuringiensis* var. *kurstaki*) at a low dosage (30 - 37 BIU/ha), or on foliage treated at a high dosage (90 BIU/ha). On Day 7 (indicated by arrow), all surviving larvae were placed on untreated foliage.


Control
Low Btk
High Btk
% Survival
100
80
60
40
20
0
A
0
2
4
6
8
10
12
Days


% Survival
100
80
60
40
20
0
B
0
2
4
6
8
10
12
Days

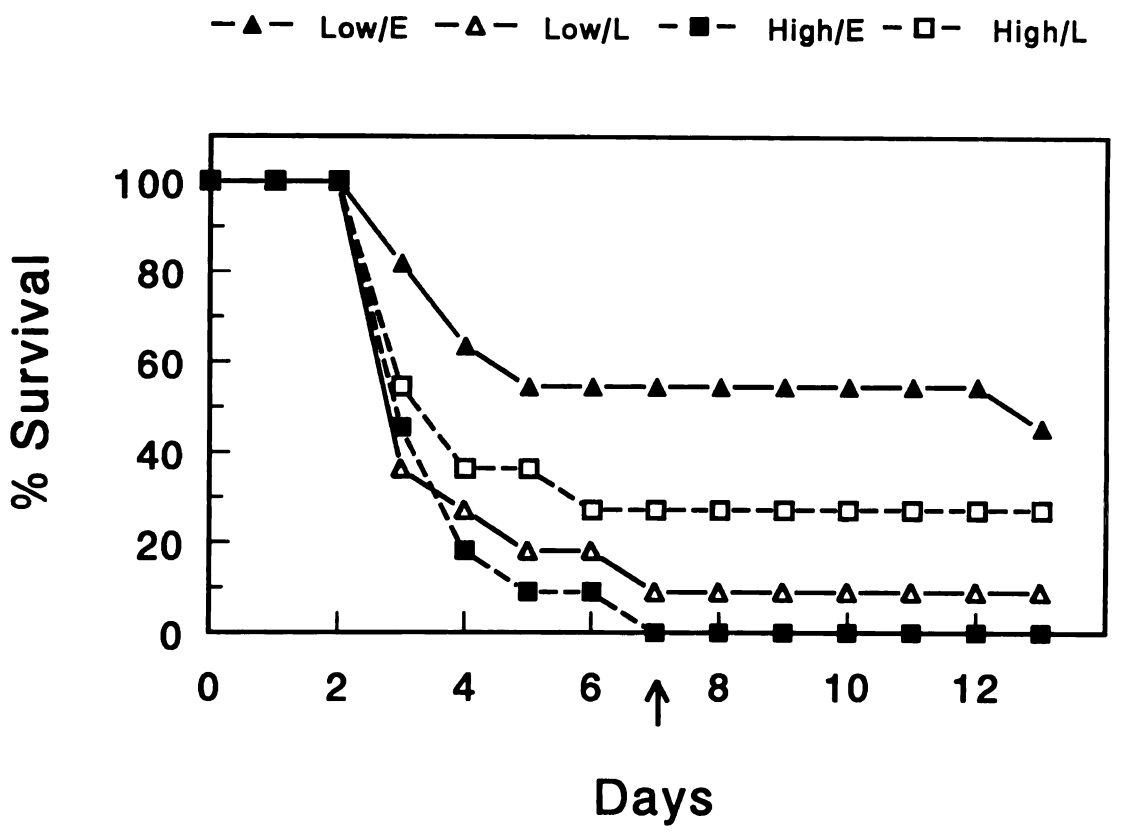


Figure 2.3. Survival over 13 days of early (1st, 2nd; E) and late (3rd, 4th; L) instar Karner blue reared on lupine foliage treated with low (30 - 37 BIU/acre) or high (90 BIU/acre) dosages of *Btk*. On Day 7 (indicated by arrow), all surviving larvae were placed on untreated lupine foliage. No further mortality occurred after day 13.

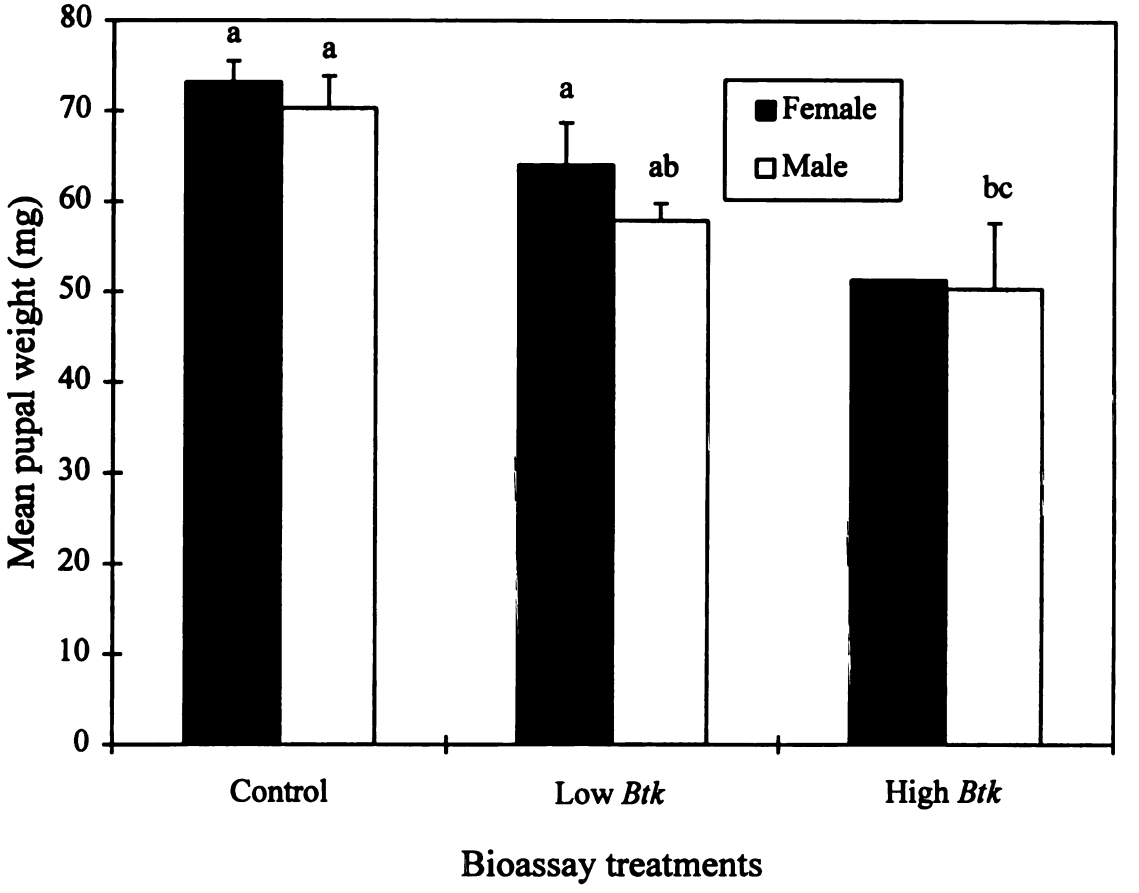


Figure 2.4. Mean pupal weight (mg) (+ 1 SE) 2 days after pupation of surviving female and male Karner blue larvae used in the *Btk* bioassay. There were 8, 4, and 1 female survivors, and 7, 2, and 2 male survivors on control, low *Btk* (30 - 37 BIU/ha) and high *Btk* (90 BIU/ha) treatments, respectively. For within-gender comparisons, bars with the same letters were not significantly different by ANOVA at $p < 0.05$ (female pupal weight for the high *Btk* treatment was not included in ANOVA).

CHAPTER 3

The Endangered Karner Blue Butterfly (Lepidoptera: Lycaenidae) in Michigan Oak Savanna: Associations among Butterfly Abundance and Habitat Variables

Abstract

The Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov) is an endangered species occupying oak savanna and pine barren habitats. Local habitat requirements of the butterfly appear to be adequate seasonal supply of wild lupine (*Lupinus perennis* L.), the sole larval foodplant, and adult nectar sources, microclimatic variation provided by shading of woody plants, and ant-tending of Karner blue larvae. An integrated study was conducted in oak savanna sites in southern Michigan to investigate habitat suitability for the butterfly with respect to those habitat requirements. In 1993 and 1994, six and seven Karner blue-occupied sites in Allegan State Game Area (Allegan Co), respectively, were surveyed during the spring and summer Karner blue flight periods to assess relative population sizes. Nectaring was also recorded. Indirect estimates of summer larval abundance were made through feeding damage surveys. Select habitat variables, e.g., wild lupine density and frequency, density and frequency of flowers during spring and summer Karner blue flight periods, and percentage canopy cover and frequency, were quantified for each site. Larval surveys were conducted to assess the quality of lupine used by larvae for feeding, to observe ant-tending, and to indirectly estimate female oviposition on lupine in different shade conditions. The relationships among Karner blue

abundance and several of the habitat variables were analyzed. There were significant positive associations between butterfly abundance and lupine density ($r > 0.8$) and frequency ($r > 0.7$) in both years, suggesting that lupine plays a significant role in Karner blue population dynamics. Karner blue abundance was not significantly correlated with flower density and diversity measures, or percentage canopy cover and frequency. Summer Karner blue abundance was highly correlated with percentages and frequencies of larval feeding damage ($r > 0.9$), suggesting that feeding damage may be used to estimate adult population size. Some summer flower species that were favored one year for nectaring were not available the other year, while some flower species that were used less for nectaring were available consistently in both years. It may be important to have a diversity of nectar sources in the Karner blue landscape due to these random phenological differences. Summer Karner blue larvae fed on lupine leaves that appeared to be less senesced than the overall clump. Karner blue larvae were found in both partial shade and in open areas, which suggests that females use both shade conditions equally for oviposition. Ant-tending was observed for almost 100 percent of the larvae found in 1993, and for 82 - 89 percent of the larvae in 1994. Thirteen species of tending ants from three subfamilies were identified. The dominant tending species was *Formica obscuripes*.

Introduction

The endangered Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov; Lepidoptera: Lycaenidae) is restricted to early successional, xeric oak savanna and pine barren habitats of the central and northeastern United States (Ewert and Ballard 1990).

Addition of the Karner blue to the federal endangered species list in December 1992 was the result of rangewide population declines (USFWS 1992). Like other invertebrate species in the United States and elsewhere, loss of habitat associated with human settlement has been the major cause of the butterfly's decline (New 1993). The primary means of preserving this species is habitat conservation (Pyle 1976; Pyle et al. 1981), to maintain remaining savannas and barrens as well as restore degraded areas. The Karner blue has become a symbol for conservation of the threatened savanna and barren landscapes and the other unique species they support (Ewert and Ballard 1990), as well as for invertebrate conservation. Like all endangered species, conservation of the Karner blue is mandated by the Endangered Species Act of 1973, which requires designation and conservation of critical habitat (Pyle et al. 1981; USFWS 1992). Understanding of Karner blue ecology and the critical habitat factors required by the butterfly is needed to form sound management plans for effective species and habitat conservation.

The Karner blue has declined an estimated 99 percent over its historical range from eastern Minnesota to New Hampshire in the past 100 years, with most of the decline occurring in the last 10 to 20 years (Schweitzer 1989; USFWS 1992). The species is extirpated in Massachusetts, Ohio, Ontario, and Pennsylvania (and most likely Illinois), and occurs as a few small localized populations in Indiana, Minnesota and New Hampshire (USFWS 1992; Haack 1993; Baker 1994). Michigan, Wisconsin and New York have the largest populations, and the best opportunities for species conservation (Baker 1994).

Karner blue populations occur on sandy post-glacial lake and outwash plains which support wild lupine (*Lupinus perennis* L.), the sole foodplant of larvae, along with other xerophytic, fire-successional savanna or barren vegetation (Bleser 1992). The butterfly's range approximates the northern limits of its larval foodplant (USFWS 1992). The savanna and barren landscapes are characterized by open canopy with an understory of grasses and other herbaceous plant species, historically maintained by fire (Nuzzo 1985; Givnish et al. 1988). In eastern states, the Karner blue is closely associated with grassy openings of fire-climax pine/oak barrens (Dirig 1994). In the Midwest, the butterfly's habitat represents the transition between native western prairies and eastern deciduous forests, taking the form of oak savanna and oak/pine barren communities (Shuey 1994).

The vast, historic savanna and barren landscapes have been drastically reduced and fragmented since European settlement, from activities such as agriculture, commercial and residential development, off-road vehicle use, timber production, and fire suppression (USFWS 1992; Haack 1993; Shuey 1994). Of the 11 - 13 million hectares of oak savanna that once covered the Midwest, only two percent remains (Nuzzo 1985). The Albany Pine Bush in New York, at one time famous for its Karner blue population numbering 100,000, was reduced from 25,000 acres to 2,500 acres by the mid-1980's (Givnish et al. 1988). Currently, most populations of Karner blue in New York number fewer than one hundred butterflies (Sommers and Nye 1994). Disturbances, such as fire, historically perpetuated lupine by preventing encroachment of the overstory and woody vegetation (Givnish et al. 1988; Shuey 1994). Current fire-suppression practices in remnant savanna and barren habitats often result in the exclusion of lupine and other

herbaceous plants necessary to the Karner blue, by allowing fire-intolerant species to shade in the openings (Lane 1994a; Wilsmann 1994).

Destruction, modification and fragmentation of Karner blue habitat as a result of development and fire suppression has impacted butterfly populations at both the landscape and local, or patch, level. At the broader scale, the Karner blue was thought to exist as metapopulations, or dynamic clusters of populations (Givnish et al. 1988). Individuals of these populations could disperse and shift among a patchy landscape, to colonize new areas created by fire, recolonize areas where populations had gone extinct, and thus maintain gene flow (Givnish et al. 1988). Currently, the majority of extant Karner blue populations are small and separated by unsuitable intervening habitat or by distances which prevent successful dispersal, disrupting the metapopulation regime (Shuey 1994). On a local scale, extreme disturbance and fire suppression have reduced the suitability of habitat patches for survival and reproduction of butterfly populations (Givnish et al. 1988; Lane 1994a; Shuey 1994).

The Karner blue overwinters in the egg stage and has two generations per year (Schweitzer 1989). Larvae of both spring and summer generations feed solely on wild lupine, and are tended by various species of ants, which feed on the sugary, protein-rich fluid emitted by specialized larval glands, and provide protection for the larvae in return (Dirig 1994; Schweitzer 1989; Savignano 1990b). Spring generation larvae hatch in late April and feed for approximately three weeks (Bleser 1992; Lane 1992). The spring adult flight period is from late May to early June, and adults live for 5 to 7 days (Schweitzer 1989). Eggs are laid on or near lupine plants (Dirig 1994; Schweitzer 1989). Summer generation adult flight occurs mid-July to mid-August, and butterfly numbers are usually

higher than in spring flight (Bleser 1992; Lane 1992). Eggs that will overwinter are laid on vegetation near senescing or senesced lupine. Adults of both generations require nectar, and utilize a variety of native and exotic flowering plants (Packer 1987; Lawrence and Cook 1989; Schweitzer 1989; Haack 1993). Moderate levels of interspersed canopy cover in the habitat appear to provide butterfly adults and larvae with shelter from daytime temperatures, as well as providing microclimate heterogeneity (Leach 1992; Lane 1994). Like other Lycaenidae, the Karner blue has low vagility, and butterflies rarely disperse more than 1 km (Fried 1987; Lawrence and Cook 1989; Cushman and Murphy 1993; Bidwell 1994).

Karner blue management has concentrated on improving habitat quality to stabilize local populations, with the eventual goal of restoring metapopulation dynamics in the landscape (Lane 1994b; Shuey 1994). Successful conservation of individual Karner blue populations requires that key, local habitat needs are met (Packer 1987; Bleser 1992). Past studies have suggested that the availability of lupine, nectar sources, microclimate heterogeneity provided by minimal shading and tending ants are critical components in the Karner blue habitat (Packer 1987; Savignano 1987, 1990a,b; Lawrence and Cook 1989; Bleser 1992; Lane 1992, 1994a; Leach 1992). However, the associations, relative importance and interactions of Karner blue with these aspects of its habitat are not fully understood, and require further examination in an integrated autecological study. Our primary objective was to investigate associations among Karner blue abundance and several components of the butterfly's habitat, primarily lupine density and frequency, flowering plant density, and percentage canopy cover and frequency, in an integrated study. We also investigated the extent of ant-tending of Karner blue larvae, the influence

of shading on female oviposition and use of lupine foliage by summer larvae. Our goal was to further elucidate aspects of the butterfly's ecology and habitat suitability to guide future research and management.

Methods & Materials

Study sites: This study was conducted during the spring and summer of 1993 and 1994 in Allegan State Game Area (Allegan County) in southwest Michigan (Figure 3.1). The Game Area is located on sandy deposits, from the Pleistocene glaciers, comprising outwash plains, lake plains and moraines (USDA 1987). Presettlement vegetation consisted of eastern white pine (*Pinus strobus* L.) forests, oak savannas and prairies which were maintained by fire (Wilsmann 1994). With pioneer settlement, the Game Area was altered by logging, fire suppression, and a brief period of cultivation practices (Lawerence and Cook 1989). Currently, ca. 7 percent of Allegan State Game Area is oak savanna and interspersed oak openings (Wilsmann 1994).

Six Karner blue-occupied sites were studied in 1993, and seven sites were studied in 1994 (the six sites from 1993 plus one additional site, the 'Park'). Four sites represented remnant oak savanna habitat (Table 3.1), and were most likely farmed for a brief period in the early 1900's (John Lerg, Allegan State Game Area, pers. comm.). These sites were located within a 2.6-km^2 area in a northern region of the Game Area. Sites were separated from one another by ca. 0.6 - 1.8 km of interspersed woodland and dirt roads. The other three sites were narrow openings created within the last 20 t0 25 years for game management (Table 3.1), and were located within a 1.3-km^2 area, ca. 3.6 km south of the remnant oak savanna sites. The 'Jay' and 'Pipe' sites were separated by

less than 0.2 km, and were both ca. 0.6 km from the 'Horseshoe'. The three created sites and the 'Park' site were surrounded completely by forested habitat, while the '48N89', 'Marsh', and 'Square' sites were bordered by forest on three sides and a road on one side. All Karner blue study sites were located on well-drained, fine sand soils of the Oakville association, with 0 - 6 % slope (USDA 1987).

We selected sites with a range of butterfly population sizes, based upon preliminary surveys by the Michigan Natural Features Inventory. We intended to include unoccupied sites in the study; however, all sites selected to represent unoccupied habitat were later found to be occupied by Karner blue.

Two Karner blue sites in the Huron-Manistee National Forest in southcentral Oceana County (Figure 3.1) were used for collection of tending ant species of Karner blue larvae (described below) in addition to the Game Area sites.

Karner blue adult abundance estimates: Karner blue adult abundance in each study site was estimated from timed-area transect counts of adults that were conducted weekly during the 1993 summer flight period, and the 1994 spring and summer flight periods. Methods used to estimate population sizes were analogous to the those developed by Pollard (1977) and Thomas (1983). However, sampling effort (e.g. the amount of time spent per survey per site) was standardized based on the area of each site.

In each site, we established a transect route which traversed the entire site. The three created openings were narrow, no more than ca. 30 meters wide in any one spot; therefore, the transect route for each created site followed a direct line from one end of the site to the other. In each of the four remnant oak savanna sites, we partitioned the entire site into ca. 30-meter wide strips, and then established a transect route which went

through the strips, alternating direction from one strip to the next. From preliminary surveys, we determined that it took ca. 20 minutes to walk, at a moderate pace, a transect route which traversed a 1 hectare opening. Based upon this and the size of the study sites, the amount of time spent for each survey was 60 minutes in the 'Marsh', 50 minutes in '48N89' and 'Park', 30 minutes in the 'Square', and 20 minutes in the 'Horseshoe', 'Jay' and 'Pipe'.

Each transect survey was conducted by two people, walking at the same pace within adjacent halves of the 30 m wide strips. Ten-meter buffers were maintained between surveyors. Karner blue adults seen within 3 to 4 meters on either side of the transect were recorded. Data on male/female, nectaring and wing wear were also recorded. Surveys were conducted between 10 am to 1 pm and 2 to 6 pm, and were not conducted if the temperature was below 20°C or if it was raining.

Numbers of adults counted during each survey were standardized across sites by converting to numbers of adults counted per person hour. The highest standardized count obtained in each site was used as the estimate of Karner blue abundance for that site during that specific flight period.

During the 1993 summer flight period, sites were surveyed twice each week when weather permitted, with surveys 2 days apart. During the 1994 spring flight period, sites were surveyed once every 4 to 7 days. For both flight periods, the order in which sites were surveyed was selected randomly each survey date. During the 1994 summer flight period, sites were surveyed twice every 6 to 7 days, with both surveys in each site occurring on the same day, once in the morning and once in the afternoon. For each 1994 summer survey date, the order in which sites were surveyed in the morning was selected

randomly, and then reversed for the afternoon surveys. The highest of the two daily counts was used to determine the adult abundance estimate for each survey date in each site.

In 1994, we documented the beginning and end of the spring and summer flight periods in each site by initiating butterfly surveys 1 to 2 weeks prior to estimated adult emergence to get zero counts, and continuing surveys through the flight period until zero counts were again obtained.

Indirect estimates of Karner blue larval abundance: From 28 to 30 June 1994, abundances of summer generation Karner blue larvae were indirectly estimated through quadrat (1-m^2) surveys for feeding damage on lupine. In each study site, 20 lupine clumps were chosen by randomly selecting points, and walking a randomly chosen direction until the first lupine plants were encountered. A 1-m^2 quadrat was then placed over the lupine clumps, and the numbers of lupine stems in the quadrat and the numbers of stems with window feeding damage, made by summer generation Karner blue larvae, were counted. The average percentage of lupine stems (per m^2) with feeding damage and feeding damage frequency (proportion of quadrats with damage) were calculated for each site.

Lupine density, frequency, flowering and quality: From 2 to 4 June 1993 and 3 to 8 June 1994, density of lupine stems was estimated in each study site using a transect - quadrat method (Bonham 1989) (surveys done in conjunction with spring flowering plant and percentage canopy cover surveys, below). The number of transects per site was based upon site area. We randomly located 25-m transects throughout each site, at a density of one transect per 1000 m^2 in 1993 and one transect per 850 m^2 in 1994. Lupine

stems were counted in six 1-m^2 quadrats placed at regular intervals along each transect (Bonham 1989). For each site, the lupine density estimate was calculated as the average number of lupine stems per m^2 per transect, and lupine frequency was calculated as the proportion of transects with lupine stems.

From 13 May to 10 June 1994, flowering phenology of lupine was monitored on 6 different days through quadrat (1-m^2) surveys. In each study site, six lupine clumps were randomly chosen using the same method as for larval feeding damage surveys (above). Only lupine clumps that occupied 1/4 or more of the quadrat were sampled. The numbers of lupine stems and flower spikes in each quadrat were counted. The stage of flowering was recorded for each flower spike using the following scale:

0	=	no flowers on spike open
< 1/4	=	flowers beginning to expand and show color
1/4	=	1/4 of flowers on spike open
1/2	=	1/2 of flowers on spike open
3/4	=	3/4 of flowers on spike open
1	=	all flowers on spike open
Seed	=	all flowers done, seed pods present
Bare	=	bare flower spike, no flowers or seed pods present

Average percentages of lupine stems (per m^2) with flower spikes and flower spikes at each stage of bloom were calculated.

To examine the quality of lupine used by summer generation larvae, we surveyed lupine clumps in late June 1994, 1 week after the first senescent lupine stems were observed. Twenty lupine clumps were chosen in each site using the same quadrat method as for larval feeding damage surveys (above). The 1-m^2 quadrat was then placed over the lupine plants, and an overall estimate of senescence for all lupine foliage in the quadrat was made. A visual senescence scale of 1 to 5 was used to rate foliage, with 1

representing foliage with no apparent signs of senescence, 2 to 4 representing foliage with increasing amounts of discolored and necrotic areas, and 5 representing complete senescence. All larvae observed in the quadrat were measured and a senescence rating was made for the leaves occupied by larvae.

Spring and summer flower density, frequency, nectaring and diversity: In 1993 and 1994, flowering plant density was surveyed during peak spring and summer Karner blue flight periods (Table 3.2), using the same transect - quadrat design used for lupine surveys (Bonham 1989). Since butterfly surveys were not conducted during the 1993 spring flight period, the peak flight period was estimated from casual observations of butterfly numbers.

In each quadrat, we counted the numbers of stems of different plant species in flower at the time of the survey. Stems that were done flowering or had only unopened buds were not counted. As with lupine density, the overall mean number of flowering stems per m^2 per transect was calculated for each site, along with overall flower frequency (proportion of transects with flowers; all species combined). In addition, averages of each flower species were calculated and used to calculate diversity and dominance indices (below) for each site. Nectaring by Karner blue adults was recorded during the butterfly surveys.

Shannon's diversity index (H'), and Simpson's dominance index (expressed as the reciprocal, 1/D) (Margurran 1988) were calculated for spring and summer flowering plants in each site in 1993 and 1994. To calculate Simpson's index, flower density estimates were converted to number of stems per 100 m^2 to avoid negative values. One of the assumptions of the Shannon diversity index, that all species from a community

were included in the sample (Margurran 1988), was not met; some flower species were not encountered in the transect surveys.

