THE CONSEQUENCES OF A MANAGEMENT STRATEGY FOR THE ENDANGERED KARNER BLUE BUTTERFLY

Bradley A. Pickens

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Committee:
Karen V. Root, Advisor
Helen J. Michaels
Juan L. Bouzat

ABSTRACT

Karen V. Root, Advisor

The effects of management on threatened and endangered species are difficult to discern, and yet, are vitally important for implementing adaptive management. The federally endangered Karner blue butterfly (Karner blue), *Lycaeides melissa samuelis* inhabits oak savanna or pine barrens, is a specialist on its host-plant, wild blue lupine, *Lupinus perennis,* and has two broods per year. The Karner blue was reintroduced into the globally rare black oak/lupine savannas of Ohio, USA in 1998. Current management practices involve burning 1/3, mowing 1/3, and leaving 1/3 of the lupine stems unmanaged at each site. Prescribed burning generally kills any Karner blue eggs present, so a trade-off exists between burning to maintain the habitat and Karner blue mortality. The objective of my research was to quantify the effects of this management strategy on the Karner blue.

In the first part of my study, I examined several environmental factors, which influenced the nutritional quality (nitrogen and water content) of lupine to the Karner blue. My results showed management did not affect lupine nutrition for either brood. For the second brood, I found that vegetation density best predicted lupine nutritional quality, but canopy cover and aspect had an impact as well. Relatively lower host-plant nitrogen during the second brood was accompanied by a higher adult foraging rate, which suggests a trade-off of nutritional resources during these different life stages. For the second part of my study, I used surveys and behavior observations to quantify how the Karner blue responded to management treatments. Second brood females and males were more abundant in burned management units, and behavior observations revealed Karner blues avoided ovipositing in unmanaged management units. These management units were unburned for at least four years and were often characterized by a high

leaf litter depth (>3.5 cm). Recolonizations of Karner blues from source populations within 120 meters was rapid, and this suggests the rotation of management units is appropriate at this scale. Therefore, burning areas with a high leaf litter depth will cause minimal harm to the Karner blue population, and will likely benefit the many threatened and endangered species in this rare ecosystem.

I dedicate this study to my parents, Walt and Lorali, for always supporting me in all my endeavors and instilling in me the love of travel and exploration.

"I only went out for a walk and finally concluded to stay
out till sundown, for going out, I found, was really going in."
-John Muir

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Identifying Factors Affecting Nutrition for the Endangered Karner Blue Butterfly, *Lycaeides melissa samuelis*

ABSTRACT

The Karner blue butterfly (Karner blue), *Lycaeides melissa samuelis,* is a federally endangered species recently reintroduced into the globally rare black oak/lupine savannas of Ohio, USA. The Karner blue is bivoltine, and the larvae feed exclusively on the leaves of wild blue lupine, *Lupinus perennis*. Currently in Ohio, annual management of occupied Karner blue habitat invokes prescribed burning, mowing, and leaving areas unmanaged. In my research, I studied the environmental characteristics involved in predicting habitat quality for the Karner blue, in terms of nutritional quality of *L. perennis* leaves. I also quantified adult nutritional sources for each of the two Karner blue broods. Host-plant nitrogen is widely known to affect Lepidoptera larval growth rates, survival, and adult fecundity. Water content of host-plant leaves has also been found to affect Lepidoptera larvae growth rates. I measured host-plant nitrogen content for the first brood and combined water and nitrogen content to evaluate the nutritional quality of lupine for the second brood larval stage. My results for the first brood larval stage found no significant difference in nitrogen levels between burned, mowed, and unmanaged management treatments. In the water-limited second brood, I used Principle Components Analysis (PCA) to combine lupine leaf nitrogen and water content into a single variable to indicate the overall nutritional quality of lupine for Karner blue larvae. Second brood lupine nutritional quality was best explained by vegetation density, followed by canopy cover and aspect. When host-plant nitrogen was lower in the second brood larval stage, Karner blue adults compensated by spending significantly more time foraging. When evaluating or restoring habitat for this species, I recommend the consideration of second brood nectar sources, aspect, and vegetative structure of the community, including the use of historical accounts of species

composition. Management with the aim of restoring or maintaining a moderate level of native herbaceous species will positively affect the quality of lupine for Karner blues, and will likely conserve other species in this endangered community.

INTRODUCTION

The restoration of a natural community, including particular species within it, is difficult to accomplish when detailed knowledge of historical vegetation, ecology, and disturbances are not well documented, nor well represented. The interactions of a species and its environment may be critical to species persistence (Ebenman & Jonsson 2005; Ellison et al. 2005; Estes & Palmisano 1974), and yet, may not be well represented in isolated remnants or degraded communities. This is a fundamental problem in restoration ecology, since the first step of restoring a community or population is to identify historical conditions (Fiedler & Groom 2006).

Historically, butterflies have received considerable attention through collection and study. Additionally, butterflies have been identified as being useful indicator species for the state of the environment (Ehrlich 2003; Ockinger et al. 2006) and species richness (Fleishman et al. 2005); plus they can often be tied to ecological processes, such as disturbance, because many are early successional species. General ecological factors involved in habitat suitability for Lepidoptera include habitat area and isolation from conspecifics (Hanski et al. 1996; WallisDeVries 2004), local habitat quality (Bergman & Kindvall 2004; Lane & Andow 2003), and nectar sources (Schultz & Dlugosch 1999). Field studies of local habitat quality often correlate butterfly abundance with vegetation structure, which affects the host-plant's abundance and/or nutritional quality. Factors such as canopy cover (Bergman 2001; Lane & Andow 2003), prescribed burning (Schultz & Crone 1998), grazing or vegetation density (Anthes et al. 2003;

Ellis 2003; Moilanen & Hanski 1998; WallisDeVries 2004), and aspect (Fleishman et al. 1997) have been identified as individually influencing the local habitat quality for butterfly species.

Experimental lab studies have consistently pointed towards two mechanisms responsible for host-plant quality for butterflies. Host-plant nitrogen levels have been found to increase larvae growth rates (Mevi-Schutz et al. 2003; Tabashnik 1982), be correlated with larvae survival (Lincoln et al. 1982; Ravenscroft 1994), increase egg production (Boggs 2003), and increase the number of host-ants protecting larvae (Baylis & Pierce 1991; Billick et al. 2005). Host-plant water content appears to be similarly associated with positive relationships in larvae growth rates (Lincoln et al. 1982; Mevi-Schutz et al. 2003; Tabashnik 1982), and water is generally thought to aid in larval digestion of nutrients (Grundel et al. 1998a). On the other hand, recent lab studies have confirmed that adult Lepidoptera, which consume nectar amino acids, have an increased fecundity and lifespan compared to adults fed only water (Fischer & Fiedler 2001). Other studies have shown female butterflies prefer nectar with high amino acid content (Erhardt & Rusterholz 1998; Rusterholz & Erhardt 2000), but species tend to differ in their dependence on host-plant nitrogen and nectar nutrient intake for egg production (O'Brien et al. 2004). Overall, few studies have related host-plant nutrition (nitrogen and water content) with host-plants found in the field (but see Grundel et al. 1998a). To my knowledge, no studies have compared the importance of multiple environmental variables on the nutritional quality of host-plants.