Canopy cover and shade: Average percentage of canopy cover in each site was estimated in late June 1994 after leaves were fully expanded using a transect-intercept method (Bonham 1989). Transects were located randomly, at a density of one 25-m transect per 850 m^2. The number of meters along each transect with direct canopy cover was recorded, along with the species of each tree intersecting the transect. Only trees 1.5 m in height or taller were included. The amount of cover along each transect was expressed as a percentage and the overall mean percentage of canopy cover per site was determined. Overall canopy cover frequency (proportion of transects with canopy cover; all species combined) was calculated.

To indirectly investigate oviposition by Karner blue females on lupine plants growing in different shade conditions, surveys for Karner blue larvae were conducted weekly in each of the sites prior to the summer adult flight period in 1993, and the spring and summer flight periods in 1994. Larval searches were done from 10 am to 6 pm, and varied in duration from 1 to 3 hours per site, based upon lupine density. For each survey, randomly chosen lupine clumps were examined for evidence of larval feeding. When feeding damage was found, the lupine foliage was searched thoroughly for larvae. Growing conditions of lupine plants occupied by larvae were estimated as either open (i.e., never shaded) or partially shaded (i.e., shaded for some part of the day by tree trunks, foliage, etc.), and plants were flagged for relocation. During subsequent larval searches, new plants were searched for additional larvae.

Ant-tending of larvae: In summer 1993 and spring and summer 1994, data on ant-tending of Karner blue larvae were collected while conducting larval surveys (described above) in Allegan State Game Area. For each larva found, presence or absence of tending ants and larval length were recorded, and ant specimens were collected for identification. Some ants were also collected during preliminary surveys in spring 1993 in Allegan State Game Area. In addition, tending ant specimens were collected in two Karner blue sites in the Huron-Manistee National Forest in spring 1993 and spring and summer 1994.

Statistical analysis: All analyses were conducted with SYSTAT, Version 5.0 (Wilkinson 1990), at the $p < 0.05$ level of significance. In each study year, differences among sites in lupine density, spring and summer flower densities, percentage larval feeding damage, percentage flower spikes, percentage of spikes at each stage of bloom, and percentage canopy cover were evaluated usng one-way analysis of variance (ANOVA) and Tukey's HSD multiple comparison. Weekly estimates of the percentage lupine stems with flower spikes were also compared among sites by repeated measures.

Estimates of lupine density, flowering plant density and percentage canopy cover were log-transformed, and percentage feeding damage estimates were arcsine-transformed, before analysis (Little and Hills 1978). After transformation, the normality assumption of homogeneous variances (Bartlett test of homogeneity of variances) was not met for lupine density estimates (both years) and marginally for percentage canopy cover. These data were analyzed with the nonparametric Kruskal-Wallis test in addition to ANOVA.

Associations among Karner blue abundance, lupine density and frequency, spring and summer flowering plant densities, percentage canopy cover, and diversity indices (H', 1/D) were evaluated using Pearson's correlation analysis. Only summer Karner blue abundance estimates were available for 1993 correlations. Separate 1994 correlation analyses were conducted using spring and summer Karner blue abundance estimates. Also, associations between 1994 summer adult abundance and percentage feeding damage and feeding damage frequency were analyzed. The critical value of significance (for a one-tailed test) of correlation coefficients (r) was 0.729 ($v = 4$, $p < 0.05$) for 1993 comparisons among sites, and was 0.669 ($v = 5$, $p < 0.05$) for 1994 site comparisons (Zar 1974).

For each site in 1994, analyses were conducted to investigate the associations between transect estimates of percentage canopy cover and corresponding transect estimates of lupine density and spring flower density.

Results

Karner blue adult abundance: In 1993, the Karner blue spring flight period occurred from 25 May to 27 June, and the summer flight occurred from 8 July to 10 August, based upon first and last observations of adult butterflies in study sites and other Karner blue-occupied areas in the Allegan State Game Area. In 1994, the spring flight period occurred from 19 May to 18 June, and the summer flight period occurred from 27 June to 12 August.

Butterfly surveys were conducted in the study sites from 7 July to 3 August (Julian Date (JD) 188 - 215) in summer 1993 (Figure 3.2), from 16 May to 22 June (JD 136 - 173) in spring 1994 (Figure 3.3), and from 24 June to 17 August (JD 175 - 229) in summer 1994 (Figure 3.3). For all flight periods, the first butterfly counts were low and dominated initially by male butterflies. After counts peaked (Figure 3.2, 3.3), late counts were dominated by female butterflies.

During the 1993 summer flight, Karner blue numbers on most sites peaked at approximately the same time, except for the 'Square', which peaked ca. 5 days earlier (Figure 3.2). In spring 1994, Karner blue abundance peaked at the same time in late May for the '48N89', 'Marsh', 'Park', and 'Square' sites, and 1 week later for the 'Horseshoe', 'Jay', and 'Pipe' sites (Figure 3.2). In summer 1994, the 'Horseshoe', 'Jay', 'Marsh',and 'Square' sites peaked at the same time mid-July, and the '48N89', 'Park', 'Pipe' sites peaked 6 days later (Figure 3.2). Overall peak summer counts were obtained within a similar range of calendar dates and degree days in 1993 as in 1994 (Table 3.2); however, calendar dates of peak counts in individual sites varied from one year to the next (Figure 3.4).

The 'Jay' site consistently had the greatest adult Karner blue abundance (adults per hour), followed by the 'Pipe', 'Square' and 'Horseshoe' sites (Table 3.3). The 'Marsh' site had the lowest abundance in 1993, and the 'Park' site (only used in 1994) had the lowest abundance in 1994 (Table 3.3). Summer abundance estimates in each site were higher in 1994 than in 1993. Counts for summer flight were consistently higher

than counts for spring flight in all sites in 1994 (Figure 3.3). Peak summer abundance was approximately two to three times greater than peak spring abundance (Table 3.3).

Indirect estimates of summer Karner blue larval abundance: Percentages of lupine stems with summer larval feeding damage differed significantly among sites ($F = 6.487$; $df = 6$, $p < 0.001$) (Table 3.4). The 'Pipe' and 'Jay' sites had the highest percentages of feeding damage, as well as the highest feeding damage frequencies (Table 3.4). The 'Horseshoe' site had the third highest percentage and frequency of feeding damage, followed by the 'Square' (Table 3.4).

Lupine density, frequency, flowering and quality: The 'Jay', 'Pipe' and 'Square' sites consistently had the highest lupine densities, and the 'Horseshoe' had the lowest densities in both years (Table 3.3). Lupine density estimates in the '48N89', 'Horseshoe', 'Marsh' and 'Pipe' sites were similar from year to year, but varied somewhat in the 'Jay' and 'Square' (Table 3.3). Lupine density differed significantly among sites in 1993 ($F = 17.698$; $df = 5$, $p < 0.001$) and 1994 ($F = 18.606$; $df = 6$, $p < 0.001$). Based upon multiple comparison tests, sites could be grouped into one of two statistically differing lupine density levels, high lupine density ('Jay', 'Pipe' and 'Square') or low lupine density ('48N89', 'Horseshoe', 'Marsh', and 'Park') (Table 3.3). Results of the non-parametric Kruskal-Wallis test were consistent with ANOVA results. Lupine density estimates differed significantly in 1993 (test statistic = 46.4; $df = 5$, $p < 0.001$) and 1994 (test statistic = 60.2; $df = 6$, $p < 0.001$).

As with lupine density, lupine frequencies were highest in the 'Jay', 'Pipe' and 'Square' sites, and lowest in the 'Horseshoe' in both years (Table 3.5). For each site, frequency estimates were similar in both years (Table 3.5).

In general, sites did not differ widely in lupine flowering phenology (Table 3.6). On 13 May 1994, the majority of lupine flower spikes in study sites had not begun to open; 4 days later, all sites had a small percentage of flower spikes that were showing some color (Table 3.6). On 20 May, sites did not differ significantly in percentage bloom for any stage (Table 3.6). By 27 May, all sites had a percentage of flower spikes at each stage of flowering from 0 to 1, full bloom (Table 3.6). Peak lupine bloom (the greatest percentage of spikes with all flowers open) occurred on 1 June; however, several sites had high percentages of spikes that had not begun to bloom (Table 3.6). The 'Horseshoe' site had consistently high percentages of unopened flower spikes from 27 May to 1 June, while percentages of unopened flower spikes rose during that period for the 'Square' and 'Marsh' sites (Table 3.6). By 10 June, the majority of lupine spikes were done flowering and had seed pods or were bare (Table 3.6). Repeated measures analysis of weekly percentages of lupine stems with flower spikes revealed that the '48N89' and 'Park' sites had significantly greater percentages of flowering lupine stems per area than all other sites ($F > 15.67$; $df = 1$, $p < 0.003$), but the other sites did not differ significantly from each other ($F < 3.10$; $df = 1$, $p < 0.5$).

On 28 June 1994, 46 summer generation Karner blue larvae were found in study sites during quadrat surveys of lupine senescence. Seven larvae were 0.5 cm or smaller, 20 larvae were 0.6 to 1 cm long, and 19 larvae were 1 to 1.6 cm long. Of the 46 quadrats with larvae, the numbers of quadrats with each overall-senescence rating were: *rating 1* =

3 quadrats; *rating 2* = 24 quadrats; *rating 3* = 15 quadrats; and *rating 4* = 4 quadrats, with 7 quadrats also containing some completely senesced lupine stems. Of the 46 leaves occupied by larvae, *rating 1* = 27 larvae; *rating 2* = 16 larvae; and *rating 3* = 3 larvae. Larvae tended to occupy leaves appearing less senesced than overall lupine in the clumps.

Flowering plants: In spring and summer 1993 and 1994, some plant species were observed flowering in the sites but were not represented in the transect surveys due to extremely low densities (Table A6.1, A6.2). Transect surveys to determine spring and summer flowering plant densities were conducted within similar ranges of degree days from 1993 to 1994 (Table 3.2).

Overall densities of spring flowers ranged more widely among sites in 1994 than in 1993 (Table 3.3). Spring flower densities differed significantly among sites in 1993 ($F = 3.819$; $df = 5$, $p < 0.004$) and 1994 ($F = 14.846$; $df = 6$, $p < 0.001$). The dominant spring flower species in 1993 and 1994 surveys in all sites were wild lupine (*Lupinus perennis*), mouse-ear hawkweed (*Hieracium pillosella*), and sheep sorrel (*Rumex acetosella*), in addition to dewberry (*Rubus* sp.) in 1994 (Table 3.7, 3.8). These flower species also had consistently high frequencies among sites in the above years, especially for mouse-ear hawkweed (Table 3.9). In both years, '48N89' and 'Square' sites had the highest overall spring flower densities (Table 3.3), mostly because of high densities of this hawkweed species (Table 3.7, 3.8). In addition, increased flower density from 1993 to 1994 in the '48N89', 'Marsh' and 'Square' sites, and decreased density in the 'Jay' site were largely the result of changes in the abundance of mouse-ear hawkweed (Table 3.7,

3.8). Overall frequencies of spring flowers were high in both years, but frequencies were generally lower in 1994 (Table 3.5).

The ranges of summer flower densities were similar in both years (Table 3.3); densities differed significantly among sites in 1993 ($F = 3.980$; $df = 5$, $p < 0.003$) and 1994 ($F = 6.3$; $df = 6$, $p < 0.001$). The dominant summer flowers encountered in the 1993 and 1994 surveys across sites were flowering spurge (*Euphorbia corollata*) and St. Johnswort (*Hypericum perforatum*); horsemint (*Monarda punctata*) in 1993, and mouse-ear hawkweed in 1994 (Table 3.10, 3.11). Of these, only flowering spurge and mouse-ear hawkweed had consistently high frequencies among sites for the years considered (Table 3.12). The 'Horseshoe' site had the highest overall densities in both years, primarily as a result of large abundances of hoary alyssum (*Berteroa incana*) and spotted knapweed (*Centaurea maculosa*), which were rare or nonexistent in other sites (Table 3.10, 3.11, 3.12). As with spring flower densities, changes in mouse-ear hawkweed abundance (Table 3.10, 3.11) explained the increase in summer flower densities from 1993 to 1994 for '48N89' and 'Square' (Table 3.3). Overall frequencies of summer flowers were generally higher in 1994 than 1993 (Table 3.5), and differences between 1993 overall spring and summer flower frequencies for some sites were most likely explained by a change in mouse-ear hawkweed frequency, as above (Table 3.9, 3.12).

Changes in numbers of flower species encountered in transect surveys per site were not consistent from 1993 to 1994; in some sites, the number of species increased, while in others, the number decreased (Table 3.15). When survey results from all sites were combined, numbers of spring and summer flower species were higher in 1994 than in 1993 (Table 3.16).

In 1993, the 'Horseshoe' site had the highest spring and summer Shannon diversity (H') and spring Simpson's dominance (1/D) values, as well as the highest number of flowering plant species (Table 3.15). The 'Marsh' had the next highest number of summer flower species and the highest summer 1/D (Table 3.15). The 'Square' site had the fewest species and values of H' and 1/D in spring, and the 'Jay' had the lowest values for those categories in the summer (Table 3.15).

In 1994, the 'Pipe' site had the highest H' and 1/D values in both seasons (Table 3.15). The '48N89' site had the lowest H' in the spring and summer, and the lowest 1/D value in the spring (Table 3.15). The 'Park' site, with the fewest summer flower species, also had the lowest 1/D value in the summer (Table 3.15). In contrast to 1993, the lowest 1994 values of diversity and dominance were not consistently associated with the lowest numbers of species (Table 3.15). The highest diversity and dominance values were associated with the highest number of species in spring 1993 and summer 1994 (Table 3.15).

In spring 1994, Karner blue adults were observed nectaring on eight flower species. Nectaring was observed most frequently on cinquefoil (*Potentilla* spp.), dewberry, mouse-ear hawkweed and wild lupine (Table 3.16). The latter three were also dominant species in spring transect surveys (Table 3.7, 3.8, 3.9).

In the summers of 1993 and 1994, Karner blue adults were observed nectaring on 19 and 21 flower species, respectively, with nectaring most frequently observed on butterfly weed (*Asclepias tuberosa*), flowering spurge, horsemint and spotted knapweed (Table 3.16, A7.1, A7.2). Nectaring was observed ca. 80 times on goat's rue (*Tephrosia virginiana*) and lance-leaved coreopsis (*Coreopsis lanceolata*) in 1994, but almost no

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nectaring was observed on these species in 1993 (Table 3.16). It appeared that these two species were past peak bloom in 1993 when summer Karner blue began flying, so no flowers of goat's rue and few coreopsis blooms were available. In support of this observation, combined density estimates (estimates from all sites added together) for each of these species were slightly higher in 1994 than in 1993 (for coreopsis, 0.31 vs. 0.01 stems per m^2, respectively; for goat's rue, 0.21 vs. 0 stems per m^2, respectively). Frequencies of these species were also higher in 1994 than in 1993 (Table 3.12).

Woodland sunflower (*Helianthus divaricatus*) and yellow hawkweed species (*Hieracium* spp.) were used for summer nectaring to lesser extents in 1993 and 1994, respectively (Table 3.16). All of the summer nectar sources mentioned above, with the exception of goat's rue and woodland sunflower, were encountered in both 1993 and 1994 transect surveys (Table 3.16). However, only flowering spurge in both years, and horsemint in 1993 had consistently high density estimates among sites. Spotted knapweed had a high density and frequency estimate only in the 'Horseshoe' site (where most of nectaring observations were made) (Table 3.10, 3.11, 3.12). Butterfly weed was consistently rare among the sites.

Canopy cover: The dominant tree species in the sites were black oak (*Quercus velutina* Lamarck), white oak (*Quercus alba* L.), black cherry (*Prunus serotina* Ehrhart) and sassafras (*Sassafras albidum* (Nuttall) Nees) (Table A8). Overall percentage canopy cover and frequency estimates in the 'Horseshoe' site were extremely low (Table 3.1). All other sites had a percentage canopy cover estimate of at least 20 percent and canopy cover frequency of at least 0.70 (Table 3.1). The 'Jay' site had the greatest cover estimate, but the 'Marsh' had the greatest frequency estimate (Table 3.1). Percentage

canopy cover was significantly different among the sites ($F = 5.33$; $df = 6$, $p < 0.001$), primarily due to the low 'Horseshoe' cover estimate. The six other sites differed significantly from the 'Horseshoe', but were not significantly different from each other (Table 3.1). The among-site difference in percentage canopy cover was also significant when tested with the non-parametric Kruskal-Wallis test (test statistic = 23.01; $df = 6$, $p < 0.001$).

At least 30 percent of transects in each site (and all of the transects in 'Horseshoe' site) had a percentage canopy cover of less than 10 percent (Figure 3.5). Most of the remaining transects in each site had percentage canopy cover of 11 to 70 percent; however, a few transects had cover greater than 70 percent (Figure 3.5).

Karner blue larvae and shade: Of 46 larvae observed in summer 1993, 65 percent were found on lupine in the open, and the other 35 percent were found in partially shaded conditions. Of 69 larvae observed in spring 1994, 39 percent were found on lupine growing in open conditions, and 61 percent were on lupine in partial shade. Of 198 summer larvae found in 1994, 62 percent were in the open, and 38 percent were in partially shaded conditions.

Ant-tending of Karner blue larvae: In summer 1993, all but one Karner blue larva was ant-tended at the time of observation (Table 3.15). In spring 1994, 83 percent of larvae were tended, and 17 percent were untended (Table 3.15). In summer 1994, 89 percent of larvae were tended, and 11 percent were untended (Table 3.15). Presence or absence of ants was not related to larval length. Ant-tending was observed for larvae of all lengths, from 0.2 to 1.9 cm; untended larvae also ranged in length from 0.2 to 1.9 cm (Table 3.15). Thirteen species of tending ants from three subfamilies were identified

from the collected specimens (Table 3.16). One of the predominant tending ant species was *Formica obscuripes* Forel (Table 3.16).

Associations among Karner blue abundance and larval feeding damage estimates: In 1994, summer Karner blue adult abundance was highly correlated with the percentage of lupine stems with summer larval feeding damage ($r = 0.97$) (Figure 3.6). Adult abundance was also highly correlated with the frequency of larval feeding damage ($r = 0.96$) (Figure 3.7).

Associations among Karner blue abundance and habitat variables: In summer 1993 and spring and summer 1994, there was a significant positive correlation of $r > 0.8$ between Karner blue abundance and lupine density (Figure 3.8, 3.9 and 3.10). Lupine frequency was also significantly correlated with summer adult abundance in 1993 ($r = 0.78$) (Figure 3.11) and 1994 ($r = 0.75$) (Figure 3.12).

Summer Karner blue abundance was not significantly associated with summer flower densities in either year (1993, $r = - 0.14$; 1994, $r = - 0.06$), nor was 1994 summer abundance correlated with 1994 spring flower density ($r = - 0.30$). Spring butterfly abundance in 1994 was not significantly correlated with 1994 spring flower densities ($r = - 0.33$), or with 1993 summer flower densities ($r = - 0.16$).

Karner blue abundance was not significantly correlated with numbers of flower species, flower diversity (H'), or flower dominance (1/D) for spring and summer of either year. However, in 1994, correlations of spring and summer Karner blue abundance with spring H' were only marginally insignificant (Figure 3.13, 3.14, respectively).

There was no significant correlation between Karner blue abundance for summer 1993, spring 1994 and summer 1994 and percentage canopy cover ($r = 0.52, 0.20, 0.23,$

respectively), or canopy cover frequency (r = 0.10, - 0.01, 0.12, respectively). Percentage canopy cover was not significantly associated with lupine density in either 1993 (r = 0.64) or 1994 (r = 0.35). The decrease in 'r' from 1993 to 1994 was due to the addition of the 'Park' site.

In both years, there was a significant negative correlation between percentage canopy cover and summer flower densities (Figure 3.15, 3.16). However, when the 'Horseshoe' site was removed from comparison, the 1993 correlation became positive and not significant (r = 0.49), and the 1994 association remained negative but was also no longer significant (r = - 0.56). A similar association occurred between percentage canopy cover and 1993 numbers of summer flower species (Figure 3.17), which disappeared when the 'Horseshoe' was removed (r = - 0.02).

For all sites, there was no significant correlation between transect estimates of percentage canopy cover and lupine density. For comparisons between transect estimates of percentage canopy cover and spring flower density, there was a significant negative correlation for the 'Jay' site (df = 12, r = - 0.57; critical r = 0.53) (Figure 3.18), but associations for all other sites were not significant.

Discussion

Habitat destruction and alteration have been the overwhelming causes of invertebrate species declines (Hafernik 1992; New 1993; New et al. 1995). Like other Lycaenidae, the Karner blue may be particularly susceptible to environmental changes, and thus endangerment, because of its limited dispersal ability, dependence on one larval hostplant found only in early successional habitats, and association with ant species

which may have patchy distributions and be impacted, as well, by altered habitat (Cushman and Murphy 1993). Habitat conservation has emerged as the primary means of preserving the Karner blue (Givnish et al. 1988; New et al. 1995), and autecology studies are only just beginning to reveal aspects of the butterfly's habitat requirements. As with other Lepidoptera, larval and adult resources are presumed to be the basic prerequisites (Wiklund et al. 1977) of the Karner blue (Schweitzer 1989). However, overall habitat suitability is most likely determined by a complex suite of components, interacting in both time and space (Singer 1972). Microclimate heterogeneity provided by canopy cover and ant-tending appear to be two additional components determining habitat suitability for the Karner blue (Packer 1987; Leach 1992; Savignano 1994).

Karner blue larvae depend solely on wild lupine; therefore, it must be present in some amount to support butterfly populations. In both years of this study, we found a strong, positive correlation between abundance of Karner blue and lupine density, as has been found by other researchers (Givnish et al. 1988; Lawrence and Cook 1989; Grundel 1994; Savignano 1994), as well as a strong correlation with lupine frequency. These associations suggest that the amount and spatial distribution of lupine play a key role in Karner blue population dynamics. However, studies done by Bleser (1992) in Wisconsin and Lane (1992, 1994) in Minnesota did not show a consistently positive correlation between density of lupine and Karner blue, and those researchers concluded that some other variable was a limiting factor.

Savignano (1990a) suggested that Karner blue abundance and distribution may be impacted by asynchronous timing of egg hatch and lupine development in the spring, and early senescence of lupine in the summer. Swengel (1995) concluded that significant

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hatching of Karner blue eggs prior to emergence of adequate lupine was unlikely. However, larval starvation caused by early hostplant senescence has been documented for some Lepidoptera other than the Karner blue (Ehrlich et al. 1980; Weiss et al. 1988). Our observations of summer generation Karner blue larvae suggest that larvae may be able to select individual lupine leaves of higher quality than the average quality of the overall clump. Leaf quality may be affected by secondary plant compounds, nitrogen content, leaf toughness and age, and can impact larval performance (Feeny 1970; Rausher 1981).

Mechanisms governing the positive association between Karner blue abundance and lupine density are not known. The absolute amount of lupine does not appear to be limiting, since the majority of lupine plants are not occupied by larvae (Lawrence and Cook 1989), supporting the contention that herbivorous insects are rarely food limited (Dethier 1959b; Hairston et al. 1960). However, lupine density and distribution may function in the ability of larvae to find suitable food (Dethier 1959b). Hostplant location is especially critical for larvae emerging in the spring. Newly emerged spring larvae have only a short time after hatching to find lupine leaves (Lane and Welch 1994; Swengel 1995). Larvae are more likely to encounter lupine stems when the plants are more abundant and randomly distributed. The same would be true for spring or summer generation larvae, which often rest for part of the day in the litter and must relocate lupine stems (Grundel 1994). Denser patches of lupine may also diffuse density-dependent mortality of larvae, including parasitism and predation, and disease. Abundance of hostplants would help to counteract any mistakes made in hostplant choice by ovipositing females (Dethier 1959a). In addition, lupine density may play a role in Karner blue

female ovipositional behavior. Females may prefer to oviposit in areas with concentrated plant resources (Root 1973).

Availability of nectar sources is an important requirement for the survival of both spring and summer Karner blue adults. In some areas and in some years, nectar plants, rather than lupine, may be the limiting factor for butterfly populations (Clench 1967; Murphy 1983). Scarcity of nectar plants, especially during the summer flight period, have been attributed with lower Karner blue numbers than would have been expected for a particular site (Schweitzer 1989; Bleser 1992). Some qualitative studies reported that absence of suitable nectar sources as a result of drought prevented establishment of Karner blue populations in areas where adequate lupine was present (Packer 1987; Schweitzer 1989). A previous study in Allegan State Game Area found a positive correlation between abundance of nectar plants and Karner blue (Lawrence and Cook 1989). However, in our study, we did not observe an association between butterfly abundance and flower density, suggesting that the minimal requirement for nectar was met in all sites and nectar was not a limiting factor during the years of study.

Lepidoptera vary in their dependence on adult resources. Many moth species do not feed, while many female butterfly species require nectar sources for egg maturation and oviposition (Murphy et al. 1983). Female butterflies in the genus *Euphydryas* can produce many eggs without feeding (Murphy et al. 1983). However, Murphy et al. (1983) found that nectar consumption increased female lifespan and fecundity, allowing females to lay more eggs later into the season. Larvae hatching from these late eggs were unlikely to survive in most years due to hostplant senescence; however, Murphy et al.

(1983) proposed that survival of late larvae in rainy years increased butterfly numbers, providing a significant buffer against extinction in dry years. The dependence of Karner blue females on nectar sources for egg production has not been investigated, but would aid in further understanding Karner blue population dynamics.

The distributions of nectar sources in relation to lupine may impact the oviposition behavior of Karner blue females. Murphy et al. 1984 and Grossmueller and Lederhouse (1987) found that female butterflies preferred oviposition hostplants that were in areas with high densities of preferred nectar plants.

We observed Karner blue adults utilizing a variety of nectar sources. As others have reported (Packer 1987; Lawrence and Cook 1989; Bleser 1992; Haack 1993; Lane 1994), the two most widely used nectar sources were butterfly weed, which was consistently rare in all the sites, and horsemint. Two other flower species, goat's rue and coreopsis, were used heavily in one year, but were not phenologically available to butterflies in the other year. Flower species such as flowering spurge and mouse-ear hawkweed, while not used as extensively as butterfly weed for nectaring, were the most abundant flowers in the sites in both years. These less-preferred but abundant flower species may be especially important if they are predictably in flower during the Karner blue flight periods. The ability of Karner blue to utilize a variety of nectar sources would help to buffer the butterfly from temporal dissociations of flowering time of particular nectar sources with the adult flight period (Carey 1994).

Microclimatic conditions have been shown to be important in determining habitat suitability for butterflies (Ehrlich et al. 1980; Dobkin et al. 1987; Weiss et al. 1988). Shade in limited amounts could provide microclimatic variation important for Karner

blue adults and larvae, as well as lupine (Givnish et al. 1988; Bleser 1992; Leach 1992; Lane 1994). Tree-canopy shade reduces understory temperatures (Belsky et al. 1993). Karner blue adults and larvae, like other butterflies, may require shady microhabitats to escape hot mid-day temperatures (Lawrence and Cook 1989; Bleser 1992). Some studies found Karner blue to be more abundant in sites with interspersed sun and shade versus large xeric openings (Lawrence and Cook 1989; Leach 1992). We did not observe an association between Karner blue abundance and percentage canopy cover in our study. Study sites with low and high butterfly abundance had similar percentages of canopy cover. However, this suggests that canopy cover of 20 to 30 percent is not a limiting factor for Karner blue, and that in the sites with low butterfly abundance, some other factor was limiting.

Tree-canopy shade reduces soil- and foliage- moisture loss (Belsky et al. 1993). Thus, shading may increase the amount of time which lupine is available to summer generation larvae, providing a buffer to population losses in dry years (Carey 1994). Lawrence and Cook (1989) observed lupine to desiccate prematurely in dry, sunny openings. In certain years, significant mortality of summer generation Karner blue larvae could result if lupine senesces before larvae finish development, as reported by Ehrlich et al. (1980) for the checkerspot butterfly. Lupine has been found to persist longer under semi-closed canopies than in open areas (Hess 1983; Leach 1992), which would provide hostplants for larvae for a longer period of time into the summer. And higher lupine densities and frequencies may translate into a wider variety of microclimates occupied by lupine, and a greater likelihood of some plants being shaded.

In our study, we found Karner blue larvae on lupine in both partially shaded and open areas, as did Lawrence and Cook (1989), suggesting that female Karner blue adults unpreferentially use lupine plants in different shade conditions for oviposition. However, conflicting data from other studies suggest oviposition preference for lupine plants in partial shade (Packer 1987) as well as in open habitats (Savignano 1990a; Bleser 1992). These data say nothing of survival of larvae in the different shade conditions. Results from a laboratory study (Grundel 1994) suggest that summer generation Karner blue larvae develop more quickly on lupine leaves from plants growing in partial shade versus the open, perhaps due to decreased leaf quality of the sun-exposed lupine plants (Rausher 1981; Dudt and Shure 1994). However, some of this difference may be mediated by the fact that larvae in the sunnier microenvironments would develop faster than in shadier microclimates (Weiss et al. 1988).

Myrmecophilous associations of lycaenid larvae have been well documented (New 1993). Though associations can range from commensalism to larval predation on ant broods, the relationship is more often a facultative mutualism (Atsatt 1982; Pierce 1985), such as with the Karner blue (Savignano 1990b). Savignano (1987, 1990a,b) and Packer (1987) found that larval survival was greater for Karner blue larvae that were ant-tended than those that were not, suggesting that ants reduce the levels of larval mortality due to parasitism and predation (Savignano 1990b).

We identified thirteen species of tending ants, many of which have been reported tending Karner blue larvae in New York (Savignano 1994) and Ontario (Packer 1987). *Formica obscuripes* Forel was a common and aggressive tending species. In our study, ant-tending was observed for 82 percent or more of the Karner blue larvae in Michigan,

similar to ant-tending percentages reported by others (Packer 1987; Savignano 1989). More than 50 percent of larvae less than 0.5 cm were tended, which was surprising since Savignano (1990b) reported that first and second instar Karner blue lack fully developed ant-associated organs. Ant-tending appears to be a significant aspect of Karner blue ecology in Michigan. Although Karner blue larvae can develop successfully without tending ants (Savignano 1990b; Chapter 2), benefits of ant-tending may be important in years when parasitoid and predator populations are high, or when other factors make Karner blue populations more vulnerable to extinction. Past extirpations of other lycaenids have been correlated with the disappearance of protective ant species due to unfavorable habitat conditions or management practices (Packer 1987; New 1993). Many of the species of tending ants we identified build nests above ground in logs and stumps (Wheeler et al. 1994), and may be more prone to disturbance. Impacts of management on ant species should be considered.

In this study, we estimated Karner blue abundance from weekly surveys throughout the entire flight period. This methodology allowed us to identify peak flight. Some sites peaked at different calendar dates from one year to the next, emphasizing the necessity of conducting surveys throughout the entire flight period each year. We also indirectly estimated larval abundance through larval feeding damage surveys, and found that the results from these surveys were highly positively correlated with adult estimates of abundance. These results are consistent with Swengel's (1995) results of positive association between larval and adult abundance.

Our results suggest that lupine density is a significant factor in determining the population dynamics of the Karner blue. Nectar sources are also important; however, the

low densities of flowers present in our study sites over the two years appeared to meet some minimum requirement, and were not a limiting factor. Since some flower species differed in their availability to Karner blue from year to year, a diversity of nectar sources in the Karner blue habitat would help to buffer this phenomenon. The exact role of canopy cover could not be determined from our study; sites with low and high abundances of butterflies and lupine had similar canopy cover. However, this finding suggests that 20 to 30 percent canopy cover does not limit Karner blue populations or lupine, and may provide a benefit of microclimatic variation in various ways including prolonging the availability of lupine to the butterfly. Karner blue larvae in Michigan are predominantly ant-tended, and ant-tending also appears to be a significant factor in the butterfly's survival, and thus in habitat suitability.

Current management activities for the Karner blue in different states are focused on improving and maintaining habitat suitability for local butterfly populations (Baker 1994). The primary goals of habitat management are to increase amounts of lupine and nectar plants by decreasing woody vegetation through hand-cutting, mowing and prescribed fire (Baker 1994; Shuey 1994). Management activities also include restoration of Karner blue-unoccupied oak savanna and pine barren habitats, which are often adjacent to existing butterfly populations in the hopes of expanding the butterfly's range (Baker 1994). In Ohio and Ontario where the Karner blue is now extirpated, habitat once occupied by the species is being restored for future Karner blue reintroductions (Baker 1994; Packer 1994). Some states are involved in Karner blue propagation through captive rearing (Lane and Welch 1994), and lupine and nectar plant propagation and planting (Baker 1994). Our results support management activities which increase lupine

densities and frequencies, maintain a diversity of nectar sources, and maintain habitat heterogeneity created by low levels of canopy cover.

Many questions regarding Karner blue ecology still need to be answered in understanding the gradient between habitat suitability and unsuitability for this butterfly species (Haack 1993). Future research activities need to address topics such as the ability of butterflies to disperse through different types of intervening habitats, and the role of lupine and nectar source density and distribution in Karner blue population dynamics. On the local scale, the Karner blue requires some minimum level of lupine and nectar sources to survive. Our results suggest that more lupine is beneficial for Karner blue populations; however, the same may not be true for nectar sources once the minimum requirements are met. Impacts of management activities on tending-ant species also need to be explored.

Habitat suitability of an invertebrate can be difficult to identify through short-term investigations, which do not reveal complex interactions or effects of sporadic climatic events. This is especially true in dynamic habitats such as the savannas and barrens that were historically maintained by natural processes (Shuey 1994). Long-term studies, like those on the checkerspot butterfly, provide extremely useful information regarding a species' ecology and habitat suitability (Ehrlich and Murphy 1987), and should be persued for Karner blue. Ultimately, long-term viability of Karner blue populations will depend upon restoration of metapopulation dynamics in the threatened oak savanna and pine barren landscapes, allowing for local extinctions and recolonizations (Givnish et al 1988; Shuey 1994). The Karner blue serves as a symbol for savanna /barren conservation and management at both the species and ecosystem levels.

Table 3.1. Size, origin, recent disturbance, average percentage canopy cover (± SE), and frequency of canopy cover (proportion of transects with canopy cover; all tree species combined) of Karner blue study sites in Allegan State Game Area.

Site[1]	Size (ha)	Origin	Recent disturbance	Ave. percentage canopy cover (± SE)	Canopy cover frequency
48N89	2.5	remnant oak opening	burned in '92	19.7 (± 4.5) a	0.70
Horseshoe	0.8	created opening	none	0.6 (± 0.6) b	0.09
Jay	0.9	created opening	burned in '90, '92	33.7 (± 7.9) a	0.77
Marsh	3.0	remnant oak opening	none	24.7 (± 3.1) a	0.91
Park	2.5	remnant oak opening	none	32.6 (± 5.6) a	0.70
Pipe	0.8	created opening	mowed in '92	22.8 (± 7.2) a	0.85
Square	1.5	remnant oak opening	none	19.8 (± 5.2) a	0.79

NOTE: Means followed by the same letter are not significantly different by Tukey's HSD multiple comparisons test ($p < 0.05$) on log-transformed (log[x+1]) data.

[1] Sites were used for study in 1993 and 1994, except the 'Park' site, which was used only in 1994.

Table 3.2. Dates and degree days of peak counts for spring and summer Karner blue flight periods, and of spring and summer flower density surveys for study sites, 1993 and 1994.

		Peak adult flight		Flower density surveys	
Year	Season	Date	Degree days (base 50°F)[1]	Date	Degree days (base 50°F)[1]
1993	Spring	*not recorded*	-- -- --	2 - 4 June	331 - 340
	Summer	15 - 22 July	1125 - 1260	21 - 22 July	1247 - 1260
1994	Spring	25 May - 2 June	256 - 338	3 - 8 June	346 - 415
	Summer	13 - 19 July	1138 - 1262	15 - 21 July	1178 - 1314

[1] Degree days (base 50°F) based upon degree day accumulation since March 1st for Fennville, Michigan, published in the Michigan State University Crop Advisory Team (CAT) Alert Newsletter, Fruit Edition. Degree days calculated using the Baskerville-Emin method (Baskerville & Emin 1969).

Table 3.3. Estimations of Karner blue abundance based upon peak counts of adults, and mean values (± SE) of lupine density and flowering plant density for Karner blue study sites, 1993 and 1994.

Year	Study site	Karner blue abundance (adults / hr)		Lupine density (± SE) (stems / m^2)	Flowering plant density (± SE) (stems / m^2)	
		Spring	Summer		Spring	Summer
1993	48N89		19.0	2.2 (± 1.5) a	9.3 (± 2.1) a	0.8 (± 0.8) a
	Horseshoe		41.0	0.2 (± 0.1) a	3.7 (± 0.9) b	3.2 (± 1.2) b
	Jay		164.0	18.1 (± 4.6) b	10.3 (± 3.1) ab	0.9 (± 0.3) a
	Marsh		7.0	2.5 (± 0.8) a	4.7 (± 1.1) ab	0.6 (± 0.1) a
	Pipe		100.0	11.7 (± 2.6) b	4.7 (± 0.9) ab	0.8 (± 0.7) a
	Square		64.0	16.7 (± 4.9) b	9.8 (± 2.1) a	0.6 (± 0.2) a
1994	48N89	10.5	32.4	2.2 (± 1.0) a	32.6 (± 6.3) d	1.9 (± 0.3) ab
	Horseshoe	32.7	68.6	0.1 (± 0.1) a	2.1 (± 0.4) ab	2.9 (± 1.0) a
	Jay	100.0	199.0	13.7 (± 2.9) b	3.7 (± 1.0) ab	1.3 (± 0.2) abc
	Marsh	12.0	22.0	2.3 (± 0.6) a	7.3 (± 1.5) ac	1.1 (± 0.3) bc
	Park	7.3	19.8	0.7 (± 0.3) a	1.9 (± 0.6) b	0.7 (± 0.2) c
	Pipe	93.0	125.7	12.0 (± 4.1) b	3.7 (± 1.2) abc	0.6 (± 0.1) bc
	Square	57.2	90.8	13.7 (± 3.1) b	15.1 (± 3.3) cd	2.3 (± 0.4) a

Table 3.3 (cont'd)

NOTE: For each variable in each year, means followed by the same letter are not significantly different by Tukey's HSD multiple comparisons test ($p < 0.05$) on log-transformed (log[x+1]) data.

Table 3.4. Average number of lupine stems (per m^2) (± SE), average percentage of lupine stems (per m^2) (± SE) with larval feeding damage, and feeding damage frequency (proportion of quadrats with feeding damage), from quadrat surveys of Karner blue summer generation larval feeding damage in Allegan State Game Area study sites, 1994.

Site	Ave. no. lupine stems / m^2 (± SE)	Ave. percentage of lupine stems / m^2 (± SE) with larval feeding damage	Feeding damage frequency
48N89	18.6 (3.6)	4.7 (2.3) a	0.30
Horseshoe	18.4 (3.8)	10.7 (3.1) ab	0.60
Jay	27.3 (4.2)	23.6 (4.5) b	0.95
Marsh	15.3 (2.5)	5.4 (2.2) a	0.40
Park	12.0 (2.1)	6.1 (2.5) a	0.35
Pipe	22.2 (3.9)	18.5 (4.9) b	0.85
Square	18.6 (3.0)	9.5 (3.1) a	0.60

NOTE: Means of percentage lupine stems with damage followed by the same letter are not significantly different by Tukey's HSD multiple comparisons test ($p < 0.05$) on arcsine-transformed data.

Table 3.5. Frequencies (proportions of transects occupied) of lupine and of overall spring and summer flowers from transect surveys in Allegan State Game Area study sites, 1993 and 1994.

	Lupine frequency		Overall spring flower frequency		Overall summer flower frequency	
Study site	1993	1994	1993	1994	1993	1994
48N89	0.41	0.37	1.0	1.0	0.82	1.0
Horseshoe	0.14	0.09	1.0	0.91	1.0	1.0
Jay	1.0	1.0	1.0	0.85	0.64	1.0
Marsh	0.50	0.54	1.0	0.97	0.53	0.68
Park	---	0.32	---	0.68	---	0.57
Pipe	0.92	0.90	1.0	0.80	0.50	0.90
Square	0.82	0.85	1.0	0.95	0.56	0.95

Table 3.6. Average percentage of lupine stems (per m^2) with flower spikes and average percentage of lupine flower spikes (per m^2) at different stages of bloom (no buds open (0) - flower spike in full bloom (1); seed pods present (Seed); flower spike bare (Bare)) from weekly quadrat surveys of lupine flowering phenology in Allegan State Game Area study sites, spring 1994.

Date	Study site	Ave. percentage of lupine stems (per m^2) with flower spikes	Ave. percentage of lupine flower spikes (per m^2) at each stage of bloom							
			0	< 1/4	1/4	1/2	3/4	1	Seed	Bare
5/13	48N89	69.4 a (4.4)	97.9 a (2.4)	2.2 a (2.4)						
	Horseshoe	36.8 b (7.3)	100 a (0)	0 a						
	Jay	48.6 ab (7.3)	100 a (0)	0 a						
	Marsh	52.5 ab (8.2)	98.5 a (0.8)	1.5 a (0.8)						
	Park	68.7 a (7.0)	95.8 a (2.9)	4.2 a (2.9)						
	Pipe	39.9 b (5.7)	100 a (0)	0 a						
	Square	49.9 ab (8.7)	97.1 a (2.0)	2.9 a (2.0)						

Table 3.6 (cont'd)

Date	Study site	Ave. percentage of lupine stems (per m^2) with flower spikes	Ave. percentage of lupine flower spikes (per m^2) at each stage of bloom							
			0	< 1/4	1/4	1/2	3/4	1	Seed	Bare
5/17	48N89	77.3 a (4.3)	82.5 a (9.1)	17.2 ab (8.8)	0.3 a (0.4)					
	Horseshoe	57.5 ab (9.3)	90.2 a (4.3)	9.3 ab (3.9)	0.5 a (0.2)					
	Jay	51.0 ab (12.5)	99.0 a (1.0)	1.0 ab (1.0)	0 a					
	Marsh	44.2 ab (12.4)	85.4 a (4.9)	14.6 ab (4.9)	0 a					
	Park	70.1 a (5.3)	91.3 a (3.5)	8.7 ab (3.5)	0 a					
	Pipe	44.2 ab (8.6)	99.7 a (0.3)	0.3 a (0.3)	0 a					
	Square	22.0 b (5.9)	78.5 a (8.5)	21.5 b (8.5)	0 a					

Table 3.6 (cont'd)

Date	Study site	Ave. percentage of lupine stems (per m^2) with flower spikes	Ave. percentage of lupine flower spikes (per m^2) at each stage of bloom							
			0	< 1/4	1/4	1/2	3/4	1	Seed	Bare
5/20	48N89	67.9 a (3.0)	60.8 a (9.7)	26.8 a (6.0)	7.4 a (2.7)	3.4 a (2.5)	1.6 a (0.9)	0 a		
	Horseshoe	52.9 a (13.9)	68.8 a (7.8)	23.8 a (6.2)	6.0 a (2.4)	1.4 a (0.7)	0 a	0 a		
	Jay	51.3 a (10.4)	78.8 a (13.2)	16.2 a (10.1)	4.2 a (2.9)	0.8 a (0.6)	0 a	0 a		
	Marsh	46.0 a (9.4)	39.2 a (8.5)	33.7 a (6.3)	17.2 a (5.8)	4.2 a (2.6)	4.8 a (2.4)	0.8 a (0.9)		
	Park	68.7 a (11.2)	54.6 a (14.1)	28.1 a (6.8)	8.5 a (4.4)	6.3 a (4.4)	2.9 a (2.1)	0 a		
	Pipe	40.4 a (8.5)	83.8 a (7.0)	12.7 a (5.0)	3.3 a (1.9)	0.7 a (0.7)	0 a	0 a		
	Square	46.0 a (8.3)	50.2 a (6.6)	31.0 a (4.1)	14.1 a (5.7)	3.6 a (2.7)	1.2 a (1.3)	0 a		

Table 3.6 (cont'd)

Date	Study site	Ave. percentage of lupine stems (per m^2) with flower spikes	Ave. percentage of lupine flower spikes (per m^2) at each stage of bloom							
			0	< 1/4	1/4	1/2	3/4	1	Seed	Bare
5/27	48N89	77.0 a (3.5)	14.9 a (2.4)	10.9 ab (3.4)	21.6 a (3.4)	25.9 a (2.8)	17.8 ab (4.3)	8.9 ab (5.1)		
	Horseshoe	44.3 b (7.8)	40.1 b (4.7)	9.1 ab (4.9)	8.3 a (3.5)	5.5 b (2.2)	3.3 a (1.8)	33.1 ac (22.4)		
	Jay	49.7 bc (5.6)	16.1 a (4.2)	11.6 ab (3.5)	13.4 a (2.6)	18.7 ab (4.7)	30.8 b (6.0)	9.4 ab (3.7)		
	Marsh	69.7 ab (5.5)	5.2 a (1.8)	3.4 a (1.7)	9.3 a (2.0)	15.4 ab (5.3)	21.7 ab (3.6)	45.1 c (4.1)		
	Park	63.0 ab (9.8)	14.8 a (7.4)	9.1 ab (2.1)	15.0 a (3.8)	14.2 ab (2.4)	23.5 ab (6.5)	25.3 abc (6.8)		
	Pipe	65.0 ab (5.0)	20.3 ab (7.0)	20.4 b (4.5)	17.2 a (3.8)	19.6 ab (5.2)	16.8 ab (6.2)	5.8 b (2.5)		
	Square	73.6 ac (6.5)	20.0 ab (6.4)	4.6 a (2.0)	11.8 a (4.0)	19.4 ab (3.5)	24.8 b (7.3)	18.7 ab (9.5)		

Table 3.6 (cont'd)

Date	Study site	Ave. percentage of lupine stems (per m^2) with flower spikes	Ave. percentage of lupine flower spikes (per m^2) at each stage of bloom							
			0	< 1/4	1/4	1/2	3/4	1	Seed	Bare
6/1	48N89	63.6 ab (6.0)	6.0 a (2.3)	0.5 a (0.6)	2.5 a (2.8)	5.5 a (3.5)	14.9 b (4.8)	70.6 ab (10.1)		
	Horseshoe	28.7 a (9.0)	48.0 bc (13.6)	9.4 b (4.7)	5.4 a (3.9)	0 a	2.8 ab (3.0)	34.4 b (14.0)		
	Jay	54.9 ab (3.2)	11.7 a (3.8)	3.7 ab (1.3)	2.2 a (1.6)	3.7 a (1.9)	5.4 ab (3.1)	73.4 a (6.0)		
	Marsh	44.8 ab (14.1)	36.0 abc (15.8)	0.2 a (0.2)	0.2 a (0.2)	0.2 a (0.2)	1.7 a (0.7)	61.7 ab (15.0)		
	Park	71.2 b (8.6)	17.1 ab (7.8)	0.4 a (0.4)	1.4 a (1.2)	1.0 a (0.8)	3.7 ab (1.6)	76.5 a (6.8)		
	Pipe	49.5 ab (10.3)	12.4 ab (3.4)	1.3 a (1.0)	2.3 a (1.1)	3.2 a (2.2)	11.5 ab (4.7)	70.0 ab (4.8)		
	Square	40.4 ab (12.6)	61.1 c (5.6)	0.3 a (0.3)	0 a	0 a	0.5 a (0.5)	38.2 ab (5.7)		

Table 3.6 (cont'd)

Date	Study site	Ave. percentage of lupine stems (per m^2) with flower spikes	Ave. percentage of lupine flower spikes (per m^2) at each stage of bloom							
			0	< 1/4	1/4	1/2	3/4	1	Seed	Bare
6/10	48N89	70.3 a (2.6)	0 a					13.1 a (8.2)	46.6 a (9.3)	40.3 a (13.8)
	Horseshoe	14.7 c (3.0)	0 a					0 a	0 b	100 c (0)
	Jay	38.5 b (7.8)	0 a					5.1 a (3.5)	33.1 a (10.1)	61.8 ab (12.4)
	Marsh	42.5 b (6.2)	0 a					0.4 a (0.5)	3.0 b (1.7)	96.6 c (2.0)
	Park	73.9 a (7.0)	0 a					6.0 a (3.7)	37.5 ab (19.5)	56.6 ab (18.0)
	Pipe	21.8 bcd (5.8)	0 a					13.0 a (8.7)	43.6 a (12.2)	43.5 a (16.6)
	Square	68.2 ad (7.2)	0.3 a (0.4)					3.1 a (1.2)	16.6 ab (12.7)	83.3 bc (10.4)

NOTE: For each week and each category, means followed by the same letter are not significantly different by Tukey's HSD multiple comparisons test ($p < 0.05$).

Table 3.7. Densities of individual flower species from transect surveys in Allegan State Game Area study sites, spring 1993.

Flowering plant species	No. flower stems / 10 m^2 by site					
	48N89	Horseshoe	Jay	Marsh	Pipe	Square
Comandra umbellata (Bastard Toadflax)	6.3	0	0.4	3.1	0	0.5
Hieracium aurantiacum (Orange Hawkweed)	0.5	0	0	0	0	0
Hieracium pillosella (Mouse-ear Hawkweed)	54.0	8.1	33.9	19.5	9.3	79.5
Krigia virginica (Dwarf Dandelion)	0.5	4.6	9.3	0.3	4.3	0.6
Linaria canadensis (Blue Toadflax)	0	1.2	2.4	0.5	0.3	0
Lithospermum canescens (Hoary Puccoon)	0.1	0	0	0	0	0
Lotus corniculatus (Birdsfoot Trefoil)	0	0.2	0	0	0.4	0
Lupinus perennis (Wild Lupine)	3.9	0.4	54.3	3.4	27.4	14.4
Potentilla sp. (Cinquefoil sp.)	0	8.9	0.7	0	2.8	0
Rubus sp. (Dewberry)	0.1	0.4	0.8	0	0	0
Rumex acetosella (Sheep Sorrel)	27.5	12.4	2.1	21.3	2.2	2.6
Viola pedata (Birdfoot Violet)	0.2	0.7	0.1	0	0	0

Table 3.8. Densities of individual flower species from transect surveys in Allegan State Game Area study sites, spring 1994.