In this study, I examined the ecology of the Karner blue butterfly (Karner blue), *Lycaeides melissa samuelis,* and its host-plant, wild blue lupine, *Lupinus perennis,* in the globally rare black oak/lupine savannas of northwest Ohio (NatureServe 2006). The Karner blue was listed as a federally endangered species in 1992 after being extirpated from five U.S. states

and Ontario, Canada (U.S. Fish and Wildlife Service 2003). The Karner blue was extirpated from Ohio by 1989 after extensive loss and degradation of Midwestern oak savanna. Nuzzo (1986) states that only 0.02% of the historical oak savanna remains in the Midwestern U.S., including the Oak Openings region in Ohio. Since 1998, Karner blues have been reintroduced into Ohio with cooperation from The Nature Conservancy, Toledo Zoo, Ohio DNR, Michigan DNR, U.S. Fish and Wildlife Service, and other organizations. However, it is unknown if extirpation in Ohio was caused by oak savanna loss or habitat degradation, so understanding management and restoration implications are critical to maintain and expand the Karner blue population in Ohio and throughout their range.

In a lab setting, Grundel et. al. (1998a) have already confirmed that the amount of feeding time Karner blue larvae need before pupation is significantly lowered on lupine with higher leaf nitrogen and water content, and this probably translates into less predation or parasitism in the field. Based on research findings from several studies (Grundel et al. 1998a, b; Lane & Andow 2003; Maxwell 1998), management for the Karner blue has focused primarily on creating a heterogeneous canopy cover in order to promote lupine with a high nutritional content during differing weather conditions. However, other environmental variables have not been tested and compared to the impact of canopy cover. The first objective of my study was to compare the influence of herbaceous vegetation structure, canopy cover, topography, and prescribed burning on the host-plant quality for the Karner blue. The second goal of my study was to examine changes in Karner blue foraging behavior and to quantify the nutritional content of lupine for the two Karner blue broods.

SPECIES OF INTEREST, STUDY AREA, AND MANAGEMENT

Lupinus perennis is a perennial plant, which primarily lives in partially shaded to open areas, nutrient poor soils, and thrives in disturbed areas (U.S. Fish and Wildlife Service 2003). Reproduction begins in May when the plants produce inflorescences of blue to purple flowers. Seed pods develop by July when they dry out and pop, spreading the seeds up to five meters from the plant (Grigore & Tramer 1996). At this time, lupine can easily become senescent, especially when water is limiting during hot, dry summer conditions. Lupine can also reproduce vegetatively, so it is often impossible to differentiate between lupine plants by stems alone (Grigore & Tramer 1996). The Karner blue butterfly is a bivoltine species, with adults living an average of 3.5 days (Knutson et al. 1999). First brood larvae emerge mid-April, first brood adults emerge mid-May, second brood larvae emerge 5-10 days after oviposition, and second brood Karner blues fly during July.

Karner blues currently occupy four sites in Ohio, and these are at The Nature Conservancy's Kitty Todd Preserve located in Lucas County, Ohio (41^0 37' N, $083^0$47' W). The Toledo Airport, 10 km from Kitty Todd Preserve, reports a total precipitation average of 840 mm per year, and mean temperature averages range from $-4.5C^0$ in January to $22\ C^0$ in July (NOAA 2006). Elevation ranges from 154-254 meters, and soils are generally well-drained and sandy. Oak savanna vegetation includes an herbaceous layer and a woodland canopy generally covering between 30-80% of the area. Dominant woody vegetation includes black oak (*Quercus velutina),* northern pin oak (*Quercus ellipsoidalis),* and white oak (*Quercus alba)*. Common herbaceous species include New Jersey tea (*Ceanothus americanus)*, wild blue lupine (*Lupinus perennis)*, western sunflower (*Helianthus occidentalis)*, rough blazing star (*Liatris aspera),* butterfly milkweed (*Asclepias tuberosa),* flowering spurge (*Euphorbia*

corollata), and wild yellow indigo (*Baptisia tinctoria*). Little bluestem (*Schizachyrium scoparium)* and big bluestem (*Andropogon gerardii)* are the primary grasses.

Each Karner blue site ranged from 0.39-2.15 ha in size, and tree canopy covered 56-61% of the area at three sites, and only 4% of the fourth site. Annually, a third of each occupied site's lupine stems are burned, mowed, or left unmanaged. In this study, mowed management units had been burned 1-2 years previously. Three unmanaged management units had been burned 4 years ago, and one management unit had not been burned in 7 years due to low fuel loads at this site (South Piels). In late November 2004, two sites were burned (South Piels, Julia's Savanna) and at the end of March 2005, the other two sites were burned (Bond, Oak Dune). In March, the appropriate areas were mowed to approximately 15.2 cm (6 inches) in height, and the clippings were left on the ground.

METHODS

In order to quantify the nutritional condition of lupine for Karner blues, I sampled the percent lupine leaf nitrogen for the first and second brood larval stages. Since water is generally abundant in the spring (first brood), I only sampled leaf water content for the second brood larval stage in June. Host-plant water deficits can lead to plant senescence and lower nutritional quality at this time. I also measured the foraging time of Karner blue adult females for both broods, and related this to the nitrogen content of lupine during each brood's larval stage.

I collected lupine leaf samples on May 10th and 11th, 2005. Based on previous Karner blue emergence dates and weather conditions, May 10th was estimated as when larvae were likely to be abundant, and, larvae and larvae feeding marks were visible on lupine at this time. Technical difficulties precluded me from taking all samples on May 10th, so one site, Julia's Savanna, was sampled on the 11th. All samples were taken between 11:30 and 18:00. Rain did

start to fall as the last samples were taken from Julia's Savanna on the 11th, but all other samples were taken during mild temperatures, 21-27^{0}C (70-80^0 F) and when weather was dry for two or more days before sampling.

Lupine tissue samples were chosen by generating random numbers in Microsoft Excel 2000 (Microsoft, Redmond, WA), which were used as indicators of compass degrees and the number of paces from the center of a management unit. In large, oddly shaped management units (e.g. Oak Dune, mowed unit), the unit was broken into several different points of origin from which random samples were taken. Four leaf samples were taken from each management unit at each of the 4 sites for a total of 48 samples. Ten grams of leaf material was needed for analysis of nitrogen content. To prevent lupine mortality, only lupine plants that covered >15% of a 1 m^2 quadrat were sampled. All leaf samples were thoroughly searched during and after sampling to ensure Karner blue larvae were not captured in a sample. The tallest leaves were taken evenly from the entire 1 m^2 area, and leaf petioles were not included. Samples were placed in paper bags and sent overnight to Brookside Laboratories, Inc. (located in New Knoxville, OH) for nitrogen analysis via an ashing method. The resulting data was the percent nitrogen of the leaves by weight.

On May 12th, vegetation surveys were performed specifically on these same nitrogen sampling points. With a 1 m^2 quadrat placed at the center of each sample location, we recorded the number of lupine stems, leaf litter, and aspect. Leaf litter was taken by measuring litter depth with a measuring stick marked in centimeters. Three samples were averaged in each quadrat by reading depth in centimeters from the highest point of any dead litter reached without manipulation. In northwest Ohio, oak trees still had only buds at this time, so there was no

canopy cover during this first brood larval stage sampling. Herbaceous vegetation was also minimal at this time.

During the second brood larval stage, I sampled using the same techniques and timing I used for first brood sampling, except I chose samples based on differing aspects and canopy cover as well as management unit. For each management unit, I chose north/northwest and south/southeast slopes and randomly placed samples along these slopes. Slopes ranged from 9-36 degrees. I also placed at least one sample on a flat area for each management unit, depending on how many samples with a slope were available. Nitrogen sampling took place on June 22nd.

From these sample locations, lupine leaves (excluding petioles) were also analyzed for leaf water content using methods similar to Grundel (Grundel et al. 1998a). Samples were taken on June 20th between 13:00 and 15:30. Field weight of lupine samples were between 0.5 and 2.9 grams of leaf matter, which represented approximately 3-6 leaves. Leaves were immediately placed in pre-weighed air-tight containers and held in a cooler until all samples were processed. Upon returning from the field, leaf samples were immediately weighed with their container, and put in a drying oven (Cenco, forced circulation incubator, 400 watts), at approximately 32-46^0 C (90-115^0 F) for 48 hours. Samples were then weighed again in their containers to obtain a dry weight, and I used the formula from Grundel (1998a) as follows:

1- (dry weight/field weight) = % leaf water content

A vegetation survey of these same sample locations occurred June 24-25 and included vegetation height, vegetation density, and canopy cover. Vegetation height and density were measured using a Robel pole (Robel 1970), and measurements were made to the nearest half decimeter. I assessed height of vegetation by measuring the tallest live vegetation within 1 meter of the pole. Vegetation density was measured by standing 3 meters away from the pole, looking

at the pole from chest height, and the lowest observable number was considered the density. Density measurements were taken from the East and West of the Robel pole, then averaged together. Canopy cover was estimated visually by a trained observer. Only one sampling point had greater than 75% canopy cover, so I classified samples as either open (0-15% cover) or shaded (16-100% cover). This is modified from Lane and Andow (Lane & Andow 2003), who used a third class between 76-100% cover.

Behavior Observations

On days without precipitation and a temperature above 17 C^0 (62.5^0 F) in the shade, I used modified Pollard-Yates transects (Pollard & Yates 1993; Thomas 1983) to survey Karner blues in each management unit during both broods. One to three trained observers performed Karner blue surveys by using a zig-zagging transect throughout all areas of lupine within a particular management unit. When a Karner blue female was observed, I stopped and performed a 15-minute observation of behavior. At the beginning of the second brood, 16 observations were performed for only 10 minutes since a larger population of butterflies was anticipated during the second brood. When numbers remained low, I switched back to 15-minute observation periods in order to be consistent with the first brood. These ten minute observations accounted for only 16 of 121 second brood observations. During behavior observations, I recorded "foraging" or "not foraging" at one-minute intervals. "Not foraging" time intervals occurred when butterflies oviposited or performed any other activity. If the Karner blue foraged at any time during the minute, the minute was counted as "foraging." This accounted for brief lapses in foraging behavior, since Karner blues often spend a few seconds in-between flower resources, making it unusual that foraging occurred at exactly the 1-minute interval.

Analysis

SAS 8.01 was used for all data analysis (SAS Institute 2000). First brood percent nitrogen and vegetation structure were analyzed using a factorial ANOVA with sites and management unit as main effects. No interactions were included with vegetation structure measurements, since only four samples per management unit were taken. When values were significant, a Tukey's test was used to compare differences. In order to analyze the nutritional quality of lupine for second brood Karner blue larvae, I used Principal Components Analysis to find the best Eigenvector (PCA1) between lupine leaf nitrogen and water content. Then I analyzed environmental factors influencing the nutritional quality of lupine for the second brood (PCA1) by using Akaike's Information Criteria, corrected for small sample sizes (AIC_c). This maximum log-likelihood method of model selection quantifies model uncertainty and can provide for the analysis of all possible interacting variables (Burnham & Anderson 2002). The lowest AIC_c value represents the best model, and all other models are considered relative to the best model. Akaike's weights give the probability that the individual model is the best model given the candidate model set (Burnham & Anderson 2002). Canopy cover (shaded or open), vegetation density, aspect (south or north/flat), and whether the management unit was burned, were included in candidate models. I combined north and flat aspects because I only had 12 flat samples compared to 24 south and 26 north samples. Additionally, flat and north samples had visibly similar values. Since previous literature (Grigore & Tramer 1996) found burned areas to be higher in lupine leaf nitrogen, I included burned in the model as 0 or 1. I included two interaction variables in the candidate model set when the individual variables were included. I eliminated vegetation height from the analysis because at the time of data collection most

vegetation heights reflected only a minor part of the vegetation structure (e.g. single grass strands). Additionally, I wanted to limit the variables in my AIC model selection.

In the comparison of first and second brood host-plant nitrogen levels, I eliminated all second brood samples with a south slope, since they were disproportionate to random sampling in the area. This method provided for a conservative analysis with the second brood samples having a slightly higher nitrogen content than random sampling could have provided with some south slopes included (see Results). Canopy cover samples were in proportion to random sampling. For the foraging analysis, I first discarded any observation less than 7.5 minutes in length, since a short observation period could result in an extreme foraging rate (minutes foraging/minutes observed). I used Poisson regression, with site as a covariate, to test whether the first and second brood differed in foraging rate. Underdispersion in the Poisson model was corrected by using the SAS quasi-likelihood function to adjust the scale parameter based on the Pearson χ^2.