Flowering plant species	No. flower stems / 10 m^2 by site						
	48N89	Horseshoe	Jay	Marsh	Park	Pipe	Square
Arenaria stricta (Rock Sandwort)	5.1	0	0	0.3	0	0	0
Berteroa incana (Hoary Alyssum)	0	0.2	0	0.1	0	0	0
Comandra umbellata (Bastard Toadflax)	0.4	0	0.1	0.1	0.8	0	0.3
Erigeron annuus (Daisy Fleabane)	0	0	0	0	0.1	0	0
Euphorbia corollata (Flowering Spurge)	0	0.2	0	0	0	0	0
Helianthemum canadense (Frostweed)	0	0.2	0.4	0.1	0.1	0.5	0
Hieracium aurantiacum (Orange Hawkweed)	2.7	0	0	0	0	0	0
Hieracium pillosella (Mouse-ear Hawkweed)	281.7	6.8	10.5	28.3	12.7	12.2	119.5
Hieracium spp. (Yellow Hawkweeds)	0	0	0	0	0	0.7	0
Krigia virginica (Dwarf Dandelion)	0.1	0.5	0.4	0.3	0.1	0.3	0.1
Linaria canadensis (Blue Toadflax)	0	0	0	0.1	0	0	0.2
Lithospermum canescens (Hoary Puccoon)	0.4	0	0	0.1	0.2	0	0.3
Lotus corniculatus (Birdsfoot Trefoil)	0	0	0	0	0	0.3	0
Lupinus perennis (Wild Lupine)	21.5	0.6	14.1	10.1	0	7.5	15.8
Polygala polygama (Racemed Milkwort)	0	0.3	0.6	0	0	0	0
Potentilla sp. (Cinquefoil sp.)	0	0.3	0.5	0.1	0	3.0	0.1
Rubus sp. (Dewberry)	2.0	11.7	7.8	1.2	3.0	12.3	3.3
Rumex acetosella (Sheep Sorrel)	12.1	0	2.3	32.0	2.0	0	11.8
Tragopogon dubius (Yellow Goatsbeard)	0	0.5	0	0.1	0	0	0
Vicia cracca (Cow Vetch)	0	0	0	0.3	0	0	0

Table 3.9. Frequencies of individual flower species (proportions of transects with flowers) from transect surveys in Allegan State Game Area study sites, spring 1993 and 1994.

Flowering plant species	Year	Flower stem frequency by site						
		48N89	Horseshoe	Jay	Marsh	Park	Pipe	Square
Arenaria stricta (Rock Sandwort)	94	0.15	0	0	0.03	0	0	0
Berteroa incana (Hoary Alyssum)	94	0	0.09	0	0.03	0	0	0
Comandra umbellata (Bastard Toadflax)	93	0.36	0	0.07	0.33	---	0	0.18
	94	0.15	0	0.08	0.03	0.07	0	0.10
Erigeron annuus (Daisy Fleabane)	94	0	0	0	0	0.04	0	0
Euphorbia corollata (Flowering Spurge)	94	0	0.09	0	0	0	0	0
Helianthemum canadense (Frostweed)	94	0	0.09	0.08	0.03	0.04	0.10	0
Hieracium aurantiacum (Orange Hawkweed)	93	0.05	0	0	0	---	0	0
	94	0.11	0	0	0	0	0	0
Hieracium pillosella (Mouse-ear Hawkweed)	93	0.95	0.43	0.87	0.89	---	0.67	1.0
	94	0.93	0.55	0.46	0.83	0.32	0.50	0.90
Hieracium spp. (Yellow Hawkweeds)	94	0	0	0	0	0	0.10	0
Krigia virginica (Dwarf Dandelion)	93	0.09	0.71	0.67	0.17	---	0.58	0.27
	94	0.04	0.18	0.23	0.11	0.04	0.10	0.05
Linaria canadensis (Blue Toadflax)	93	0	0.29	0.13	0.11	---	0.17	0
	94	0	0	0	0.03	0	0	0.05

Table 3.9 (cont'd)

Lithospermum canescens (Hoary Puccoon)	93	0.05	0	0	0	---	0	0
	94	0.11	0	0	0.03	0.07	0	0.05
Lotus corniculatus (Birdsfoot Trefoil)	93	0	0.07	0	0	---	0.08	0
	94	0	0	0	0	0	0.10	0
Lupinus perennis (Wild Lupine)	93	0.27	0.07	0.93	0.39	---	0.92	0.64
	94	0.37	0.09	0.31	0.23	0	0.40	0.20
Polygala polygama (Racemed Milkwort)	94	0	0.09	0.08	0	0	0	0
Potentilla sp. (Cinquefoil sp.)	93	0	0.64	0.13	0	---	0.50	0
	94	0	0.09	0.08	0.06	0	0.60	0.05
Rubus sp. (Dewberry)	93	0.05	0.07	0.20	0	---	0	0
	94	0.19	0.73	0.54	0.34	0.39	0.70	0.65
Rumex acetosella (Sheep Sorrel)	93	0.64	0.43	0.20	0.56	---	0.17	0.09
	94	0.37	0	0.08	0.57	0.18	0	0.55
Tragopogon dubius (Yellow Goatsbeard)	94	0	0.09	0	0.03	0	0	0
Vicia cracca (Cow Vetch)	94	0	0	0	0.03	0	0	0
Viola pedata (Birdfoot Violet)	93	0.05	0.14	0.07	0	---	0	0

Table 3.10. Densities of individual flower species from transect surveys in Allegan State Game Area study sites, summer 1993.

Flowering plant species	No. flower stems / 10 m^2 by site					
	48N89	Horseshoe	Jay	Marsh	Pipe	Square
Asclepias tuberosa (Butterfly Weed)	0	0.8	0.2	0.1	0	0
Asclepias verticillata (Whorled Milkweed)	0	1.0	0	0.1	1.0	0
Berteroa incana (Hoary Alyssum)	0	5.8	0	0	0	0
Centaurea maculosa (Spotted Knapweed)	0	12.5	0	0.2	0	0
Coreopsis lanceolata (Lance-leaved Coreopsis)	0	0	0	0.1	0	0
Erigeron annuus (Daisy Fleabane)	0	0.3	0	0.1	0	0
Euphorbia corollata (Flowering Spurge)	6.0	6.2	7.1	2.2	0.8	4.0
Helianthus divaricatus (Woodland Sunflower)	0	0.2	0.2	0.1	0	0
Hieracium pillosella (Mouse-ear Hawkweed)	0.2	0.3	0	0.2	0	0.2
Hieracium spp. (Yellow Hawkweeds)	0.1	0.1	0	0.1	0	0
Hypericum perforatum (St. Johnswort)	0.2	0.3	0.8	1.4	3.1	1.1
Krigia virginica (Dwarf Dandelion)	0	0.2	0	0	0	0
Liatris cylindracea (Cylindric Blazing-star)	0.3	0.3	0	0	0	0.5
Lotus corniculatus (Birdsfoot Trefoil)	0	0	0	0	0.2	0
Monarda fistulosa (Wild Bergamot)	0.5	0	0	0	0	0
Monarda punctata (Horsemint)	0	1.2	0	1.2	2.9	0.2
Polygala polygama (Racemed Milkwort)	0.4	0.2	0	0.1	0	0
Rudbeckia hirta (Black-eyed Susan)	0	0.2	0.3	0	0	0
Tragopogon dubius (Yellow Goatsbeard)	0	0.3	0	0	0	0

Table 3.11. Densities of individual flower species from transect surveys in Allegan State Game Area study sites, summer 1994.

Flowering plant species	No. flower stems / 10 m^2 by site						
	48N89	Horseshoe	Jay	Marsh	Park	Pipe	Square
Asclepias tuberosa (Butterfly Weed)	0.1	0	0	0	0	0	0.3
Asclepias verticillata (Whorled Milkweed)	0	0	0	0	0	0	0.5
Berteroa incana (Hoary Alyssum)	0	7.0	0	0	0	0	0
Centaurea maculosa (Spotted Knapweed)	0	9.0	0	0	0	0	0
Coreopsis lanceolata (Lance-leaved Coreopsis)	0	0	0	3.0	0	0	0.1
Dianthus armeria (Deptford Pink)	0	0.3	0	0	0	0	0
Erigeron annuus (Daisy Fleabane)	0	0	0	0.3	0.1	0.3	0
Euphorbia corollata (Flowering Spurge)	7.0	2.0	6.0	0.7	4.0	2.0	7.0
Galium asprellum (Rough Bedstraw)	0.4	0	3.0	0	0	0.5	2.0
Hieracium aurantiacum (Orange Hawkweed)	0	0.2	0	0	0	0	0.1
Hieracium pillosella (Mouse-ear Hawkweed)	11.0	7.0	0.5	4.0	3.0	0.2	12.0
Hieracium spp. (Yellow Hawkweeds)	0.1	0	0.6	0	0.1	0.3	0
Hypericum perforatum (St. Johnswort)	0.1	2.0	0	0.4	0	0.7	0.8
Krigia virginica (Dwarf Dandelion)	0	0	0.5	0.1	0	0.2	0
Liatris cylindracea (Cylindric Blazing-star)	0.2	0	0	0	0	0	0
Melampyrum lineare (Cow Wheat)	0	0	0.1	0.1	0	0	0
Monarda fistulosa (Wild Bergamot)	0.5	0	0	0	0	0	0
Monarda punctata (Horsemint)	0	0	0	0.3	0	0.8	0

Table 3.11 (cont'd)

Polygala polygama (Racemed Milkwort)	0.3	0	2.0	0.6	0.1	0.2	1.0
Rosa carolina (Pasture Rose)	0	0.2	0	0	0	0	0
Rubus sp. (Dewberry)	0	0	0	0	0	0	0.1
Rumex acetosella (Sheep Sorrel)	0	0	0	0.7	0	0	0
Solanum carolinense (Horse Nettle)	0	0	0.1	0	0	0	0
Tephrosia virginiana (Goat's Rue)	0.1	2.0	0	0	0	0	0
Tradescantia ohiensis (Spiderwort)	0	0	0	0	0	0.2	0.1

Table 3.12. Frequencies of individual flower species (proportions of transects with flowers) from transect surveys in Allegan State Game Area study sites, summer 1993 and 1994.

Flowering plant species	Year	Flower stem frequency by site						
		48N89	Horseshoe	Jay	Marsh	Park	Pipe	Square
Asclepias tuberosa (Butterfly Weed)	93	0	0.10	0.09	0.03	---	0	0
	94	0.04	0	0	0	0	0	0.10
Asclepias verticillata (Whorled Milkweed)	93	0	0.10	0	0.03	---	0.13	0
	94	0	0	0	0	0	0	0.10
Berteroa incana (Hoary Alyssum)	93		0.20	0	0	---	0	0
	94	0	0.18	0	0	0	0	0
Centaurea maculosa (Spotted Knapweed)	93	0	0.60	0	0.03	---	0	0
	94	0	0.64	0	0	0	0	0
Coreopsis lanceolata (Lance-leaved Coreopsis)	93	0	0	0	0.06	---	0	0
	94	0	0	0	0.18	0	0	0.05
Dianthus armeria (Deptford Pink)	94	0	0.09	0	0	0	0	0
Erigeron annuus (Daisy Fleabane)	93	0	0.20	0	0.09	---	0	0
	94	0	0	0	0.03	0.04	0.10	0
Euphorbia corollata (Flowering Spurge)	93	0.77	0.60	0.55	0.38	---	0.38	0.50
	94	0.82	0.45	1.0	0.35	0.46	0.40	0.75
Galium asprellum (Rough Bedstraw)	94	0.11	0	0.38	0	0	0.30	0.10

Table 3.12 (cont'd)

Helianthus divaricatus (Woodland Sunflower)	93	0	0.10	0.09	0.06	---	0	0
Hieracium aurantiacum (Orange Hawkweed)	94	0	0.09	0	0	0	0	0.05
Hieracium pillosella (Mouse-ear Hawkweed)	93	0.14	0.20	0	0.12	---	0	0.06
	94	0.71	0.36	0.31	0.35	0.46	0.10	0.95
Hieracium spp. (Yellow Hawkweeds)	93	0.05	0.10	0	0.03	---	0	0
	94	0.04	0	0.15	0	0.04	0.20	0
Hypericum perforatum (St. Johnswort)	93	0.09	0.50	0.09	0.26	---	0.38	0.17
	94	0.04	0.36	0	0.15	0	0.20	0.15
Krigia virginica (Dwarf Dandelion)	93	0	0.10	0	0	---	0	0
	94	0	0	0.15	0.03	0	0.10	0
Liatris cylindracea (Cylindric Blazing-star)	93	0.05	0.10	0	0	---	0	0.11
	94	0.04	0	0	0	0	0	0
Melampyrum lineare (Cow Wheat)	94	0	0	0.08	0.03	0	0	0
Lotus corniculatus (Birdsfoot Trefoil)	93	0	0	0	0	---	0.13	0
Monarda fistulosa (Wild Bergamot)	93	0.09	0	0	0	---	0	0
	94	0.07	0	0	0	0	0	0
Monarda punctata (Horsemint)	93	0	0.10	0	0.29	---	0.13	0.06
	94	0	0	0	0.09	0	0.20	0
Polygala polygama (Racemed Milkwort)	93	0.09	0.10	0	0.03	---	0	0
	94	0.11	0	0.46	0.03	0.07	0.10	0.15
Rosa carolina (Pasture Rose)	94	0	0.09	0	0	0	0	0
Rubus sp. (Dewberry)	94	0	0	0	0	0	0	0.05

Table 3.12 (cont'd)

Rudbeckia hirta (Black-eyed Susan)	93	0	0.10	0.09	0	---	0	0
Rumex acetosella (Sheep Sorrel)	94	0	0	0	0.03	0	0	0
Solanum carolinense (Horse Nettle)	94	0	0	0.08	0	0	0	0
Tephrosia virginiana (Goat's Rue)	94	0.04	0.36	0	0	0	0	0
Tradescantia ohiensis (Spiderwort)	94	0	0	0	0	0	0.10	0.05
Tragopogon dubius (Yellow Goatsbeard)	93	0	0.10	0	0	---	0	0

Table 3.13. Numbers of flower species encountered, and Shannon's diversity index (H') and Simpson's dominance index (expressed as reciprocal, 1/D) of flowering plant species, based on transect surveys in Karner blue study sites conducted during peak spring and summer flight of Karner blue, 1993 and 1994.

		No. species		Shannon's index (H')		Simpson's index (1/D)	
Year	Site	Spring	Summer	Spring	Summer	Spring	Summer
1993	48N89	9	6	1.08	0.84	2.33	1.59
	Horseshoe	9	15	1.61	1.72	4.27	3.86
	Jay	9	5	1.19	0.67	2.58	1.45
	Marsh	6	11	1.17	1.67	2.71	5.00
	Pipe	7	5	1.24	1.32	2.52	3.34
	Square	5	5	0.60	1.01	1.46	2.09
1994	48N89	10	10	0.58	0.84	1.33	2.31
	Horseshoe	10	9	1.19	1.70	2.43	4.20
	Jay	9	8	1.47	1.46	3.62	3.35
	Marsh	14	10	1.12	1.69	2.79	4.01
	Park	8	5	1.04	0.87	2.07	2.16
	Pipe	8	11	1.48	2.07	3.73	6.36
	Square	9	10	0.74	1.31	2.07	2.80

Table 3.14. Spring and summer flowering plant species encountered (indicated by √) in transect surveys conducted during the peak of the respective Karner blue flight periods in study sites, and numbers of Karner blue adult nectaring events observed during butterfly abundance surveys in study sites, 1993 and 1994.

		Spring 1993[2]	Spring 1994		Summer 1993		Summer 1994	
Flowering plant species[1]		Transect	Transect	Nectaring	Transect	Nectaring	Transect	Nectaring
Achillea millefolium	Yarrow			--		1		--
Arenaria stricta	Rock Sandwort		√	2		--		--
Asclepias tuberosa	Butterfly Weed			--	√	166	√	245
Asclepias verticillata	Whorled Milkweed			--	√	16	√	12
Berteroa incana	Hoary Alyssum		√	1	√	--	√	--
Centaurea maculosa	Spotted Knapweed			--	√	64	√	42
Comandra umbellata	Bastard Toadflax	√	√	--		--		--
Coreopsis lanceolata	Lance-leaved Coreopsis			--	√	2	√	89
Dianthus armeria	Deptford Pink			--		1	√	--
Erigeron annuus	Daisy Fleabane		√	--	√	2	√	5
Euphorbia corollata	Flowering Spurge		√	--	√	25	√	43
Galium asprellum	Rough Bedstraw			--		--	√	--
Helianthemum canadense	Frostweed		√	--		--		--
Helianthus divaricatus	Woodland Sunflower			--	√	21		8
Helianthus occidentalis	Western Sunflower			--		5		--
Hieracium aurantiacum	Orange Hawkweed	√	√	--		--	√	2
Hieracium pillosella	Mouse-ear Hawkweed	√	√	7	√	6	√	7

Table 3.14 (cont'd)

Hieracium spp.	Yellow Hawkweeds		√	--	√	4	√	23
Hypericum perforatum	St. Johnswort			--	√	4	√	5
Krigia virginica	Dwarf Dandelion	√	√	--	√	--	√	--
Liatris cylindracea	Cylindric Blazing-star			--	√	7	√	1
Linaria canadensis	Blue Toadflax	√	√	--		--		--
Lithospermum canescens	Hoary Puccoon	√	√	1		--		1
Lotus corniculatus	Birdsfoot Trefoil	√	√	--	√	2		2
Lupinus perennis	Wild Lupine	√	√	9		--		--
Melampyrum lineare	Cow Wheat			--		--	√	--
Monarda fistulosa	Wild Bergamot			--	√	--	√	--
Monarda punctata	Horsemint			--	√	86	√	58
Polygala polygama	Racemed Milkwort		√	--	√	3	√	1
Potentilla spp.	Cinquefoil spp.	√	√	7		--		--
Rosa carolina	Pasture Rose			--		--	√	--
Rubus sp.	Dewberry	√	√	14		--	√	5
Rudbeckia hirta	Black-eyed Susan			--	√	9		11
Rumex acetosella	Sheep Sorrel	√	√	--		--	√	--
Solanum carolinense	Horse Nettle			--		--	√	--
Specularia perfoliata	Venus Looking-glass			--		--		1
Tephrosia virginiana	Goat's Rue			--		--	√	87
Tradescantia ohiensis	Spiderwort			--		1	√	--
Tragopogon dubius	Yellow Goatsbeard		√	--	√	--		--
Vicia cracca	Cow Vetch		√	--		--		1
Viola pedata	Birdfoot violet	√		1		--		--
	Total	12	20	42	19	425	25	653

Table 3.14 (cont'd)

[1] Data for all sites have been combined together; most of the flower species checked (√) were not encountered in every site, but in subsets of sites.

[2] Formal butterfly abundance surveys were not conduted; therefore, timing of transect survey was based upon casual butterfly observations, and formal nectaring observations were not made.

Table 3.15. Numbers of ant-tended and untended Karner blue larvae, by larval body length (cm), observed in Allegan State Game Area study sites, summer 1993 and spring and summer 1994.

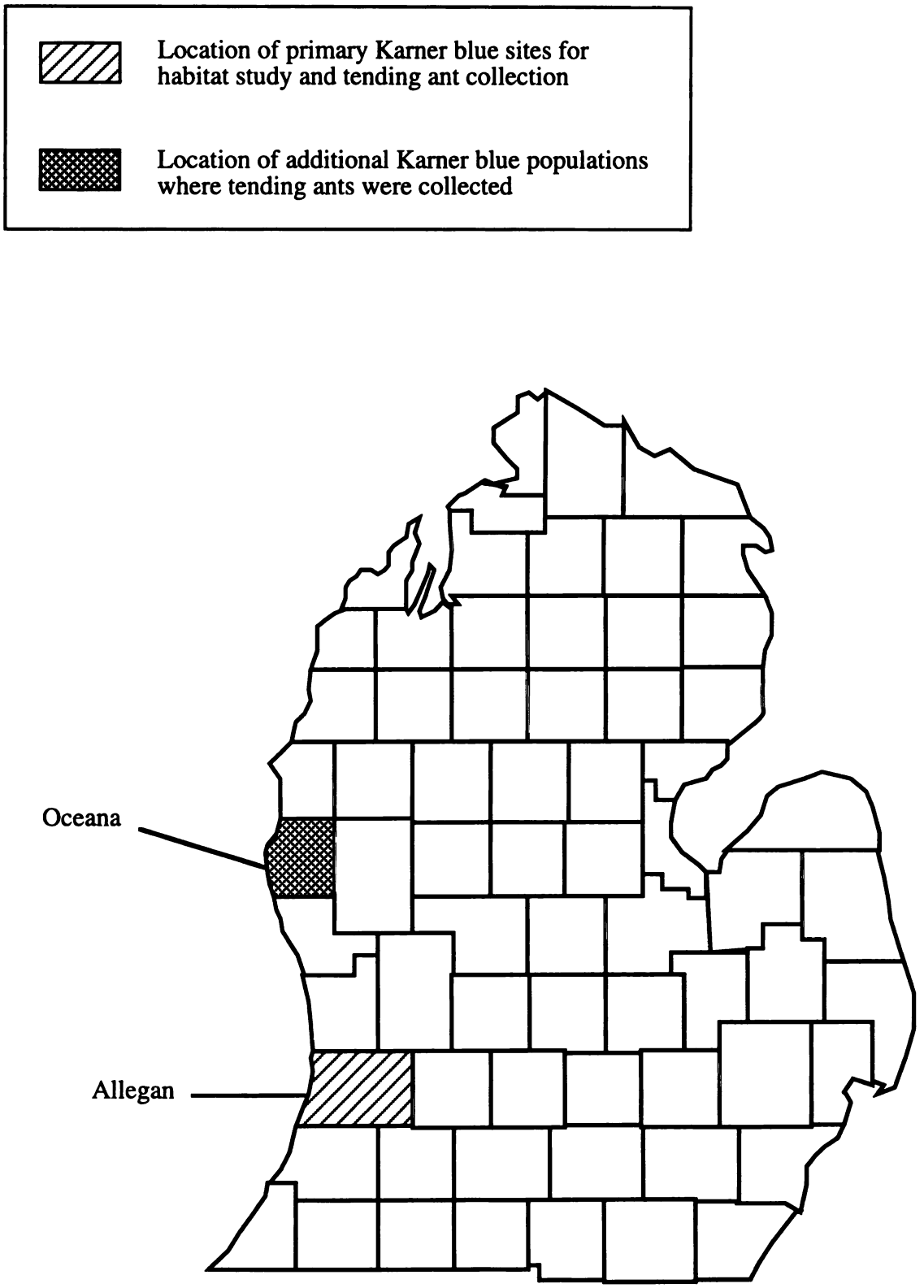
		Total no. Karner blue larvae		No. of larvae by body length (cm)							
				Tended				Untended			
Year	Season	Tended	Untended	0.2-0.5	0.6-0.9	1.0-1.4	1.5-1.9	0.2-0.5	0.6-0.9	1.0-1.4	1.5-1.9
1993	Summer	77	1	3	16	31	27	0	1	0	0
1994	Spring	57	12	25	10	17	5	5	3	2	2
1994	Summer	176	22	39	48	63	26	4	5	8	5
	Total	310	35	67	74	111	58	9	9	10	7

Table 3.16. Thirteen species of ants (Hymenoptera: Formicidae) representing three subfamilies observed tending Karner blue larvae. Ant specimens were collected in Allegan County (Allegan State Game Area) and Oceana County (Huron-Manistee National Forest), Michigan, during the 1993 and 1994 spring (Spr) and summer (Su) Karner blue larval generations.

Tending ant species[1]	Karner blue larval generation tended at time of collection	County
Subfamily **Myrmicinae**		
Crematogaster lineolata (Say)	Spr, Su	Allegan, Oceana (Su only)
Monomorium pharaonis (L.)	Spr	Oceana
Myrmica americana Weber	Su	Allegan
Myrmica fracticornis Emery	Spr, Su	Allegan
Subfamily **Dolichoderinae**		
Dolichoderus mariae Forel	Spr	Oceana
Dolichoderus pustulatus Mayr	Su	Allegan
Tapinoma sessile (Say)	Spr, Su	Allegan, Oceana
Subfamily **Formicinae**		
Formica neogatates Emery	Su	Allegan, Oceana
Formica obscuripes Forel	Spr, Su	Allegan, Oceana
Formica obscuriventris Mayr	Su	Allegan, Oceana
Formica schaufussi Mayr	Su	Allegan
Formica subsericea Say	Spr	Allegan, Oceana
Lasius neoniger Emery	Su	Allegan

[1] Ant species were determined on 14 September, 1995 by D. R. Smith, Research Entomologist, USDA Agricultural Research Service, Systematic Entomology Laboratory, Communications & Taxonomic Services Unit, Beltsville Agricultural Research Center-West, Beltsville, Maryland 20705-2350.

Figure 3.1. Map of Lower Peninsula of Michigan showing the location of Allegan State Game Area study sites (Allegan Co) and Huron-Manistee National Forest (Oceana Co).