RESULTS

I used first brood nitrogen sample vegetation plots to assess the effect of leaf litter depth on the number of lupine stems/m^2. A regression analysis showed a negative relationship between lupine stems and leaf litter depth ($n=48$, $p<0.015$, $r^2=0.12$) (Figure 1). All percent leaf nitrogen and water content data had normal distributions, so no data transformations were performed. For the first brood nitrogen sampling, there was no significant difference between management units (ANOVA, $df=2$, $F=0.27$, $p=0.77$) (Figure 2), sites ($df=3$, $F=2.73$, $p=0.058$), or the interaction of site and management unit ($df=6$, $F=0.80$, $p=0.58$). The Tukey's multiple comparison test showed the marginal significance of site was due to the difference between the highest nitrogen site, Julia's savanna (fall burn) and the lowest nitrogen site, Bond (spring burn).

For the second brood, a correlation analysis of water and nitrogen identified two outliers well outside the 95% confidence interval. These outliers were seen as outliers in further analysis and represented a low nitrogen value and a high water content value, probably caused from sampling errors. Therefore, these values were eliminated from all analysis. One additional outlier was identified, but the high vegetation density value appeared to be a result of vegetative sprouting of oak trees so the value was kept for analysis. The resulting Pearson's correlation between water and nitrogen resulted in a highly significant value ($n=52$, $p<0.0001$, $r=0.55$) indicating a positive relationship (Figure 3).

The Principal Components Analysis of leaf water and nitrogen content showed an Eigenvalue for the first axis (Eigenvector) of 1.55, which explained 78% of the variance (corresponding to a factor loading of $r=0.71$ for leaf nitrogen and water contents). The second Eigenvalue was 0.44, which explained the only 22% of the variance. Therefore, I used the first Principle Component axis (1st Eigenvector) as the dependent variable when analyzing the second brood "nutritional quality" of lupine plants to Karner blues.

The results of AIC_c analysis showed the two best models for explaining lupine nutritional quality during the second brood larval stage included vegetation density, canopy cover, and aspect ($n=58$, $r^2=0.48$ and 0.50) (Table 1). The best individual model also included the interaction of the three variables. These two best models had considerable support with a combined Akaike's weight of 0.67. Vegetation density was positively associated with lupine nutritional quality and explained 37% of the variation ($r^2=0.37$) by itself (Figure 4). Partial canopy cover increased the quality of lupine (Figure 5), and when added to vegetation density, 45% of the variance was explained. South slope and the interaction effect combined to explain 50% of the variation, with south slopes having a negative effect (Figure 6). Burned management

treatments were not included in the best AIC_c models (AIC_c <2 from the best model), and explained only 2% of the variance by itself (Figure 7). Vegetation density was not correlated with aspect (n=62, r=-0.23, p=0.07) or canopy cover (n=62, r=0.14, p=0.29). There was a significantly less host-plant nitrogen content for the second brood compared to the first brood larval stage (t-test, df=83, t-statistic=6.43, p<0.0001) (Figure 8).

Vegetation structure

Vegetation density did not differ between management units (ANOVA, df=2, F=1.09, p=0.34) (Figure 9) or sites (df=3, F=1.06, p=0.37). Leaf litter was significantly different between management units (ANOVA, df=2, F=12.16, p<0.0001) (Figure 10) and sites (df=3, F=4.41, p<0.009). A Tukey's test showed burned, mowed, and unmanaged units each differed in leaf litter depth with burned < mow < unmanaged. Julia's Savanna and Oak Dune had more leaf litter than South Piels.

Foraging

Fifty seven behavioral observations (805.5 minutes) were made during the first brood and 116 observations (1,643 minutes) during the second brood. Karner blues never foraged for greater than 7 minutes during the first brood, but several Karner blues were observed foraging for 15 minutes during the second brood. Foraging rate did not differ between sites (df=3, χ^2 =5.9, p=0.11), and the second brood foraged significantly more than the first brood (df=1, χ^2 =7.2, p<0.008) (Figure 11). Tables 2 and 3 provide a list of all plant species foraged upon by female Karner blues, and their approximate amount of use. First brood Karner blue females foraged primarily on cinquefoil (41% of total foraging time), dewberry (18%), and strawberry (11.5%). These species were common in all of the study sites (personal observation). The most utilized second brood nectar species included yarrow (22.7% of total foraging time), horsemint

(16.6%), yellow wild indigo (14.6%), and black-eyed susan (12.1%). These second brood results reflect both the abundance of nectar species and the number of Karner blues at each site. For instance, yarrow was common at the most abundant Karner blue site (South Piels) and horsemint occurred in only two small patches at South Piels. Butterfly milkweed occurred at 3 sites (excluding S. Piels) and New Jersey tea was present at only 2 sites (excluding S. Piels).

DISCUSSION

Karner blue ecology proved to be a valuable indicator of the historical structure and composition of Midwestern oak savanna. I found several interactions between the Karner blue and the environment that should be incorporated into restoration and management for oak savanna in Ohio and throughout the Karner blues' range. First, I found that lupine plants are smaller when leaf litter depth is greater, and these results are conservative, since only plants with a minimum coverage of 15% of a $1m^2$ quadrat were sampled. In fact, several plants in high leaf litter areas could not be sampled for nitrogen because of their small size and new plant samples were chosen. This result supports Grigore and Tramer's (1996) finding that lupine had higher biomass in recently burned areas, but it is critical to quantify when leaf litter accumulation impacts lupine growth. Plant size is often correlated with subsequent survival and fecundity (Gurevitch et al. 2002; Menges et al. 2006), and robust lupine plants were not found in areas with more than 3.5 cm of leaf litter depth.

The comparison of multiple environmental factors revealed that vegetation density, canopy cover, and aspect all contributed to the nutritional quality of lupine to varying extents during the second brood, while management had no direct impact for either brood. In a previous study, prescribed burning produced an increased nitrogen level in *L. perennis* (Grigore & Tramer 1996), but the difference was slight (2.31 vs. 2.25 mcg/g), and, again, other

environmental variables were not tested. Burned lupine areas did not have more lupine leaf nitrogen in this study. This discrepancy could also be explained by the general decrease in vegetation density in burned management treatments. Fire causes the loss of nitrogen in ecosystems (Gurevitch et al. 2002) and sandy soils, indicative of oak savannas (Brewer & Vankat 2004; Ricci 2006; Will-Wolf & Stearns 1999), probably leach newly available soil nutrients quickly.

For the second brood, nitrogen and water in lupine leaves were found to be correlated with each other, but not to a high degree. Both factors relate to the nutritional content of lupine plant tissue for Karner blues, but may reflect different temporal scales of influence on the plant. For instance, dehydration often causes plant leaves to temporarily curl in order to conserve water until replenished. Whereas, nitrogen may be a factor indicative of environmental conditions over the course of the growing season, phenology of individual plants (Grundel et al. 1998a), and nitrogen-fixing can be inhibited by water deficits (Engin & Sprent 1973).