Location of primary Karner blue sites for habitat study and tending ant collection
Location of additional Karner blue populations where tending ants were collected
Oceana
Allegan

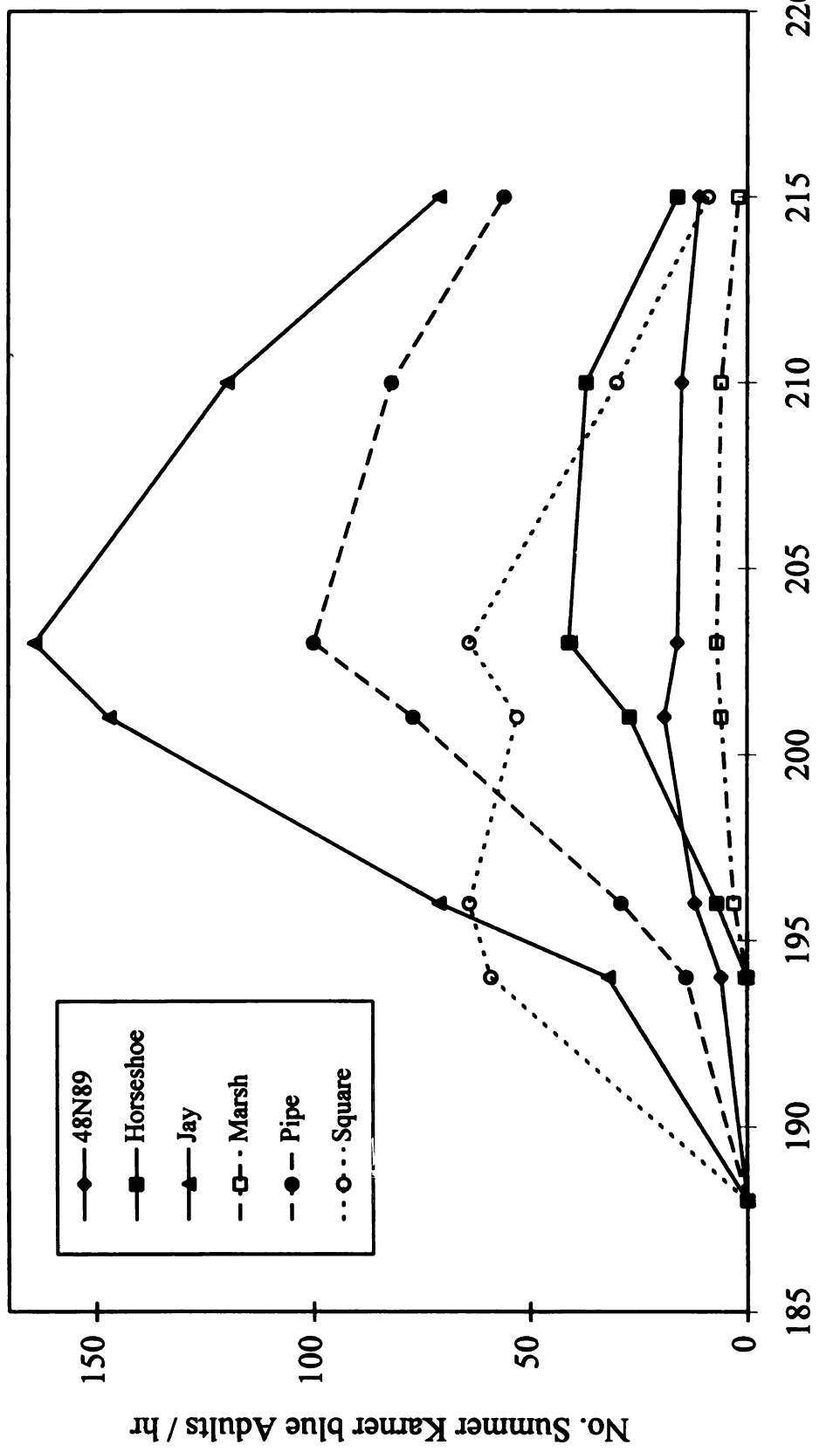


Figure 3.2. 1993 summer flight period of the Karner blue butterfly on six study sites in Allegan State Game Area (Allegan Co), Michigan.

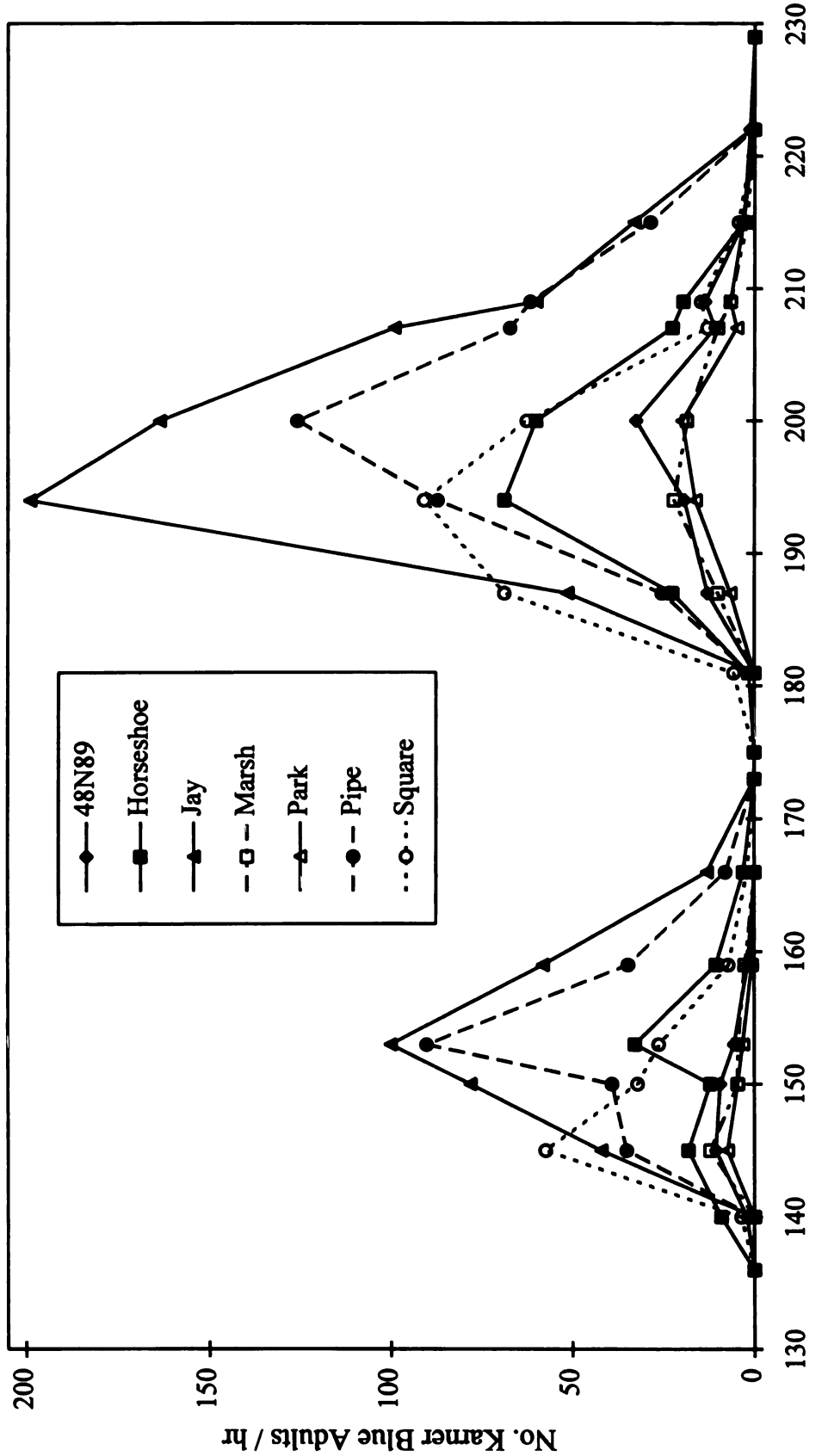


Figure 3.3. 1994 spring and summer flight periods of the Karner blue butterfly on seven study sites in Allegan State Game Area (Allegan Co), Michigan.

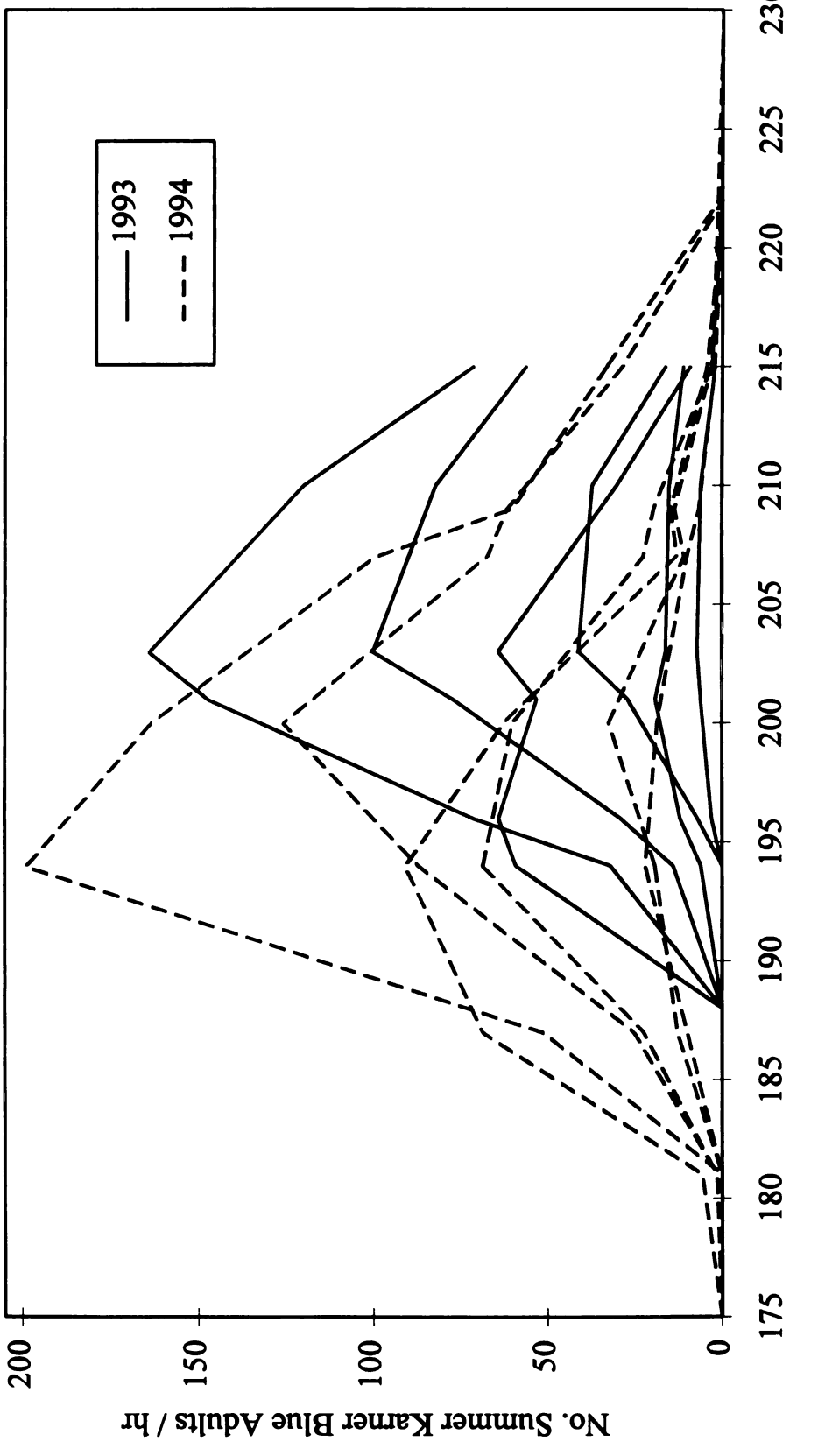


Figure 3.4. 1993 and 1994 summer flight periods of the Karner blue butterfly on six study sites in Allegan State Game Area (one site used only in 1994 not included).

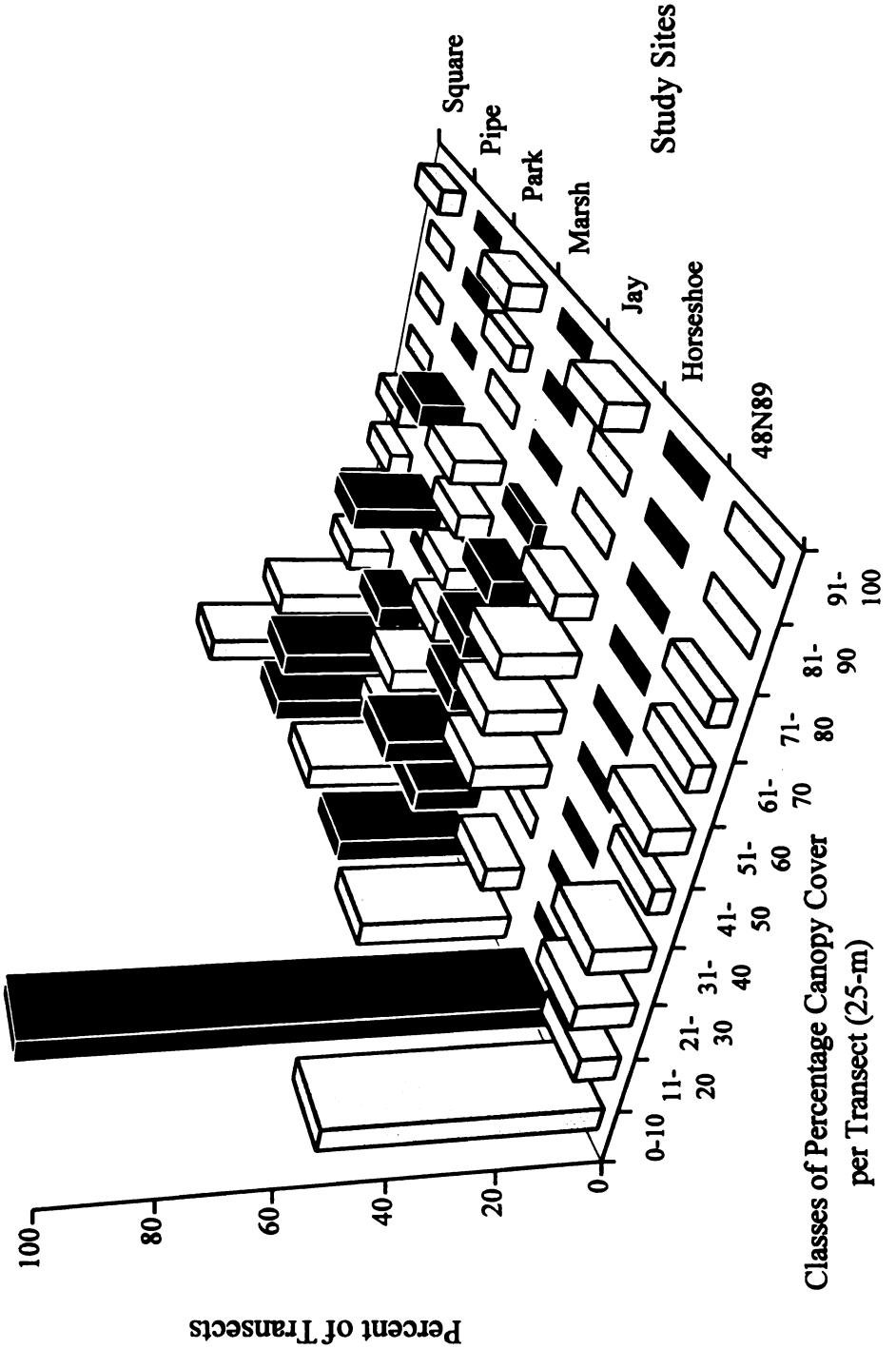


Figure 3.5. Distribution of percentage canopy cover of individual transects (25-m) from surveys in each study site.

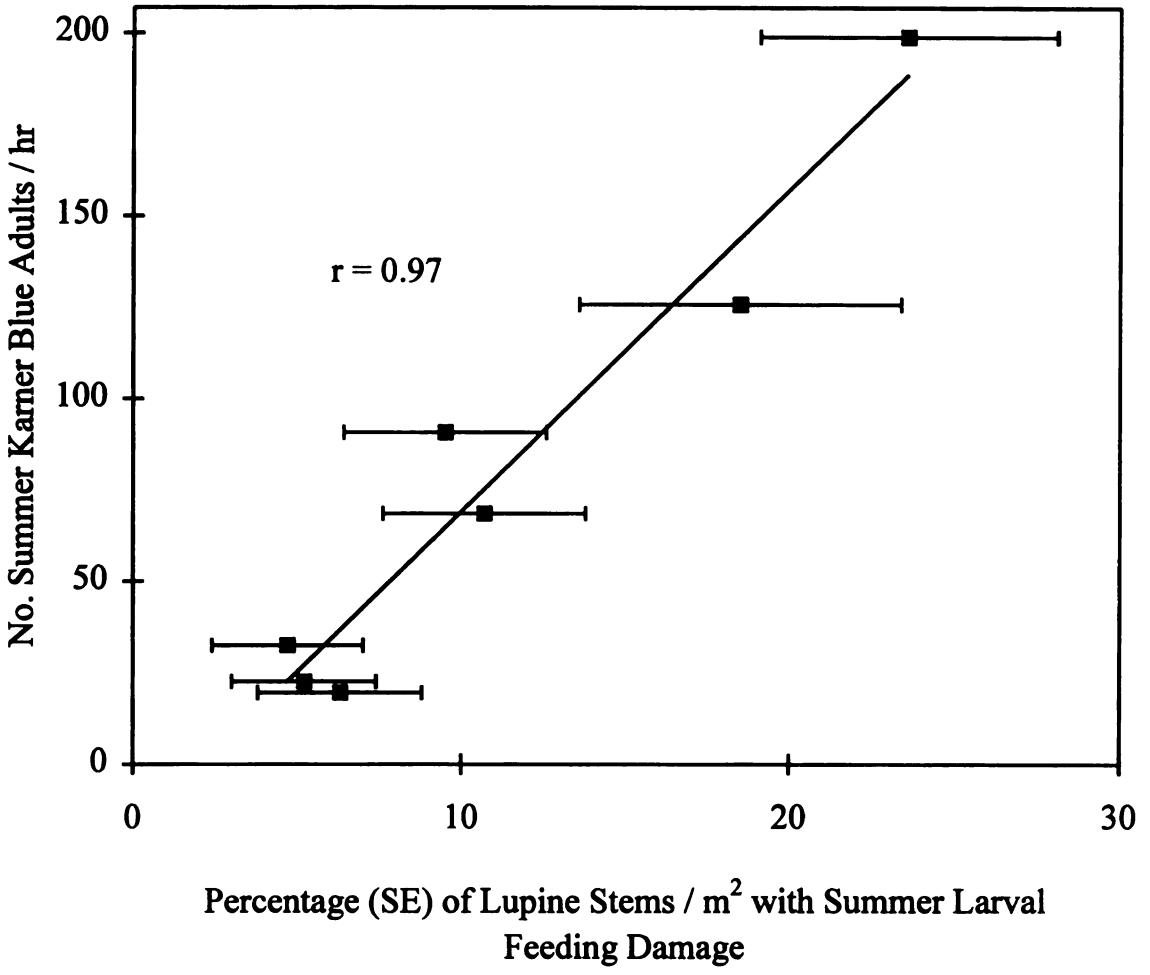


Figure 3.6. Scatterplot of 1994 summer Karner blue abundance versus percentage (SE) of lupine stems (per m^2) with summer larval feeding damage from feeding damage surveys in study sites.

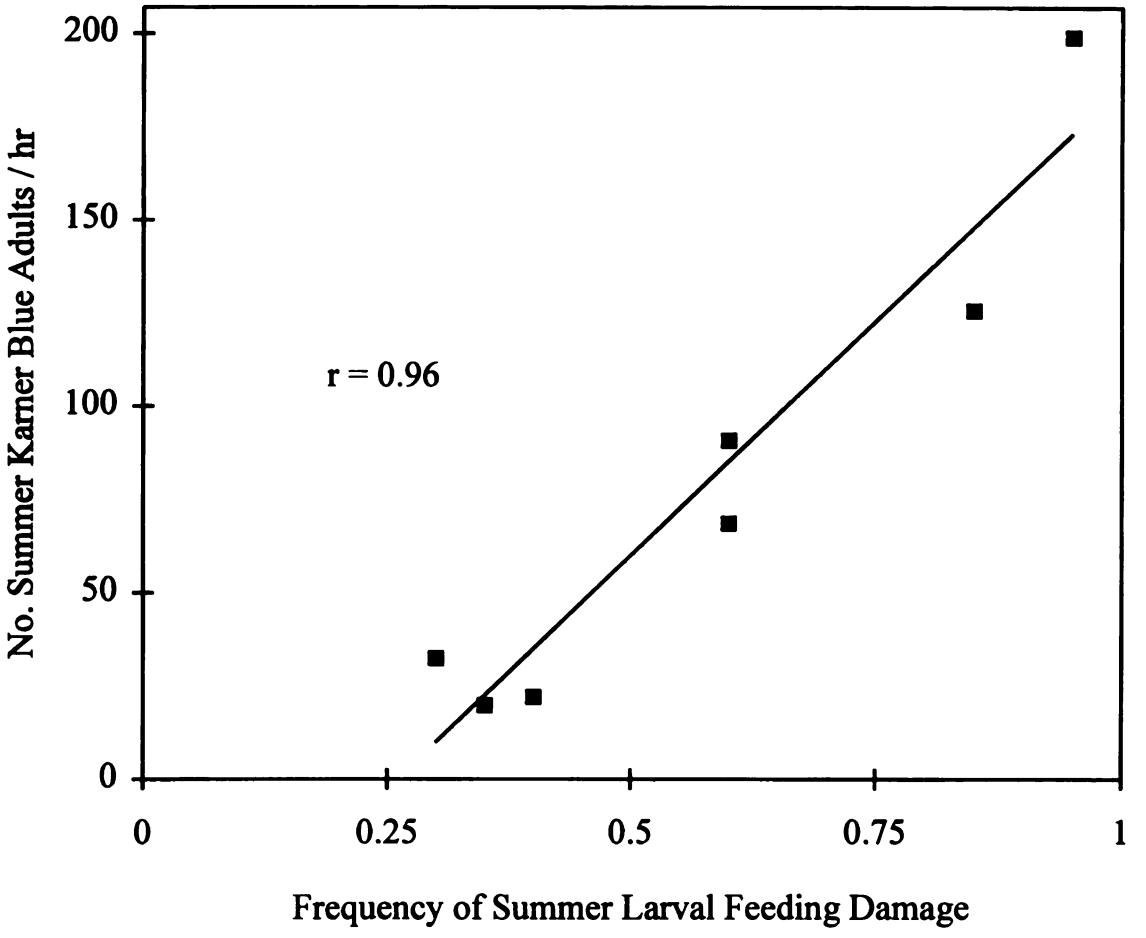


Figure 3.7. Scatterplot of 1994 summer Karner blue abundance versus frequency of summer larval feeding damage (proportion of quadrats with feeding damage) from surveys in study sites.

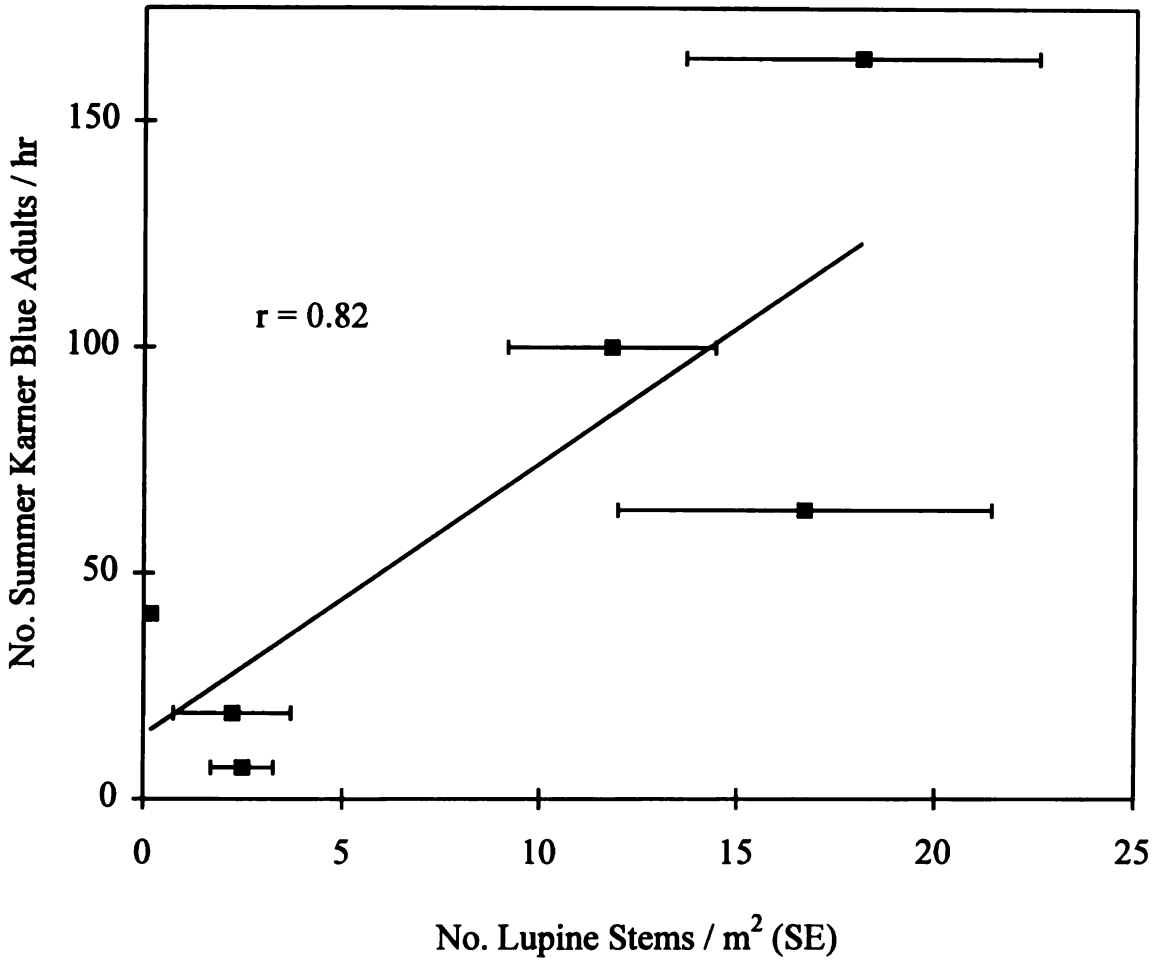


Figure 3.8. Scatterplot of 1993 summer Karner blue abundance versus 1993 lupine density estimates (SE) in study sites.

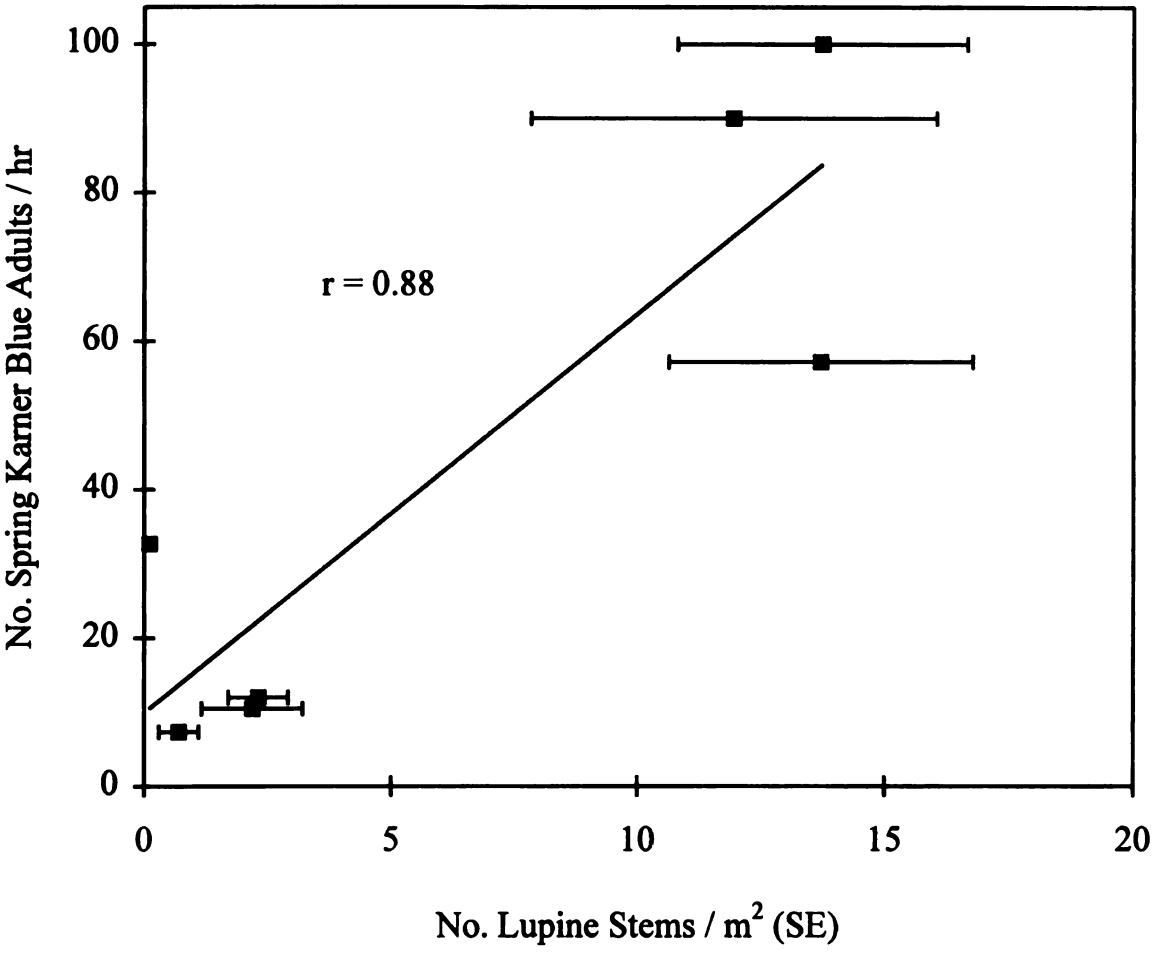


Figure 3.9. Scatterplot of 1994 spring Karner blue abundance versus 1994 lupine density estimates (SE) in study sites.

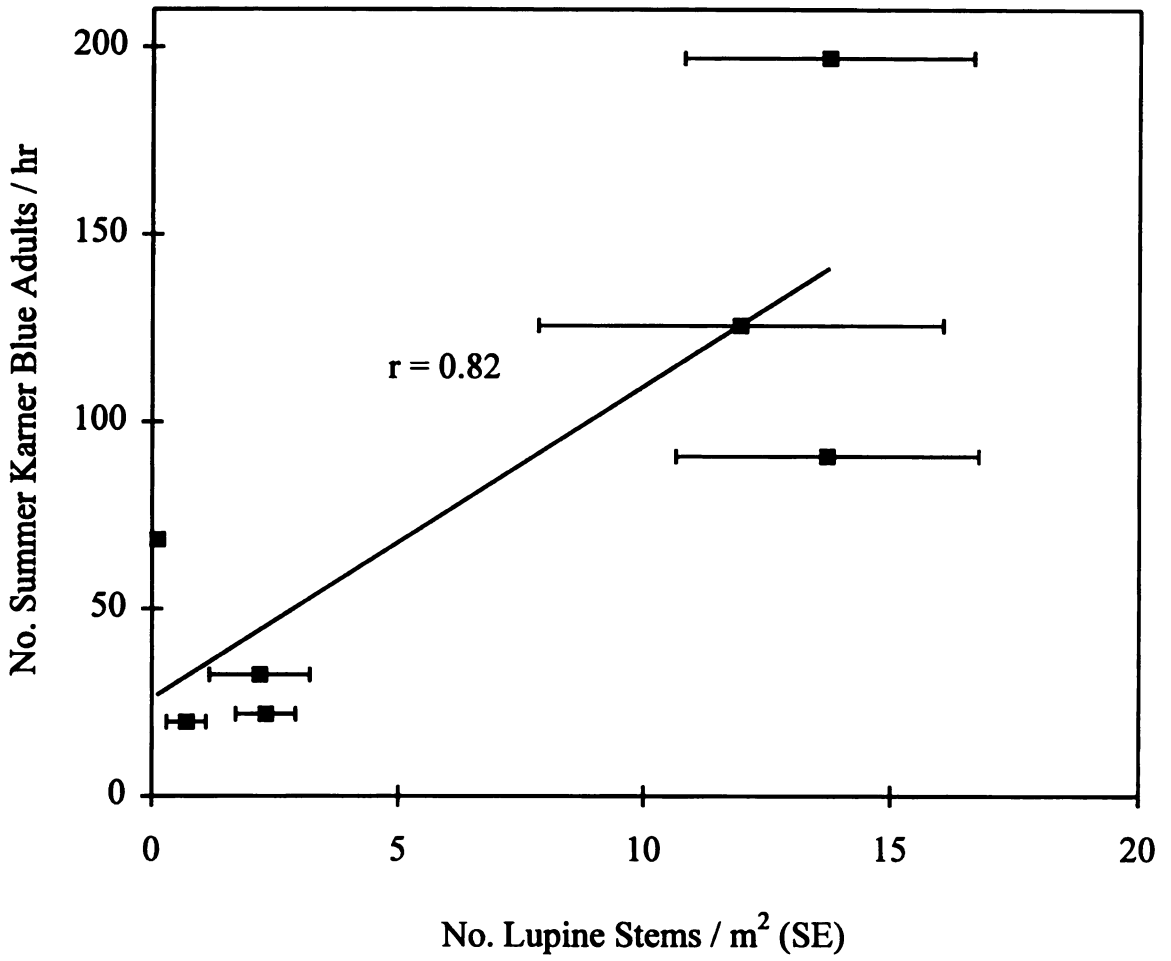


Figure 3.10. Scatterplot of 1994 summer Karner blue abundance versus 1994 lupine density estimates (SE) in study sites.

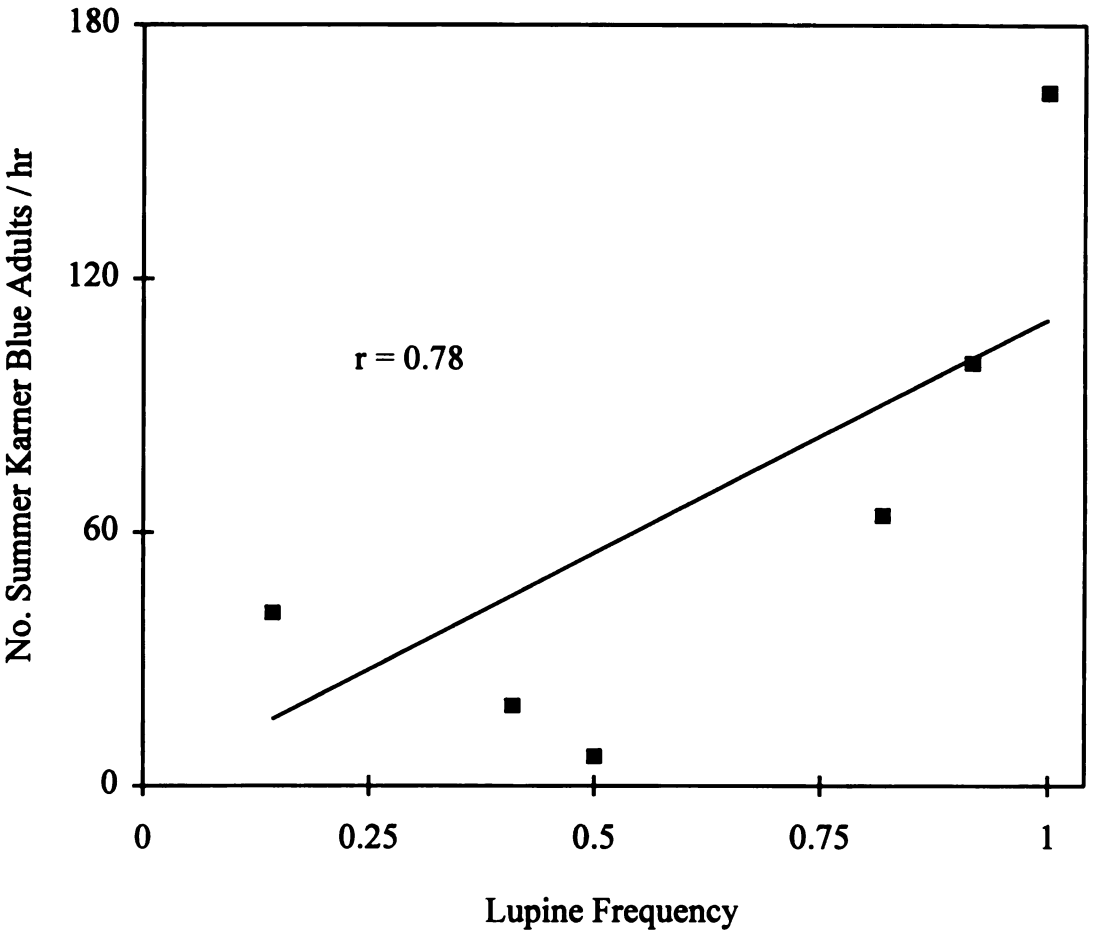


Figure 3.11. Scatterplot of 1993 summer Karner blue abundance versus 1993 lupine frequency (proportion of transects with lupine) from transect surveys in study sites.

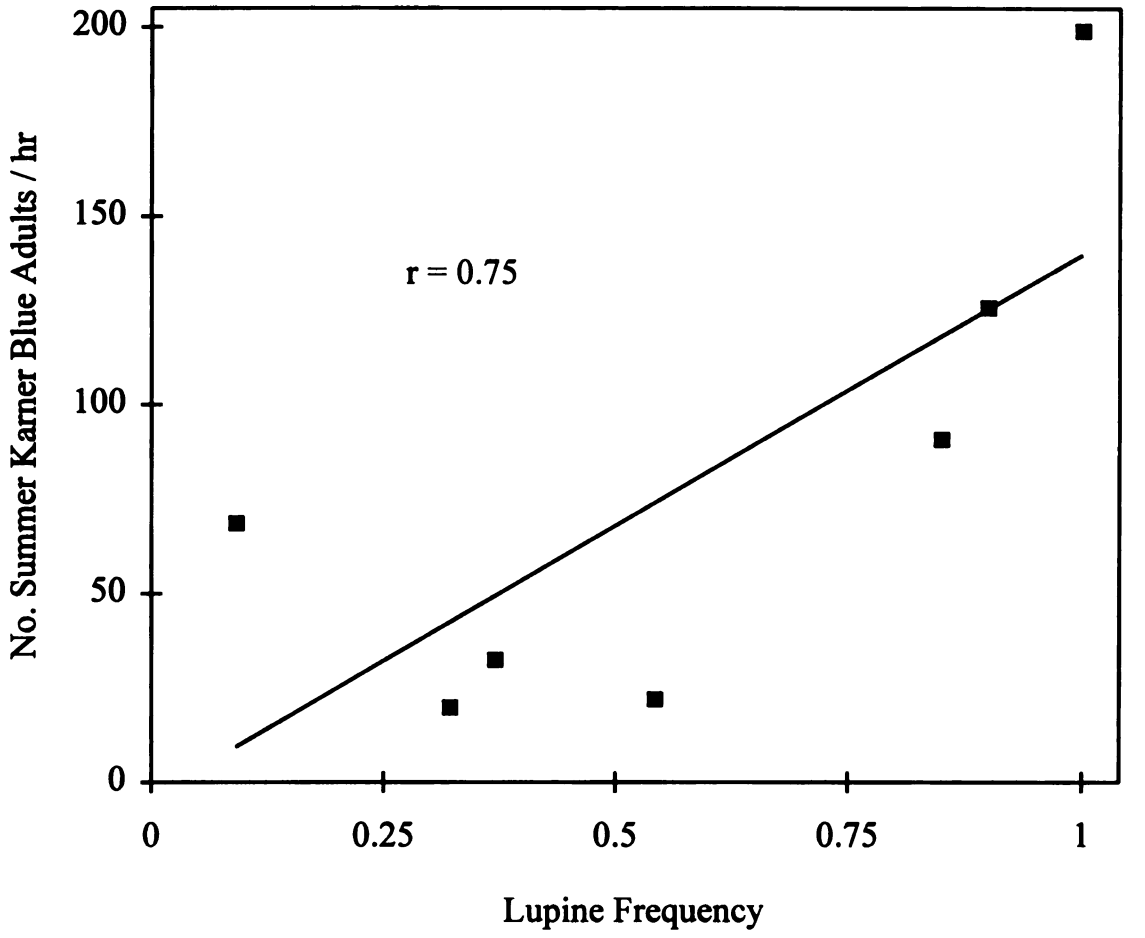


Figure 3.12. Scatterplot of 1994 summer Karner blue abundance versus 1994 lupine frequency (proportion of transects with lupine) from transect surveys in study sites.

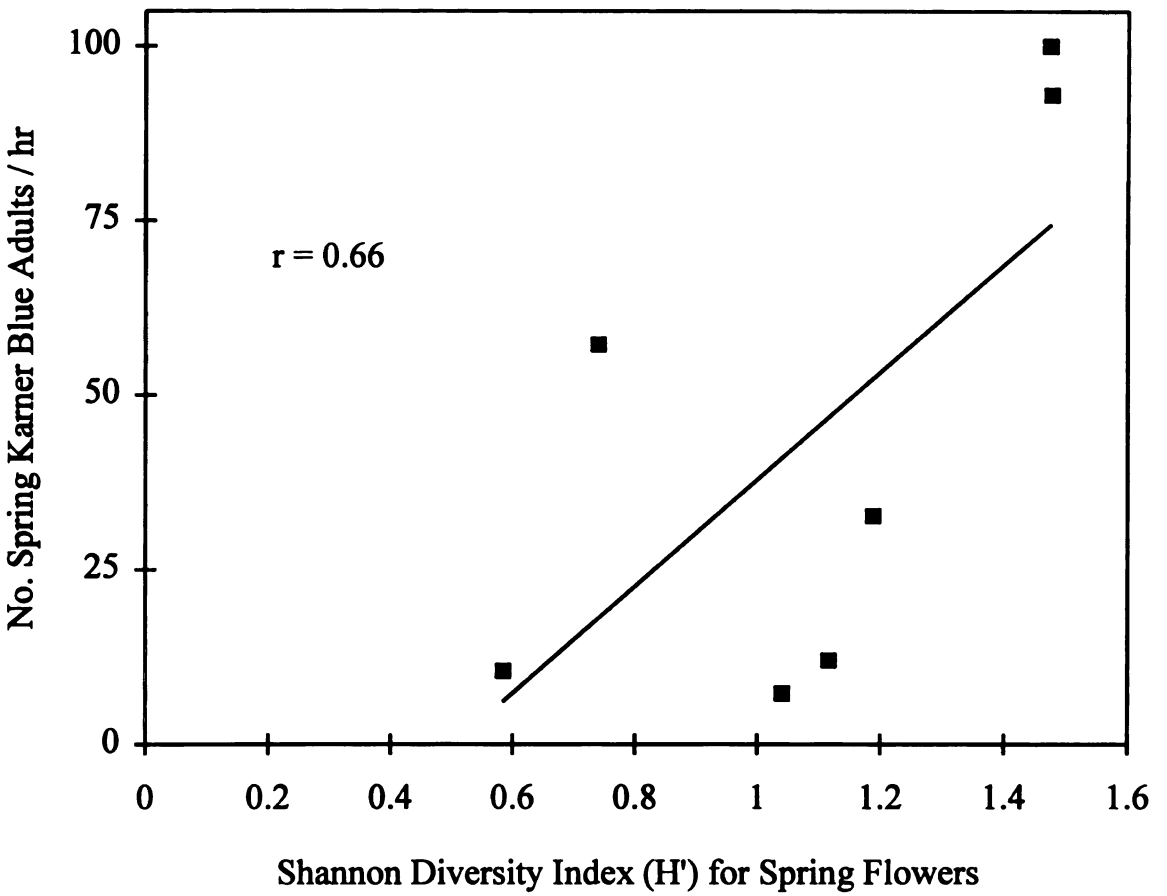


Figure 3.13. Scatterplot of 1994 spring Karner blue abundance versus Shannon diversity index (H') for 1994 spring flowering plants in study sites.

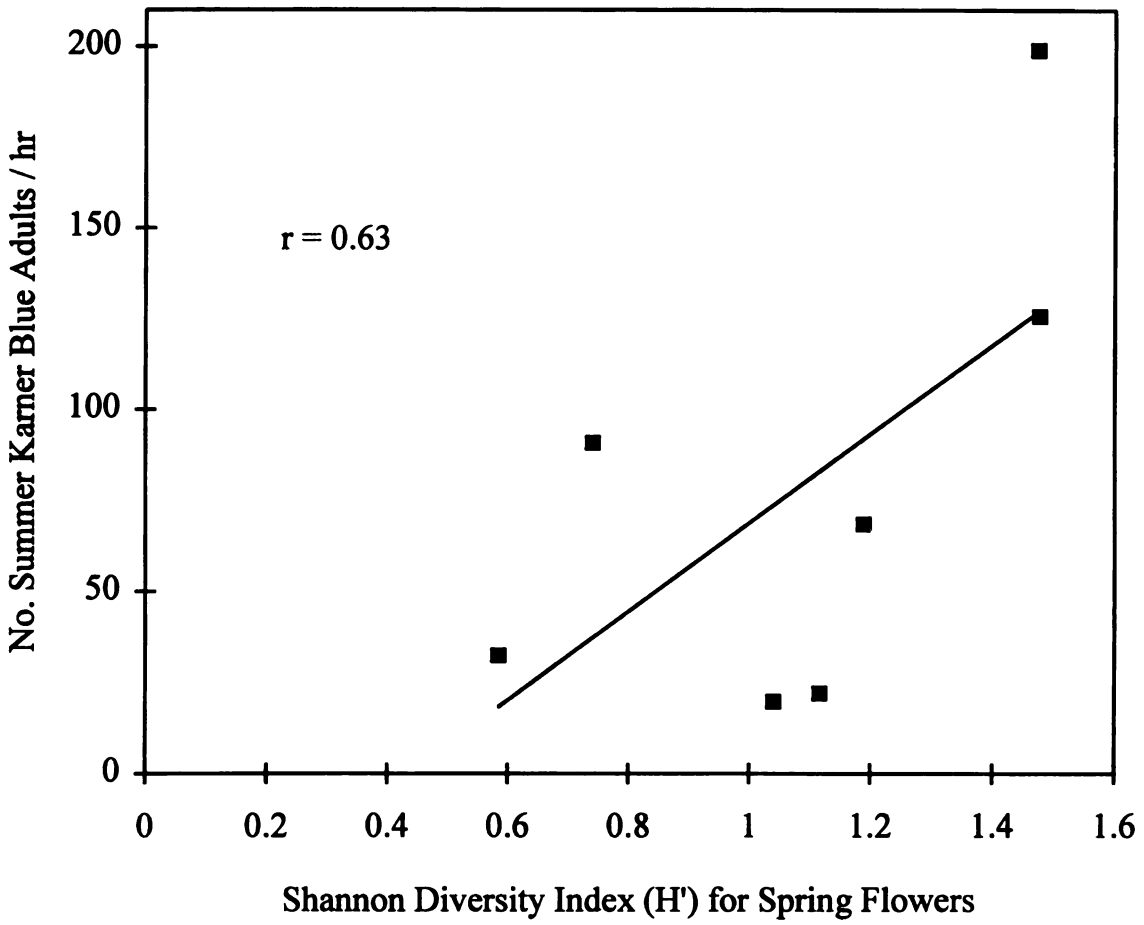


Figure 3.14. Scatterplot of 1994 summer Karner blue abundance versus Shannon diversity index (H') for 1994 spring flowering plants in study sites.

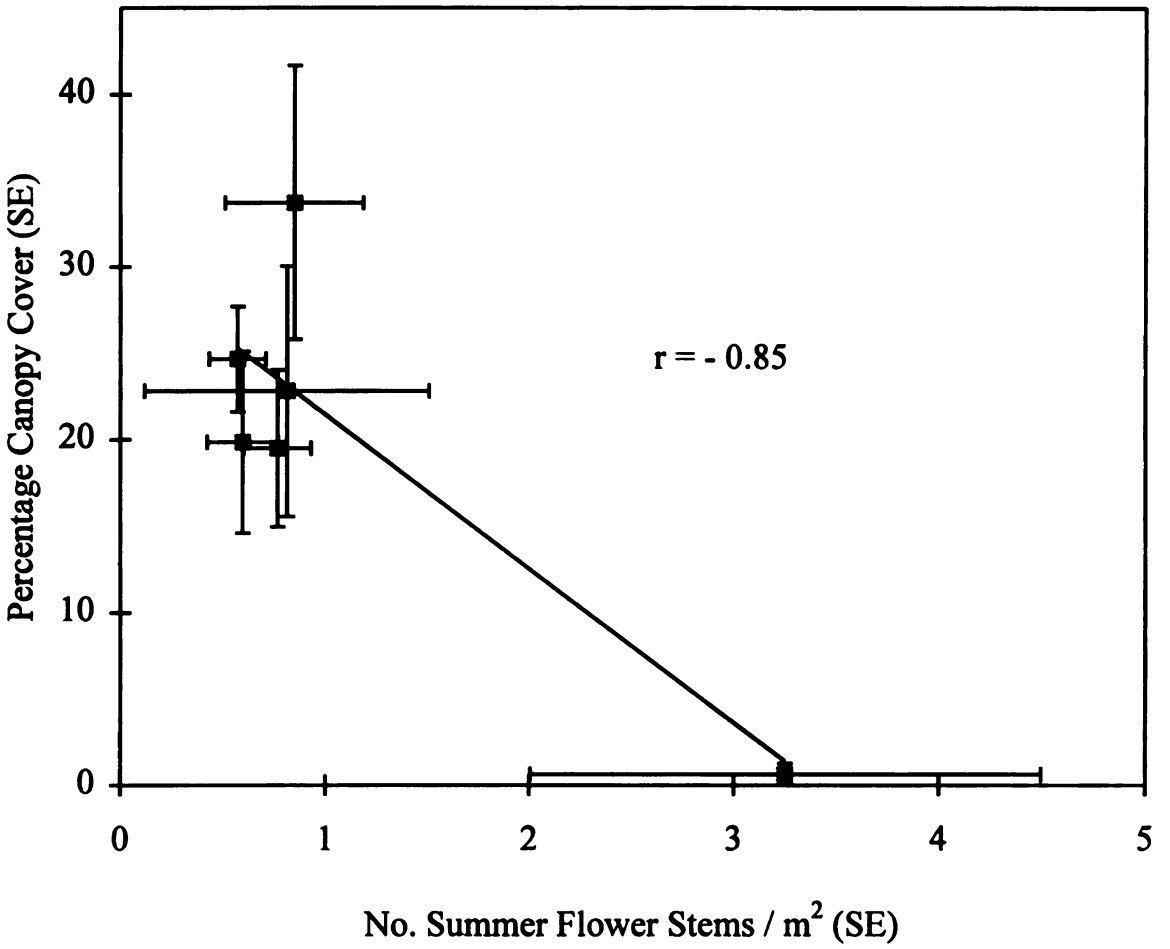


Figure 3.15. Scatterplot of percentage canopy cover (SE) versus 1993 summer flower density estimates (SE) in study sites.