Previous Karner blue studies have focused on canopy cover as a mechanism to increase nutritional quality of lupine leaves (Grundel et al. 1998a; Lane & Andow 2003; Maxwell 1998). Andow (1994) and Grundel et al. (1998a) have stated that herbaceous vegetation may be a helpful shade-producing layer to lupine, but no one had tested this theory, and consequently, management for this endangered species has been aimed at creating a heterogeneous canopy cover throughout the Karner blues' range. My study demonstrates the critical importance of initially exploring several variables before committing to single variable experimental testing. My results show that vegetation density alone explained 37% of the variance in nutritional quality of lupine for Karner blue larvae, while canopy cover contributed a relatively minor portion of the variance (8%). It should be noted that the vast majority of samples classified as

shade ranged between 20% and 55% canopy cover, which represents a habitat conducive to relatively abundant lupine plants (Lane 1999). Using canopy cover as a continuous variable could have improved my model slightly, but I wanted to avoid inaccuracies inherent to subjective visual estimates.

Vegetation density in this study was primarily associated with changes in species composition, not by more robust herbaceous plants or grazing (personal observation). A greenhouse experiment has shown that *L. perennis* leaf nitrogen content rises only slightly, 3.94% versus 3.70%, with consistent fertilization (Reich et al. 2003), so changes in soil nitrogen cannot explain the increased leaf nitrogen in areas with high vegetation density. It is more likely that reduced water loss (see Appendix Figures 1 & 4) kept leaf nitrogen levels higher in areas with dense vegetation. Dewberry (*Rubus villosus)* and bracken fern (*Pteridium aquilinum)* were the most common forbs providing herbaceous cover to lupine, and both are indicative of infertile soils. In 1928, Moseley (1928) classified each of these species as "abundant," which meant they were found in the "hundreds of thousands per square mile." Unfortunately, Moseley's accounts of the flora in the Oak Openings region did not include species associations, but the writings are valuable because they are the only description of historical native herbaceous vegetation available for the region. There is evidence that lupine associates with moderate levels of certain plant species. For example, Smallidge et al. (1996) found large *L. perennis* populations to be associated with sweet fern (*Comptonia peregrina),* in transmission line right-of-ways in New York. The same assemblage is found to support the largest population of Karner blues in the Northeastern USA at Saratoga Airport, NY where no woody vegetation is present (Forrester et al. 2005). *C. peregrina* is a shrub with a maximum height of 1 meter, which can easily provide shade to lupine.

Moseley (1928) wrote that both *C. peregrina* and *L. perennis* were abundant (see above) in the oak savanna region of Ohio. It is interesting to note that both species are nitrogen-fixers, which had a competitive advantage in nutrient poor savannas where fires were frequent. In fact, vegetation density surrounding lupine could have increased after burning if *C. peregrina* were present. Instead, *C. peregrina* is a threatened species in Ohio, and is only seen associated with lupine at the nearby Campbell Prairie and Oak Openings Preserve owned by the Metroparks of the Toledo Area. However, there are several examples, which indicate nitrogen-fixing species thrive in burned areas and decrease without burning; e.g. *Comptonia peregrina* (Niering & Dreyer 1989; Probst & Donnerwright 2003)*, Baptisa tintoria* (Niering & Dreyer 1989) and legumes such as *Tephrosia virginiana* (Dudley & Lajtha 1993) and *L. perennis* (Grigore & Tramer 1996).

Aspect was an important explanatory variable in the model, which is surprising considering the minor topography changes associated with sand dunes in the study area. The effect of topography, with south slopes being associated with low moisture, has been shown to affect host-plant phenology and larval growth rates of bay checkerspot butterflies (Fleishman et al. 1997).

Canopy cover, vegetation density, and aspect may have differing roles in different parts of the Karner blue range, but managing for higher vegetation density has certain benefits. The restoration of trees is difficult to accomplish in the time scale in which it may be necessary to sustain or restore Karner blue populations (e.g. 1-10 years). Lupine abundance declines with an increase in canopy cover (Grundel et al. 1998b), and reproduction decreases as well (Maxwell 1998). Herbaceous species observed in this study sprouted when or after lupine bloomed, and did not seem to inhibit lupine reproduction (personal observation). However, based on my

results at the Kitty Todd preserve, I cannot determine if a greater density of these particular species could inhibit lupine growth and reproduction. Therefore, large lupine populations associated with a compatible vegetation density are recommended for Karner blue populations. I found second brood nectar species to be very important for the Karner blue. This differs from a Karner blue study performed in Indiana (Grundel et al. 1998b), but my methodology differed because it accounted for brief lapses in foraging behavior. First brood nectar plant species were characterized by small plants with singular flowers, such as strawberry (*Fragaria virginiana)* and cinquefoil (*Potentilla simplex)*. During the second brood, species such as New Jersey tea (*Ceanothus americanus)*, butterfly milkweed (*Asclepias tuberosa)*, and dotted horsemint (*Monarda punctata*) provided a much more abundant floral resource for the Karner blue. Additionally, host-plant nitrogen was lower during the second brood larval stage, and adult foraging could have compensated for this loss. This finding complements lab-based studies where nutrient trade-offs between larvae and adult derived nutritional sources have been found (Mevi-Schutz et al. 2003); they found female butterfly larvae (Family Satyridinae) feeding on low nitrogen/water plants had a greater preference for high amino acid content in nectar compared to larvae raised on high nitrogen plants.

In summary, I have successfully found vegetation density, canopy cover, and aspect to play important roles in determining host-plant quality for the Karner blue. Comparing the influence of these variables led to an improved understanding of the interaction between the Karner blue and environmental characteristics. Oak savanna degradation, including the loss of nitrogen-fixing herbaceous species, could indirectly lead to declines in Karner blue populations through decreased larval nutrition. Host-plant senescence has often been blamed for lower larval survival and butterfly population declines (Fleishman et al. 1997; Lane & Andow 2003; Maxwell

1998), but the effect of senescence on overall host-plant nutritional content has rarely been quantified. Measuring host-plant quality by both nitrogen and water content provided a more robust model than either variable alone. Lower second brood nitrogen levels and inherently limited water in June-July make the Karner blue more susceptible to habitat degradation and dry weather during this time. Second brood nectar sources could buffer the Karner blue from these impacts by providing essential nutrients to adults. Therefore, a multitude of benefits can be obtained from managing and restoring the native vegetative assemblage in this ecosystem.