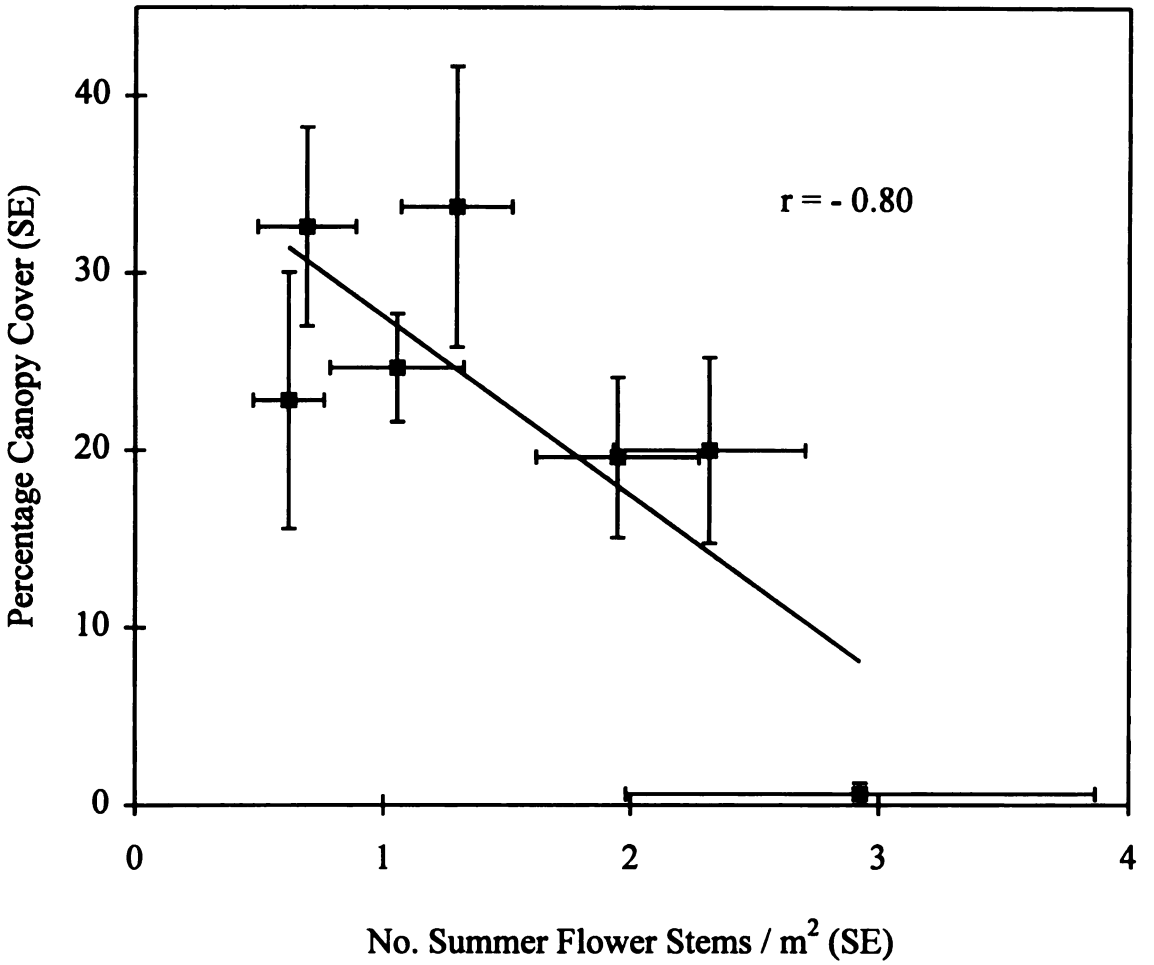


Figure 3.16. Scatterplot of percentage canopy cover (SE) versus 1994 summer flower density estimates (SE) in study sites.

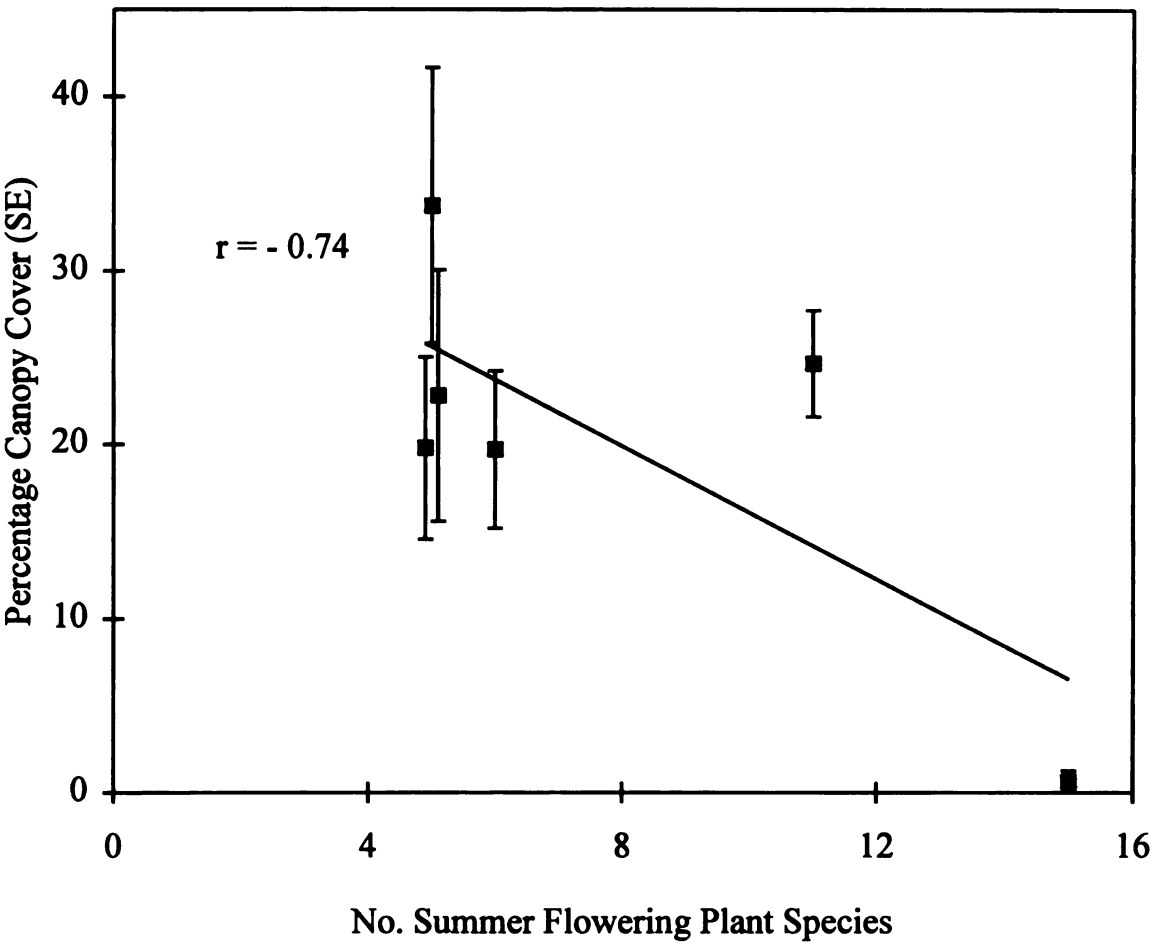


Figure 3.17. Scatterplot of percentage canopy cover (SE) versus the number of 1993 summer flowering plant species encountered in transect surveys in study sites.

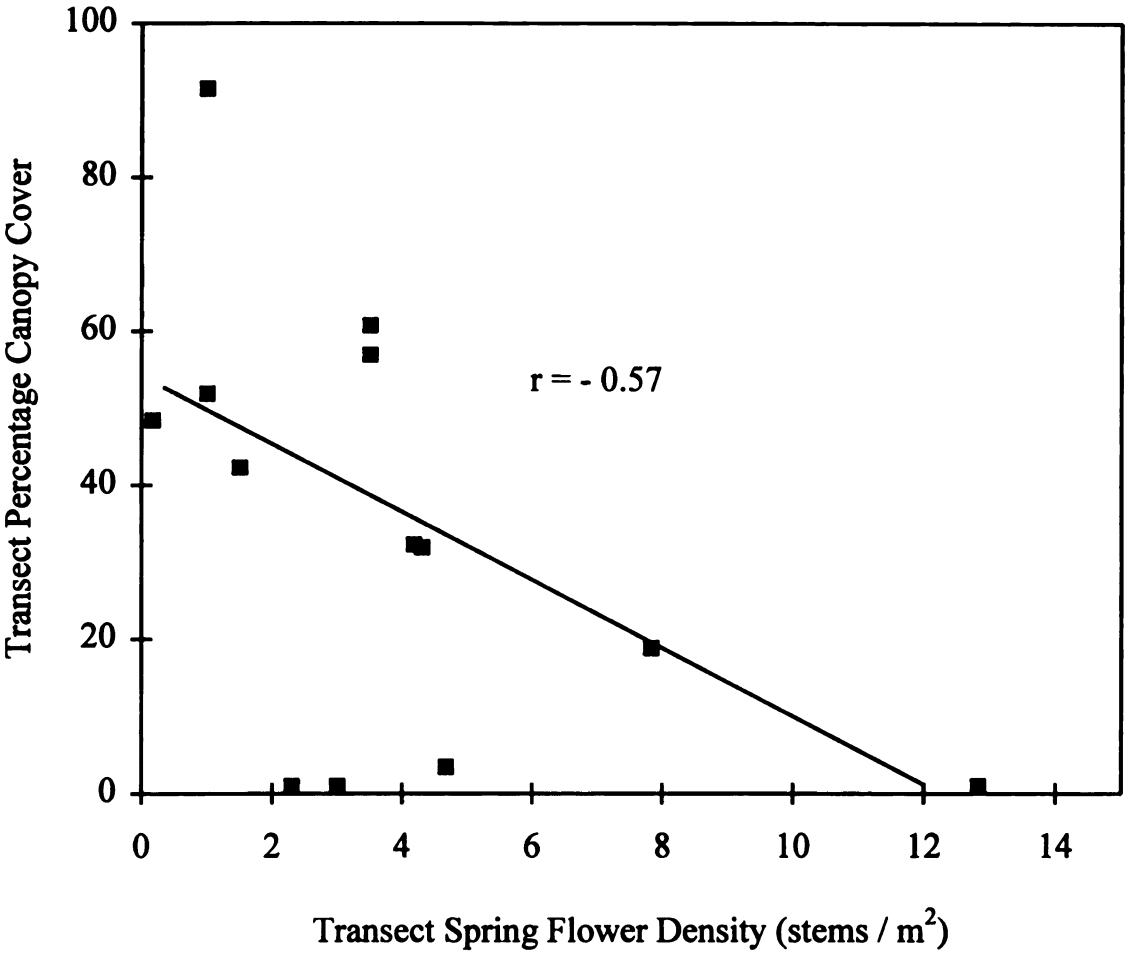


Figure 3.18. Scatterplot of 1994 transect estimates of percentage canopy cover versus 1994 transect estimates of spring flower density (stems / m^2) in the 'Jay' study site.

APPENDICES

APPENDIX 1

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1996-3

Title of thesis or dissertation (or other research projects):

The Endangered Karner Blue Butterfly (Lepidoptera: Lycaenidae) in Michigan: Habitat Suitability, Potential Impacts of Gypsy Moth (Lepidoptera: Lymantriidae) Suppression, and Laboratory Rearing.

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)
Catherine Papp Herms

Date April 25, 1996

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 2 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Lycaeides melissa samuelis Nabokov	MI: Allegan Co, Allegan State Game Area (ASGA) 6/1/94					5			MSU
	MI: Montcalm and Newaygo Co's, Huron-Manistee National Forest (HMNF) 6/9/94					5			MSU
Crematogaster lineolata (Say)	MI: Allegan Co, ASGA 5/13/93					2			MSU
	MI: Oceana Co, HMNF 5/13/94					2			MSU
Monomorium pharaonis (L.)	MI: Oceana Co, HMNF 5/20/93					1			MSU
Myrmica americana Weber	MI: Allegan Co, ASGA 6/29/93					2			MSU
Myrmica fracticornis Emery	MI: Allegan Co, ASGA 5/2/94					1			MSU
Dolichoderus mariae Forel	MI: Oceana Co, HMNF 5/13/94					1			MSU
Dolichoderus pustulatus Mayr	MI: Allegan Co, ASGA 6/22/93					2			MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Catherine Papp Herms

Date April 25, 1996

Voucher No. 1996-3

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date 4/25/96

APPENDIX 1.1

Voucher Specimen Data

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Tapinoma sessile (Say)	MI: Oceana Co, HMNF 5/20/93					1			MSU
	7/7/94					2			MSU
	MI: Allegan Co, ASGA 6/21/94					2			MSU
Formica neogatates Emery	MI: Allegan Co, ASGA 6/21/94					2			MSU
Formica obscuripes Forel	MI: Oceana Co, HMNF 5/3/94					3			MSU
Formica obscuriventris Mayr	MI: Allegan Co, ASGA 6/29/93					2			MSU
Formica schaufussi Mayr	MI: Allegan Co, ASGA 7/8/93					1			MSU
	6/21/94					2			MSU
Formica subsericea Say	MI: Allegan Co, ASGA 5/4/94					2			MSU
Lasius neoniger Emery	MI: Allegan Co, ASGA 6/21/94					1			MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Catherine Papp Herms

Date April 25, 1996

Voucher No. 1996-3

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

H. E. Evans for F. Ulke 4/25/96

Curator Date

APPENDIX 2

APPENDIX 2

1993 Federal Endangered and Threatened Species Subpermit

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Bishop Henry Whipple Federal Building
1 Federal Drive
Fort Snelling, MN 55111-4056

IN REPLY REFER TO:
FWS/AES-TE

AUTHORIZATION TO USE REGION 3 ENDANGERED AND THREATENED SPECIES PERMIT TO CARRY OUT THE FOLLOWING ACTIVITIES WITHIN THE STATE(S) OF Michigan

SUBPERMIT #93-23-I ISSUED June 2, 1993

INDIVIDUALS COVERED BY THIS SUBPERMIT:

Catherine M. Papp, Deborah G. McCullough, Thomas Ellis, and two student employees; all under the supervision of Catherine Papp.

SPECIES COVERED BY THIS SUBPERMIT:

Karner blue butterfly (*Lycaeides melissa samuelis*)

In accordance with Federal Endangered Species Permit PRT-697830, you are authorized to conduct the following take activities on the above species for scientific research, enhancement of propagation, or enhancement of survival through September 30, 1993. Any activity related to Federally listed threatened or endangered species that is not specifically permitted in this document is prohibited.

The activities allowed under this subpermit, and the conditions under which those activities must be conducted, are as follows:

1. Authorized activities to be conducted at Allegan State Game Area, Allegan County, MI, and Huron-Manistee National Forest, Manistee Unit, Newaygo and Oceana Counties, MI.

2. No specimens may be collected or removed from the wild for laboratory studies.

3. Conduct census' to determine Karner blue butterfly habitat and the population density and diversity of attending ants.

4. Census' are to be conducted at the same time of the day for all days that a census is conducted.

5. Injuries and/or mortalities may not exceed five specimens. In the event that this number is met, all permitted activities must cease. You must contact the U.S. Fish and Wildlife Service (Service) within 48 hours explaining the circumstances in writing to the following: U.S. Fish and Wildlife Service, 1 Federal Drive, Fort Snelling, MN 55111 (Attn: Carlita Shumate), and U.S. Fish & Wildlife Service, East Lansing

Field Office, 302 Manly Miles Bldg., 1405 South Harrison Road, East Lansing, MI 48823 (Attn: Susan Walker); telefax (517) 337-6899.

6. Any specimens that are killed are to be preserved according to standard museum practices, properly labeled (date, complete scientific and common name, and location where obtained), and submitted to Ms. Carlita Shumate of this office at the address above.

A copy of PRT-697830 is attached and the conditions of that permit must be adhered to. This subpermit and PRT-697830 must be in your possession while conducting authorized activities. You are reminded that necessary state and/or local permits, if applicable, must also be acquired and adhered to; this subpermit is invalid without such permits.

All specimens obtained under this subpermit remain the property of the United States Government and must be clearly identified as such.

Reporting Requirements

A full report of activities conducted under the authority of this subpermit, as well as copies of all data obtained from those activities, are due in this office by close of business 01/31/94, and to Ms. Susan Walker of the Service's East Lansing Field Office. In addition, copies of all reports and publications resulting from those data must be submitted to this office as they become available. The report required for this permit must include the following:

1. A complete discussion of field procedures, data collection methods, results, and conclusions.

2. The dates data are collected, a description of weather conditions for each day of collection, and the location (state, county, section, township, and range) of collection sites.

3. For each date data are collected, the report must specifically provide the time of day, the location and size of each sampling quadrat or plot; the number of Karner blue butterfly eggs, larvae and adults observed in each quadrat or plot; a description of any larval and adult behavior that is observed; the number, location, scientific, and common name of vascular vegetation within each plot; a description of distribution, percentage cover, and phenology of nectar and lupine plants; the percentage cover of canopy, litter, and soil layers (e.g., percentage sandy substrate) in each quadrat or plot where Karner blue butterfly eggs, larvae and adults are observed; the number of larvae observed on each lupine plant; and the scientific name of ants tending Karner blue butterfly larvae.

4. A complete description of injuries and/or mortalities to Karner blue butterflies, the dates they occurred, any circumstances surrounding the incidents, and describe steps that will be taken to reduce the likelihood of such situations from occurring in the future.

Failure to furnish any reports that are required by this permit is cause for permit revocation and/or denial of future permit applications.

All correspondence related to this subpermit should reference subpermit 93-23-I. Any questions you may have regarding this subpermit should be directed to the Region 3 Chief, Division of Endangered Species, at (612) 725-3276.

Chief, Division of Endangered Species

Attachment

cc: FWS Ft. Snelling, MN (LE)
ES Field Office TE Coordinator for E. Lansing, MI
DNR/DOC Endangered Species Coordinator for Michigan
FWS RD-5, ES Regional Office TE, Chief

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Bishop Henry Whipple Federal Building
1 Federal Drive
Fort Snelling, MN 55111-4056

IN REPLY REFER TO:
FWS/AES-TE

June 2, 1993

Miss Catherine Papp
Department of Entomology
Michigan State University
East Lansing, Michigan 48824

Dear Miss Papp:

Enclosed is your subpermit, 93-23-I, which authorizes research activities that will be carried out on Karner blue butterflies (*Lycaeides melissa samuelis*). The permit authorizes you and the individuals you included in your application to conduct most of the activities requested in your April 9, 1993, permit application. Please provide my office with the names, statement of qualifications, and resumes of the two student employees you plan to hire for this proposal within ten working days of their hire.

I have not granted authorization for you to collect and lethally take adult butterflies from the wild for the *Bacillus thuringiensis* (*Bt*) susceptibility studies for several reasons. First, my office will not authorize lethal take of this endangered species for research purposes while alternatives exist. My staff and I are being particularly cautious this year because of the large number of individuals who want to work with Karner blue butterflies, the fact that the studies are likely to affect the Karner blue butterfly throughout their range in the midwestern states, and the potential for mortality associated with some of those studies (particularly prescribed burns to restore habitat).

For the moment, I recommend conducting your study using closely-related, Lycaenidae that are not endangered species (for example, the melissa blue, *Plebjus melissa*, or tailed blue, *Everes comyntas*, butterflies). Depending on the results of your research with one of these species, you could consider resubmitting an application for a permit to conduct *Bt* susceptibility studies on the Karner blue butterfly.

Any questions regarding your application or this subpermit may be directed to me at (612) 725-3276 or to Ms. Carlita Shumate of my staff at the same telephone number.

Sincerely,

Craig Johnson
Chief, Division of
Endangered Species

Enclosure

cc: ES Field Office TE Coordinator for E. Lansing, MI
DNR/DOC Endangered Species Coordinator for Michigan
FWS RD-5, ES Regional Office TE, Chief

APPENDIX 3

APPENDIX 3

1994 Federal Endangered and Threatened Species Subpermit

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Bishop Henry Whipple Federal Building
1 Federal Drive
Fort Snelling, MN 55111-4056

IN REPLY REFER TO:
FWS/AES-TE

AUTHORIZATION TO USE REGION 3 ENDANGERED AND THREATENED SPECIES PERMIT TO CARRY OUT THE FOLLOWING ACTIVITIES WITHIN THE STATE OF Michigan

SUBPERMIT #94-23-R

ISSUED May 23, 1994
EXPIRES December 31, 1994

INDIVIDUALS COVERED BY THIS SUBPERMIT:

Catherine Papp, Deborah McCullough, Robert Haack, Leah Bauer, and one student employee; all under the supervision of Catherine Papp.

SPECIES COVERED BY THIS SUBPERMIT:

Karner blue butterfly (*Lycaeides melissa samuelis*)

In accordance with Federal Endangered Species Permit PRT-697830, you are authorized to conduct the following take activities on the above species for scientific research, enhancement of propagation, or enhancement of survival through December 31, 1994. Any activity related to federally listed threatened or endangered species that is not specifically permitted in this document is prohibited.

The activities allowed under this subpermit, and the conditions under which those activities must be conducted, are as follows:

1. Collect no more than 20 female Karner blue butterflies during the spring generation (late May through early June) to study the effects of *Bacillus thuringiensis* var. *kurstaki* (Btk) on this species. Collection is limited by the following conditions:

A. Collect no more than ten butterflies from the following locations within the Allegan State Game Area (ASGA), Allegan County, with between three and five individuals collected per site: 1. T2NR15W, Section 14 NE4NE4; 2. T2NR15W Section 2 NW4SE4; 3. T2NR14W Section 28 E2NE4; or, 4. T2NR15W Section 13 NW4NW4.

B. Collect no more than ten butterflies from the following locations within the Manistee National Forest (MNF), Montcalm, Newaygo, and Oceana Counties, with between three and five individuals collected per site: 1. T13NR16W Section 26 NW4; 2. T12NR12W Section 35 SE4; 3. T15NR12W Section 6 SW4SW4; 4. T12NR10W Section 21 NW4; or, 5. T13NR10W Section 32 NE4SE4.

C. Collect adult female butterflies using a butterfly net and transfer to small screen cages, then place in coolers and immediately transport to Michigan State University laboratory facilities at the Pesticide Research Center. Butterflies will be kept there in an environmental chamber which regulates temperature, light and humidity.

D. House captive adult butterflies in the laboratory for a period of two to five days to permit ovipositioning on cultivated wild lupine (*Lupine perennis*) and then immediately return butterflies to the place of capture.

E. House eggs and larvae in an environmental chamber which regulates day/night temperatures, dark/light cycles and humidity and rear larvae according to the protocol developed by C.P. Lane (copy on file).

2. Conduct Btk bioassay of Karner Blue butterfly larvae according to the study protocol presented in the permit application (on file), as outlined below:

A. Not more than 180 larvae will subject to the bioassay study. Two larval stages of Karner blue butterfly (second and fourth instars) will be subjected to three treatments. Thirty larvae of each stage will be subjected to each of the following treatments:
1) control consisting of untreated lupine which has been subjected to the same handling procedures as the treated lupine,
2) lupine which has been sprayed with the either the carrier of Foray 48B® or autoclaved Foray 48B®, and
3) lupine treated with Foray 48B®.

B. Keep larvae on treated or untreated lupine foliage in petri dishes for five to seven days, and check daily for lethal effects.

C. Transfer larvae surviving the treatments to fresh petri dishes with fresh lupine and monitor daily through pupation and adult emergence.

D. Return all Karner blue butterfly adults which survive the Btk bioassay to the sites of parental capture within two days of emergence.

E. Rear additional larvae produced but not used in the bioassay according to the protocol developed by C. N. Lane and release the adults within two days of emergence. Progeny must be released at the sites of original parental capture.

3. Conduct walk-through survey and monitoring activities to assess habitat characteristics of the oak savannah habitat that includes determination of Karner blue butterfly larvae presence, measuring of larvae, observation of attending ants, determination of wing wear and sex ratio of adult butterflies, and wild lupine (*Lupinus perennis*)

phenology as described under section J, Objective 1 (A)-(C), through Objective 3 of the March 14, 1994, permit application. The survey and monitoring activities shall be conducted in a manner to minimize disturbance to the Karner blue butterfly and wild lupine. Handling of adults and larvae shall be kept to a minimum.

4. Census' are to be conducted at the same time of day for all days that a census is conducted.

5. Injuries and/or mortalities may not exceed five specimens. In the event that this number is met, all permitted activities must cease. You must contact the U. S. Fish and Wildlife Service (Service) within 48 hours explaining the circumstances in writing to the following: U. S. Fish and Wildlife Service, 1 Federal Drive, Fort Snelling, MN 55111 (Attn: Carlita Shumate), U.S. Fish and Wildlife Service, Ecological Services Field Office, 302 Manly Miles Building, 1405 South Harrison Road, East Lansing, MI 48823 (Attn: Charles Wooley, Field Supervisor); telefax: 517/337-6899.