MANAGEMENT IMPLICATIONS

Karner blue habitats should be managed to minimize leaf litter depths in order to achieve more robust populations of lupine. When evaluating habitat or the restoration potential for Karner blues in oak savanna, herbaceous species density and composition during June-July should be considered. An increased vegetation density will increase the nutritional quality of lupine unless the vegetation appears to interfere with the growth of lupine. The composition and abundance of nitrogen-fixing plants should be a primary concern in this system. South slopes should be considered detrimental, and enhancing second brood nectar sources should be a high priority. The restoration of oak savanna herbaceous and woody species, along with the natural process of fire, will provide high quality habitat to the Karner blue. Restoration and management aimed at the Karner blue should assist many other species in this rare community because Karner blue habitat requires proper canopy cover and many native herbaceous species.

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TABLES AND FIGURES

Table 1. Akaike's Information Criteria (AIC) results of second brood lupine leaf nutrition (n=52). AIC_c is the AIC value corrected for small sample sizes; delta AIC_c is the difference between the best model and each individual model. Weight is Akaike's weight, or the probability of each model being the correct model, given the candidate models. Potential variables in model include vegetation density, canopy cover (shaded or open), slope (south or not south), and two interaction effects denoted by an asterisk between variables.

Four asterisks represent the two best models.

K	r^2	AIC_c	delta AIC_c	Weight	Variables in Model	
6	0.50	-9.6	0.0	0.35	veg density + canopy + slope + slope*veg density*canopy	****
5	0.48	-9.4	0.2	0.32	veg density + canopy + slope	****
4	0.45	-7.8	1.8	0.14	veg density + canopy	
5	0.45	-6.5	3.1	0.07	veg density + canopy + veg density*canopy	
5	0.45	-6.5	3.1	0.07	veg density + canopy + burned	
4	0.40	-2.7	6.9	0.01	veg density + slope	
5	0.41	-2.2	7.4	0.01	veg density + slope + burned	
3	0.37	-1.9	7.7	0.01	veg density	
4	0.38	-1.0	8.6	0.00	veg density + burned	
5	0.19	13.9	23.5	0.00	canopy + slope + burned	
4	0.17	14.1	23.7	0.00	canopy + slope	
3	0.09	17.5	27.1	0.00	canopy	
4	0.11	17.5	27.1	0.00	slope + burned	
4	0.10	18.2	27.8	0.00	canopy + burned	
3	0.07	18.5	28.1	0.00	slope	
3	0.02	21.1	30.7	0.00	burned	

Table 2. First brood nectar species use by female Karner blues. Total minutes used as well as % of use as a proportion of the total time spent foraging are listed for each species.

Common Name	Scientific Name	Total minutes used	Foraging use/ Total foraging time observed (%)
cinquefoil	*Potentilla simplex*	25	41.0
dewberry	*Rubus flagellaris*	11	18.0
strawberry	*Fragaria virginiana*	7	11.5
dwarf dandelion	*Krigia virginica*	5	8.2
chickweed	*Stellaria media*	5	8.2
violet	*Viola sp.*	3	4.9
ox-eye daisy	*Chrysanthemum leucanthemum*	3	4.9
clover	*Trifolium sp.*	2	3.3

Table 3. Second brood nectar species use by female Karner blues. Total minutes used as well as % of use as a proportion of the total time spent foraging are listed for each species.

Common Name	Scientific Name	Total minutes used	Foraging use/ Total foraging time observed (%)
yarrow	*Achillea millefolium*	56	22.7
horsemint	*Monarda punctata*	41	16.6
yellow wild indigio	*Baptisia tinctoria*	36	14.6
black-eyed susan	*Rudbeckia hirta*	30	12.1
early goldenrod	*Solidago juncea*	15	6.1
tall dandelion	*Krigia biflora?*	14	5.7
meadowsweet	*Spiraea sp.*	13	5.3
butterfly milkweed	*Asclepias tuberosa*	8	3.2
daisy fleabane	*Erigeron strigosus*	8	3.2
New Jersey tea	*Ceanothus americanus*	8	3.2
flowering spurge	*Euphorbia corollata*	7	2.8
ox-eye daisy	*Chrysanthemum leucanthemum*	7	2.8
deptford pink	*Dianthus armeria*	3	1.2
St.John's wart	*Hypericum sp.*	1	0.4

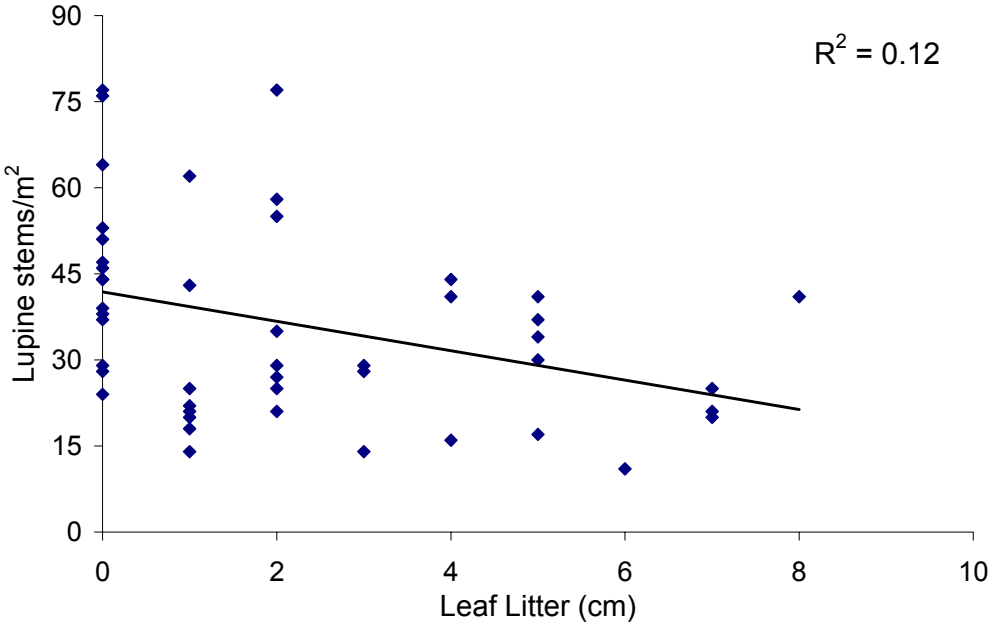

Figure 1. Lupine stems per m^2 versus leaf litter depth from first brood nutrient sampling; data was collected with a minimum plant size of 15% of $1m^2$ (n=48, $p<0.015$).

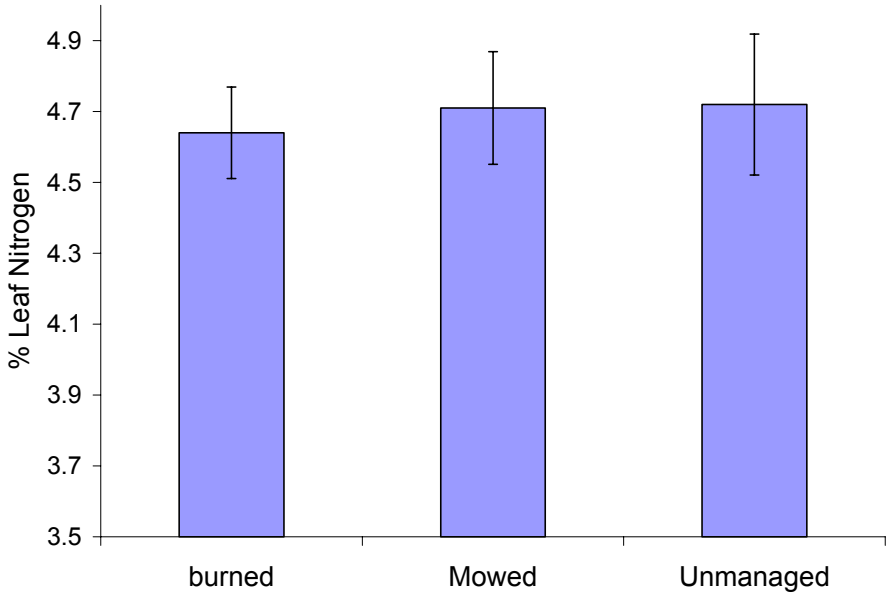

Figure 2. First brood lupine leaf nitrogen content (n=48), as percent weight, compared by management units. Error bars represent standard deviation (p=0.77).

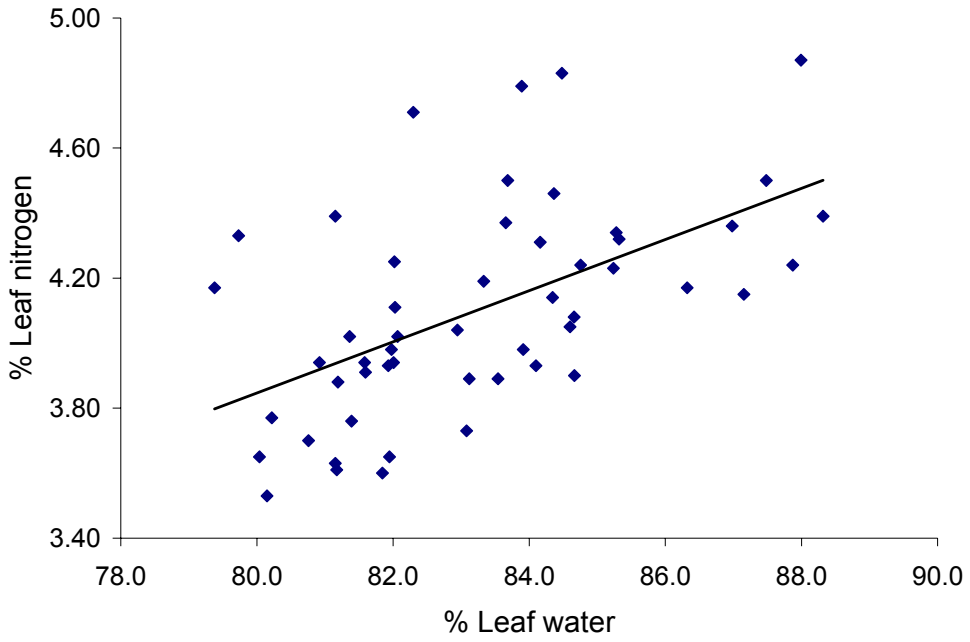

Figure 3. Lupine leaf water and nitrogen content as percent weight (n=52) for Karner blue second brood (Pearson's correlation, r=0.55, p<0.0001)

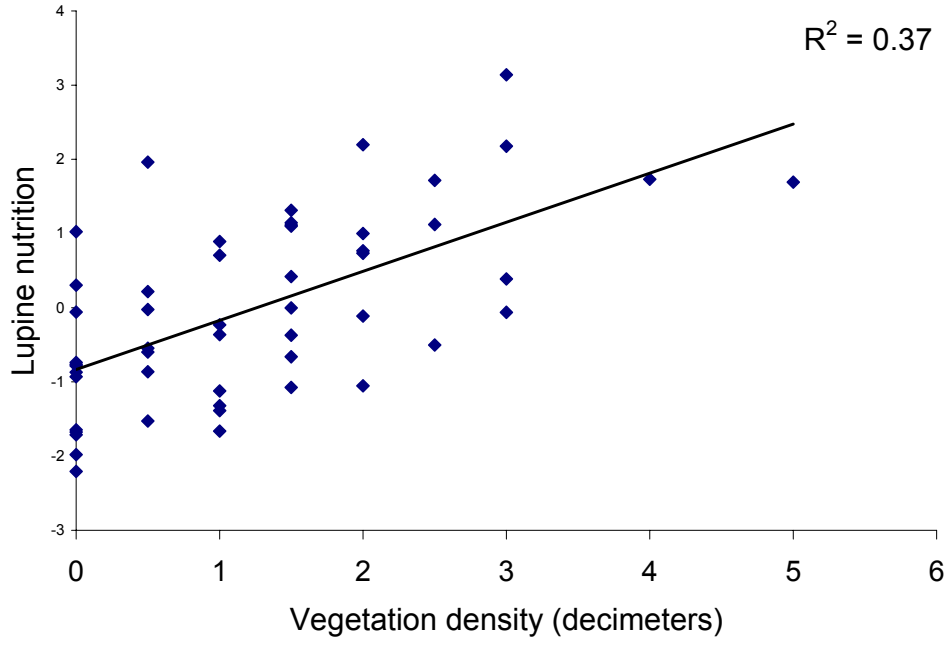

Figure 4. Lupine leaf nutrition versus vegetation density (n=58) for Karner blue second brood. Lupine nutrition represents leaf water and nitrogen content (PCA1) weighed equally.