6. Any observed intact specimens of the Karner blue butterfly accidentally killed or freshly dead shall be preserved according to standard museum practices, and properly identified and indexed [include date, complete scientific and common names, and location (include township, range, and section)]. The specimens shall be sent a public scientific museum in the State of Michigan. All specimens obtained under this subpermit remain the property of the United States Government and must clearly be identified as such. A list of specimens collected (if any) and pertinent location data shall be provided to the Service's Regional Office, Division of Endangered Species and to the East Lansing, Michigan, Field Office by December 31, 1994.

A copy of Federal Endangered Species Permit PRT-697830 is attached; you are required to adhere to the conditions of that permit. *This subpermit, Permit PRT-697830, and your permit application (signed March 14, 1994) must be in your possession while conducting authorized activities.* Be advised that necessary state and/or local permits must also be acquired and adhered to; this subpermit is invalid without such permits.

Reporting Requirements

A full report of activities conducted under the authority of this subpermit, as well as copies of all data obtained from those activities, and year-end report are due in this office by the close of business on December 31, 1994, and to the Service's East Lansing Field Office (Attn: Field Supervisor). In addition, copies of all reports and publications resulting from those data must be submitted to these offices as they become available. Failure to furnish any reports that are required by this permit is cause for permit revocation and/or denial of future permit applications. The 1994 year-end report must include the following:

1. A complete discussion of field procedures, data collection methods, results, and conclusions.

2. Legible photocopies of all field data sheets or complete summaries of all field data sheets as described under the conditions of this subpermit as well as the following analyses of studies done:

a) An assessment of the relationship between Karner blue butterfly density and lupine density.

b) An assessment of the relationship between Karner blue butterfly density and nectar source density.

c) An assessment of the relationships between Karner blue butterfly and lupine density with percentage of canopy cover.

d) An analysis of the extent and importance of ant-tending to Karner blue butterfly larvae.

e) Estimates of the population size of Karner blue butterflies at each study site.

f) A determination of whether the period when spring generation Karner Blue butterfly larvae are present coincides with the period of aerial application of Btk for gypsy moth suppression in Michigan.

g) An assessment of the susceptibility of Karner Blue butterfly larvae to Btk, at the rate and of the formulation used in gypsy moth suppression, in a controlled laboratory setting.

3. A complete description of injuries and/or mortalities to Karner blue butterflies, the dates they occurred, and any circumstances surrounding the incidents. In addition, steps should be identified to reduce the likelihood of such injuries and/or mortalities occurring in the future.

4. The ultimate disposition of injured or dead butterflies (i.e., retained, returned to location of encounter, forwarded to a state or Federal agency or educational institution, etc.). If a specimen was retained, your report must identify the location where it is being stored and the reason it is being retained. A list of specimens collected (if any) and pertinent location data shall be provided also.

All correspondence related to this subpermit should reference subpermit 94-23-R. Any questions you may have regarding this subpermit should be directed to the Region 3 Chief, Division of Endangered Species, at 612/725-3276.

/s/ John A. Blankenship

John A. Blankenship
Assistant Regional Director
Ecological Services

Attachment

SEND TO: U.S.Fish and Wildlife Service
Division of Endangered Species
1 Federal Drive
Ft. Snelling, MN 55111
Call: 612/725-3276

RECEIVED
FISH & WILDLIFE SERVICE
TE

OMB NO. 43-R1670

DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE

FEDERAL FISH AND WILDLIFE LICENSE/PERMIT APPLICATION

1. APPLICATION FOR (Indicate only one)
☐ IMPORT OR EXPORT LICENSE ☒ PERMIT

2. BRIEF DESCRIPTION OF ACTIVITY FOR WHICH REQUESTED LICENSE OR PERMIT IS NEEDED.
1. Habitat and population field studies of the endangered Karner blue butterfly (Lycaeides melissa samuelis).
2. Collection of adult female Karner blue for rearing and Bacillus thuringiensis var. kurstaki susceptibility studies.

3. APPLICANT. (Name, complete address and phone number of individual, business, agency, or institution for which permit is requested)
Catherine M. Papp
Department of Entomology
Michigan State University
East Lansing, MI 48824
(517) 355-4662

4. IF "APPLICANT" IS AN INDIVIDUAL, COMPLETE THE FOLLOWING:
☐ MR. ☐ MRS. ☒ MISS ☐ MS.
HEIGHT: 5'6" WEIGHT: 125 lbs.
DATE OF BIRTH: 05/05/67 COLOR HAIR: Br COLOR EYES: Br
PHONE NUMBER WHERE EMPLOYED: (517) 336-3494 SOCIAL SECURITY NUMBER: 381-92-3680
OCCUPATION: Master of Science Student
ANY BUSINESS, AGENCY, OR INSTITUTIONAL AFFILIATION HAVING TO DO WITH THE WILDLIFE TO BE COVERED BY THIS LICENSE/PERMIT: N/A

5. IF "APPLICANT" IS A BUSINESS, CORPORATION, PUBLIC AGENCY, OR INSTITUTION, COMPLETE THE FOLLOWING:
EXPLAIN TYPE OR KIND OF BUSINESS, AGENCY, OR INSTITUTION: N/A
NAME, TITLE, AND PHONE NUMBER OF PRESIDENT, PRINCIPAL OFFICER, DIRECTOR, ETC.
IF "APPLICANT" IS A CORPORATION, INDICATE STATE IN WHICH INCORPORATED

6. LOCATION WHERE PROPOSED ACTIVITY IS TO BE CONDUCTED
Allegan State Game Area
(Allegan County, MI)

Manistee National Forest
(Montcalm, Newaygo, and Oceana Counties, MI)

7. DO YOU HOLD ANY CURRENTLY VALID FEDERAL FISH AND WILDLIFE LICENSE OR PERMIT? ☐ YES ☒ NO
(If yes, list license or permit numbers)

8. IF REQUIRED BY ANY STATE OR FOREIGN GOVERNMENT, DO YOU HAVE THEIR APPROVAL TO CONDUCT THE ACTIVITY YOU PROPOSE? ☒ YES ☐ NO
(If yes, list jurisdictions and type of documents)
State of Michigan Endangered Species Permit, Wildlife Div., Department of Natural Resources

9. [redacted] CHECK OR MONEY ORDER (If applicable) PAYABLE TO THE U.S. FISH AND WILDLIFE SERVICE ENCLOSED IN AMOUNT OF: N/A

10. DESIRED EFFECTIVE DATE: 05/01/94

11. DURATION NEEDED: 09/31/94

12. ATTACHMENTS. THE SPECIFIC INFORMATION REQUIRED FOR THE TYPE OF LICENSE/PERMIT REQUESTED (See 50 CFR 13.12(a)) MUST BE ATTACHED. IT CONSTITUTES AN INTEGRAL PART OF THIS APPLICATION. LIST SECTIONS OF 50 CFR UNDER WHICH ATTACHMENTS ARE PROVIDED.
N/A

CERTIFICATION

I HEREBY CERTIFY THAT I HAVE READ AND AM FAMILIAR WITH THE REGULATIONS CONTAINED IN TITLE 50, PART 13, OF THE CODE OF FEDERAL REGULATIONS AND THE OTHER APPLICABLE PARTS IN SUBCHAPTER B OF CHAPTER I OF TITLE 50, AND I FURTHER CERTIFY THAT THE INFORMATION SUBMITTED IN THIS APPLICATION FOR A LICENSE/PERMIT IS COMPLETE AND ACCURATE TO THE BEST OF MY KNOWLEDGE AND BELIEF. I UNDERSTAND THAT ANY FALSE STATEMENT HEREIN MAY SUBJECT ME TO THE CRIMINAL PENALTIES OF 18 U.S.C. 1001.

SIGNATURE (In ink): Catherine M. Papp
DATE: 3/14/94

3-200
10/78

*Applicant listed in 3 above or principal officer listed in 5 above must sign.

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Bishop Henry Whipple Federal Building
1 Federal Drive
Fort Snelling, MN 55111-4056

IN REPLY REFER TO:
FWS/AES-TE

May 24, 1994

Miss Catherine M. Papp
Department of Entomology
Michigan State University
East Lansing, Michigan 48824

Dear Miss Papp:

Enclosed is your subpermit, 94-23-R, which authorizes research activities that will be carried out on Karner blue butterflies (*Lycaeides melissa samuelis*). Please provide my office with the name, statement of qualifications, and resume of the student employee you plan to hire for this proposal within 10 working days of hiring.

Any questions regarding your application or this subpermit may be directed to Mr. Robert Adair, Chief, Division of Endangered Species, or Ms. Carlita Shumate of his staff, at 612/725-3276.

Sincerely,

John A. Blankenship
Assistant Regional Director
Ecological Services

Enclosure

cc (w/enclosure):
FWS ES Field Office TE Coordinator, East Lansing, MI
Endangered Species Coordinator, Michigan DNR
Chief, ES/TE, FWS Region 5

APPENDIX 4

APPENDIX 4

1993 and 1994 State of Michigan Threatened / Endangered Species Permits

STATE OF MICHIGAN
DEPARTMENT OF NATURAL RESOURCES
WILDLIFE DIVISION
P.O. BOX 30028
LANSING, MICHIGAN 48909

Permit Number: 1446

Date Issued: May 20, 1994

THREATENED/ENDANGERED SPECIES PERMIT

BY THE AUTHORITY OF 1974 PA 203 AND THE RULES AND REGULATIONS ESTABLISHED THEREUNDER, PERMISSION IS HEREBY GRANTED TO:

Ms. Catherine M. Papp
Department of Entomology
243 Natural Science
Michigan State University
East Lansing, Michigan 48824

To conduct the scientific activities listed under special conditions on the threatened/endangered species listed below. All activities are subject to the standard permit conditions on the back of this permit.

This Permit shall be valid only on the following lands/locations:

Allegan State Game Area, Huron-Manistee National Forest.

SPECIES: *Lycaeides melissa samuelis*, Karner blue butterfly

SPECIAL CONDITIONS:

Permitted is the entry into known karner blue sites to conduct research into habitat features of oak savanna affecting butterfly distribution and population size.

Permitted is field research including the collection of up to 20 adult females and the establishment of a laboratory colony for research into their succeptibility to Bt in relation to the effcts of the Michigan MDA gypsy moth control program on non-target species.

Collect a maximum of 3-5 butterflies from each location to reduce the impact to the local population. If possible, release surplus eggs or larvae from the laboratory colony back into the areas where they were collected.

Provide the Endangered Species Coordinator a copy of the results of the research including the techniques needed to raise larvae under laboratory conditions.

UNLESS REVOKED SOONER, THIS PERMIT EXPIRES ON 12/31/94

THE DIRECTOR OF NATURAL RESOURCES

BY: Thomas F. Weise
ENDANGERED SPECIES COORDINATOR

Revised 4/92

APPENDIX 5

APPENDIX 5

1993 and 1994 State of Michigan Use Permits

STATE OF MICHIGAN
DEPARTMENT OF NATURAL RESOURCES

USE PERMIT

Issued under authority of Act 17, P.A. 1921, as amended.

PERMIT NUMBER	BLDG. INVENTORY NUMBER
A-20-94	N/A
ISSUING LOCATION (Forest, Game Area, Etc.)	
Allegan State Game Area	

Subject To The Provisions Of The Law And The Conditions Herein Contained Permission Is Hereby Granted To The Person Named To Use State-Owned Land Described For The Purpose Indicated.

PERMITTEE (Name and Address)	SOCIAL SECURITY NUMBER
Cathy Pap MSU, Dept of Entomology E. Lansing, MI 48824	(517) 336-3494

TOTAL CHARGE FOR USE OF LAND	PAY IN INSTALLMENTS OF (per)	PERMIT PERIOD
N/C	beginning N/A .19__	from May 10, 1994 .19__ to Dec 31 .1994

DESCRIPTION OF STATE-OWNED LAND

Locations on Allegan State Game Area as detailed on the attached map

AUTHORIZED LAND USE

To conduct studies on Karner blue butterflies and their habitat.

SPECIAL CONDITIONS AND/OR PENALTIES NOT CITED BELOW.

Permittee responsible to maintain Federal and State Endangered Species Permits, as applicable.

ADDRESS OF AUTHORIZED DEPARTMENT REPRESENTATIVES TO CONTACT RELATIVE TO OPERATIONS UNDER THIS PERMIT. PAY ANY INSTALLMENTS HERE.

LOCATION	NAME and STREET	CITY		ZIP CODE	PHONE NUMBER
Allegan State Game Area,	4590-48th Ave	Allegan	MI	49010	673-2430

THIS PERMIT IS SUBJECT TO THE FOLLOWING CONDITIONS AND REQUIREMENTS:

Here and after the Department of Natural Resources shall be referred to as DNR.

(1) Unless sooner terminated this permit shall expire on date indicated above.

(2) ~~Payment in the amount specified above shall be made prior to use of land indicated above or in installments as indicated above.~~

(3) Permittee shall maintain the area under permit in a clean and sightly condition.

(4) Requests for permit renewals should be made to the Department Representative thirty days prior to the expiration date of this permit. Such requests will be considered only when all stipulations in the original permit have been complied with.

(5) The rights accruing under this permit shall not be assigned or transferred without the written consent of the Department Representative.

(6) Permittee shall not commit, cause, or allow to be committed any waste of, or injury to, said premises or any part thereof, nor use the same except for the purpose indicated.

(7) Temporary improvements necessary for the efficient utilization of the said premises may be made as indicated.

(8) Improvements made by the permittee on said premises and not removed within 30 days after cancellation or expiration of this permit, and when such removal shall be requested by the Department Representative shall become attached and remain a part of the premises.

(9) The DNR reserves the right:
- (a) to dispose of any portion of the premises herein described during the term of this permit. If possible, proper notice of sale or disposition will be given permittee. However, failure to notify permittee will not affect this right.
- (b) to lease said premises for exploration and production of any or all minerals, including coal, gas, oil, sand, gravel, etc.
- (c) to grant rights-of-way and easements of any kind and nature over and across said premises, and to grant or exercise all other rights and privileges of every kind and nature not herein specifically granted.

(10) LIABILITY. Permittee hereby releases, waives, discharges and covenants not to sue, the State of Michigan, its departments, officers, employees and agents, from any and all liability to Permittee, its officers, employees and agents, for all losses, injury, death or damage, and any claims or demands therefore, on account of injury to person or property, or resulting in death of Permittee, its officers, employees or agents, whether caused by the State of Michigan, its departments, officers, employees or agents.

(11) INDEMNIFICATION. Permittee hereby covenants and agrees to indemnify and save harmless, the State of Michigan, its departments, officers, employees and agents, from any and all claims and demands, for all loss, injury, death or damage, that any person or entity may have or make, in any manner, arising out of any occurrence related to (1) this permit; (2) the activities authorized by this permit; and (3) the use or occupancy of the premises which are the subject of this permit, as well as any other state-owned lands. This indemnification and save harmless agreement shall extend to all loss, injury, death or damage, proximately caused or arising out of the negligence of the State of Michigan, its departments, officers, employees and agents.

(12) ~~Permittee and occupants are responsible for the payment of all utility bills including water, electricity, gas, etc.~~

(13) Permittee agrees to comply with all requirements herein, and, if for any reason permittee violates or neglects to fulfill such requirements, this permit shall terminate and permittee shall forfeit all rights and payments made hereunder. Should permittee remain in possession of said premise after cancellation or expiration of this permit, said permittee shall be considered as tenant or tenants holding over without permission and may be evicted from said premises.

I HAVE READ THE CONDITIONS GOVERNING THIS PERMIT AND AGREE TO ABIDE BY THEM IN THE CONDUCT OF MY OPERATIONS UNDER THIS PERMIT.

Catherine Pap — 5/11/94
PERMITTEE SIGNATURE — DATE

[signature] — Wildlife Biologist — 5/11/94
DEPARTMENT REPRESENTATIVE — TITLE — DATE

PR 1...

APPENDIX 6

APPENDIX 6

Tables of Flowering Plant Species Observed in Study Sites but Not Occuring Transect Surveys

Table A6.1. Plant species observed in flower in Allegan State Game Area study sites during the Karner blue spring flight period, but not encountered in transect surveys, 1993 and 1994.

Flowering plant species[1]		Year observed 1993	1994
Achillea millefolium	Yarrow		√
Antennaria plantaginifolia	Pussytoes	√	√
Apocynum androsaemifolium	Dogbane		√
Arabis sp.	Rock Cress		√
Claytonia virginica	Spring Beauty		√
Convolvulus spithameus	Erect Bindweed		√
Coreopsis lanceolata	Lance-leaved Coreopsis	√	√
Elaeagnus umbellata	Autumn Olive		√
Pedicularis canadensis	Wood Betony		√
Penstemon hirsutus	Hairy Beardtongue	√	
Saponaria officinalis	Bouncing Bet		√
Senecia aureus	Golden Ragwort		√
Tradescantia ohiensis	Spiderwort	√	√
Tragopogon dubius	Yellow Goatsbeard	√	
Vaccinium angustifolium	Blueberry		√
Viola pedata	Birdfoot Violet		√
Viola spp.	other Violet spp.		√
Vicia cracca	Cow Vetch	√	

[1] Some plant species listed here were not observed in every site.

Table A6.2. Plant species observed in flower in Allegan State Game Area study sites during the Karner blue summer flight period, but not encountered in transect surveys, 1993 and 1994.

Flowering plant species[1]		Year observed 1993	1994
Achillea millefolium	Yarrow	√	√
Anemone virginiana	Thimbleweed		√
Apocynum androsaemifolium	Dogbane		√
Aureolaria pedicularia	False Foxglove		√
Campanula rotundifolia	Harebell		√
Ceanothus americanus	New Jersey Tea	√	√
Daucus carota	Queen Anne's Lace		√
Desmodium rotundifolium	Prostrate Tick-trefoil		√
Desmodium spp.	Tick-trefoils		√
Dianthus armeria	Deptford Pink	√	
Gnaphalium obtusifolium	Sweet Everlasting		√
Helianthemum canadense	Frostweed	√	√
Helianthus divaricatus	Woodland Sunflower		√
Helianthus occidentalis	Western Sunflower	√	√
Hieracium aurantiacum	Orange Hawkweed	√	
Lespedeza hirta	Hairy Bush-clover	√	√
Lespedeza intermedia	Wandlike Bush-clover		√
Lespedeza violacea	Bush-clover		√
Lithospermum canescens	Hoary Puccoon	√	√
Lotus corniculatus	Birdsfoot Trefoil		√
Medicago sativa	Alfalfa	√	√
Oenothera biennis	Evening Primrose		√
Opuntia humifusa	Prickily Pear	√	√
Penstemon hirsutus	Hairy Beardtongue		√
Potentilla recta	Rough-fruited Cinquefoil	√	√
Rosa carolina	Pasture Rose	√	
Rudbeckia hirta	Black-eyed Susan		√
Solanum dulcamara	Bittersweet Nightshade	√	
Solidago juncea	Early Goldenrod	√	√
Solidago nemoralis	Gray Goldenrod	√	√

Table A6.2 (cont'd)

Solidago speciosa	Showy Goldenrod	√	√
Specularia perfoliata	Venus Looking-glass		√
Tephrosia virginiana	Goat's Rue	√	
Tradescantia ohiensis	Spiderwort	√	
Tragopogon dubius	Yellow Goatsbeard		√
Verbascum thaspus	Common Mullein		√
Vicia cracca	Cow Vetch	√	

[1] Some plant species listed here were not observed in every site.

APPENDIX 7

APPENDIX 7

Tables of Nectaring Observations of Summer Generation Karner Blue Adults

Table A7.1. Total numbers of nectaring observations per survey week (# male, # female) of summer generation Karner blue adults on individual flower species in Allegan State Game Area study sites, 1993.

Flowering plant species		No. nectaring observations of Karner blue (# male, # female) by survey week			
		13-15 July	20-22 July	28-29 July	3-4 Aug
Achillea millefolium	Yarrow	1 (1, 0)			
Asclepias tuberosa	Butterfly Weed	28 (18, 10)	9 (57, 42)	27 (19, 18)	12 (8, 4)
Asclepias verticillata	Whorled Milkweed		12 (9, 3)	3 (3, 0)	1 (0, 1)
Centaurea maculosa	Spotted Knapweed		12 (8, 4)	31 (16, 15)	21 (5, 16)
Coreopsis lanceolata	Lance-leaved Coreopsis	1 (1, 0)	1 (1, 0)		
Dianthus armeria	Deptford Pink		1 (1, 0)		
Erigeron annuus	Daisy Fleabane	1 (1, 0)	1 (1, 0)		
Euphorbia corollata	Flowering Spurge		9 (8, 1)	13 (9, 4)	3 (1, 2)
Helianthus divaricatus	Woodland Sunflower		1 (0, 1)	5 (3, 2)	15 (7, 8)
Helianthus occidentalis	Western Sunflower				5 (1, 4)
Hieracium pillosella	Mouse-ear Hawkweed	4 (3, 1)	2 (1, 1)		
Hieracium spp.	Yellow Hawkweeds		4 (2, 2)		
Hypericum perforatum	St. Johnswort		2 (1, 1)	1 (1, 0)	1 (0, 1)
Liatris cylindracea	Cylindric Blazing-star		2 (1, 1)	4 (1, 0)	1 (1, 0)
Lotus corniculatus	Birdsfoot Trefoil	1 (1, 0)	1 (0, 1)		
Monarda punctata	Horsemint	4 (4, 0)	49 (20, 29)	21 (10, 11)	12 (3, 9)
Polygala polygama	Racemed Milkwort	1 (1, 0)	2 (1, 1)		
Rudbeckia hirta	Black-eyed Susan		6 (5, 1)	3 (1, 2)	
Tradescantia ohiensis	Spiderwort	1 (1, 0)			

Table A7.3. Total numbers of nectaring observations per survey week (# male, # female, # unknown) of summer generation Ke

Table A7.2. Total numbers of nectaring observations per survey week (# male, # female, # unknown) of summer generation Karner blue adults on individual flower species in Allegan State Game Area study sites, 1994.

Flowering plant species		No. nectaring observations of Karner blue (# male, # female, # unknown) by survey week			
		6 July	13 July	19 July	26 July - 3 Aug
Asclepias tuberosa	Butterfly Weed	39 (23, 16)	122 (52, 66, 4)	80 (31, 48, 1)	4 (1, 3)
Asclepias verticillata	Whorled Milkweed			4 (4, 0)	8 (3, 5)
Centaurea maculosa	Spotted Knapweed		4 (3, 1)	19 (12, 7)	19 (4, 15)
Coreopsis lanceolata	Lance-leaved Coreopsis	8 (7, 1)	45 (15, 23, 7)	34 (16, 17, 1)	2 (1, 1)
Erigeron annuus	Daisy Fleabane	2 (2, 0)	1 (1, 0)	2 (2, 0)	
Euphorbia corollata	Flowering Spurge	8 (8, 0)	6 (4, 2)	16 (13, 2, 1)	13 (4, 9)
Helianthus divaricatus	Woodland Sunflower				8 (0, 8)
Hieracium aurantiacum	Orange Hawkweed	1 (1, 0)			1 (0, 1)
Hieracium pillosella	Mouse-ear Hawkweed		1 (0, 1)	4 (3, 1)	2 (0, 2)
Hieracium spp.	Yellow Hawkweeds	5 (2, 3)	15 (12, 3)	3 (3, 0)	
Hypericum perforatum	St. Johnswort	2 (2, 0)	1 (1, 0)	2 (1, 1)	
Liatris cylindracea	Cylindric Blazing-star				1 (0, 1)
Lithospermum canescens	Hoary Puccoon		1 (1, 0)		
Lotus corniculatus	Birdsfoot Trefoil		2 (0, 2)		
Monarda punctata	Horsemint		2 (1, 1)	32 (5, 27)	24 (3, 21)
Polygala polygama	Racemed Milkwort		1 (1, 0)		
Rubus sp.	Dewberry	5 (3, 2)			
Rudbeckia hirta	Black-eyed Susan	1 (0, 1)	4 (2, 2)	4 (2, 2)	2 (0, 2)
Specularia perfoliata	Venus Looking-glass	1 (1, 0)			
Tephrosia virginiana	Goat's Rue	22 (12, 10)	29 (12, 17)	40 (22, 18)	
Vicia cracca	Cow Vetch	1 (1, 0)			

APPENDIX 8

APPENDIX 8

Table of Percentage Canopy Cover and Frequency by Tree Species

Table A8. Percentage canopy cover (P) (± SE) by tree species and frequency (F) of tree species from transect surveys in Allegan State Game Area study sites, 1994.

	Black oak		White oak		Black cherry		Sassafras		Autumn olive	
Study site	P (± SE)	F	P (± SE)	F	P (± SE)	F	P (± SE)	F	P (± SE)	F
48N89	10.9 (± 2.9)	0.52	6.7 (± 2.7)		2.11 (± 1.2)	0.22				
Horseshoe	0.2 (± 0.3)	0.09	0.4 (± 0.4)	0.09						
Jay	15.4 (± 5.9)	0.46	16.9 (± 5.3)	0.62	1.5 (± 1.2)	0.15				
Marsh	15.7 (± 2.4)	0.83	4.0 (± 1.9)	0.23	2.2 (± 1.3)	0.14	2.6 (± 1.0)	0.29	0.1 (± 0.1)	0.03
Park	18.9 (± 4.7)	0.57	10.2 (± 3.6)	0.32	2.8 (± 1.4)	0.14	0.7 (± 0.4)	0.11		
Pipe	6.5 (± 6.8)	0.10	12.1 (± 5.1)	0.60	4.2 (± 3.6)	0.20				
Square	14.7 (± 3.5)	0.75	3.4 (± 0.6)	0.25	1.65 (± 0.9)	0.20				

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