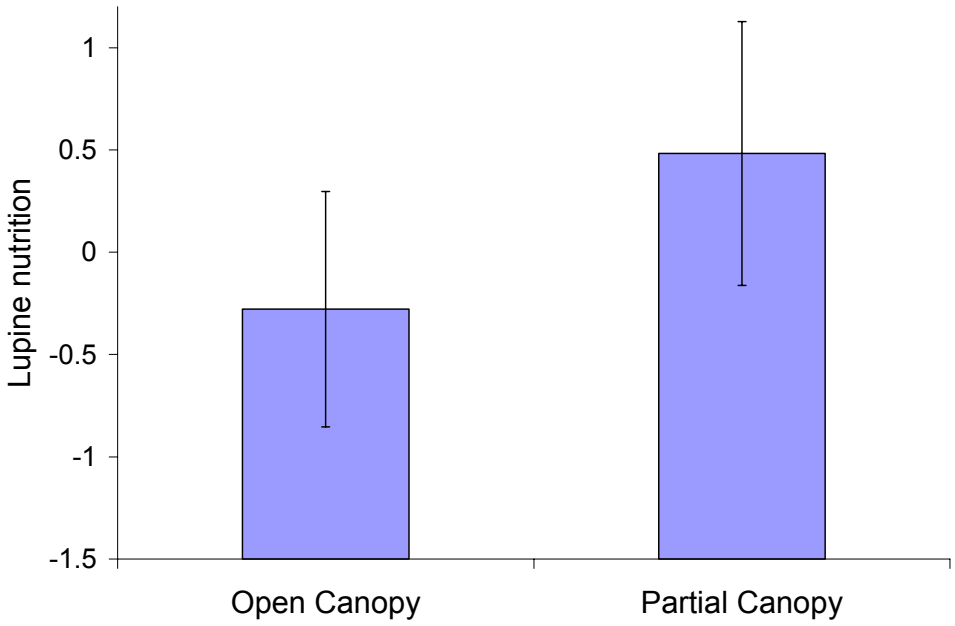

Figure 5. Lupine leaf nutrition versus canopy cover; partial canopy is >15% canopy cover and open canopy is ≤15% canopy cover, for Karner blue second brood. Error bars represent standard deviations. (Partial Canopy n=23, Open Canopy n=35). Lupine nutrition represents leaf water and nitrogen content (PCA1) weighed equally.

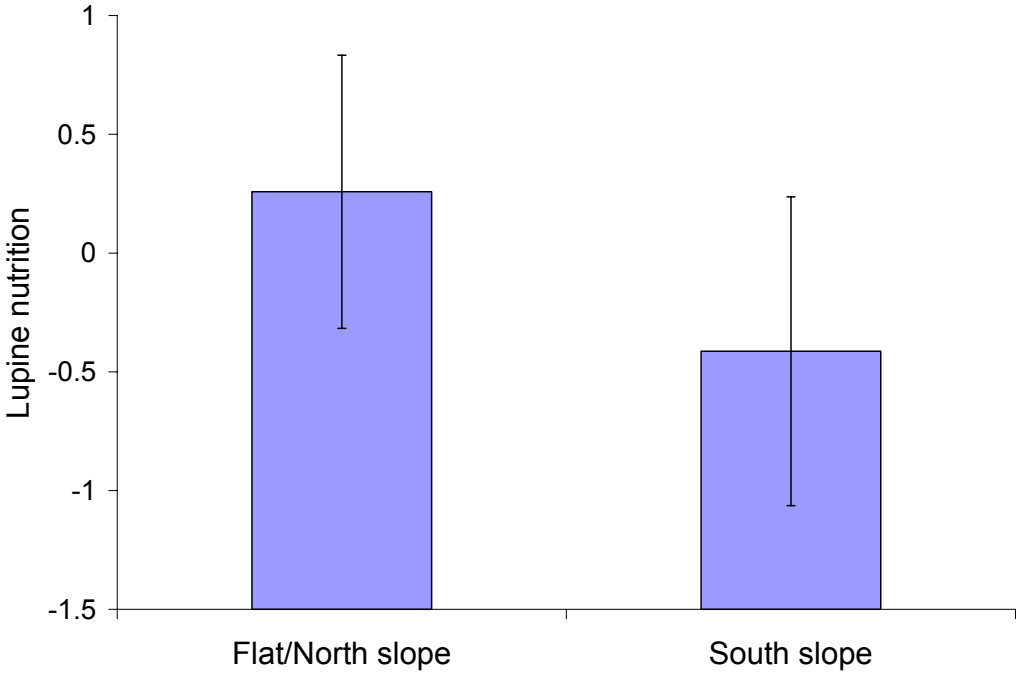

Figure 6. Lupine leaf nutrition versus aspect for Karner blue second brood.
Error bars represent standard deviations (Flat n=11, North n=25, South n=22).
Lupine nutrition represents leaf water and nitrogen content (PCA1) weighed equally.

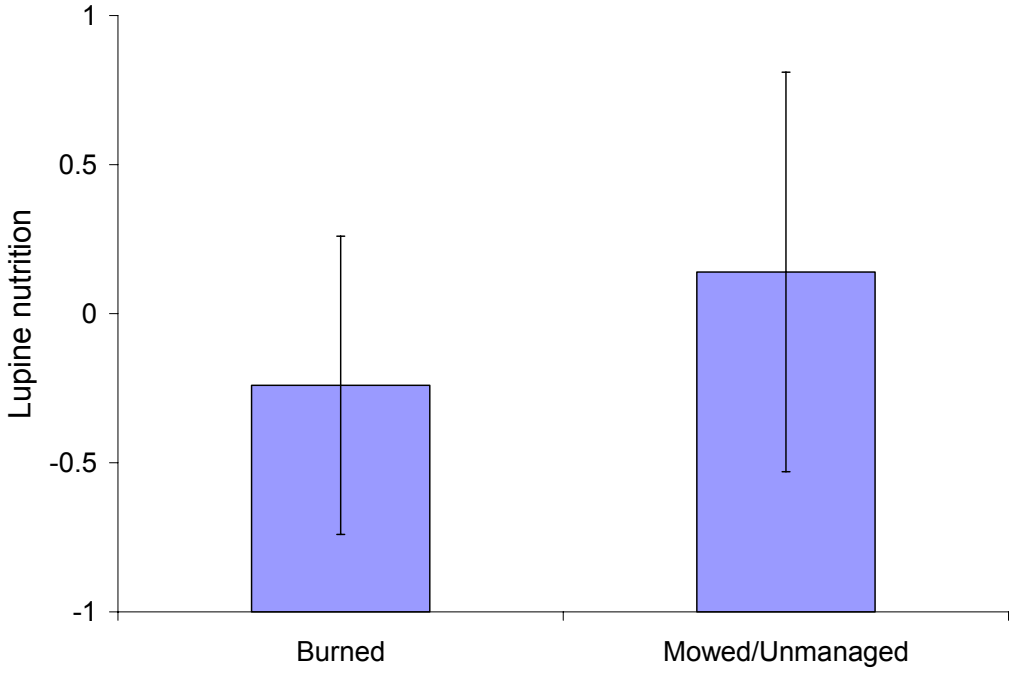

Figure 7. Lupine leaf nutrition versus management unit (n=58) for Karner blue second brood. Lupine nutrition represents leaf water and nitrogen content (PCA1) weighed equally.

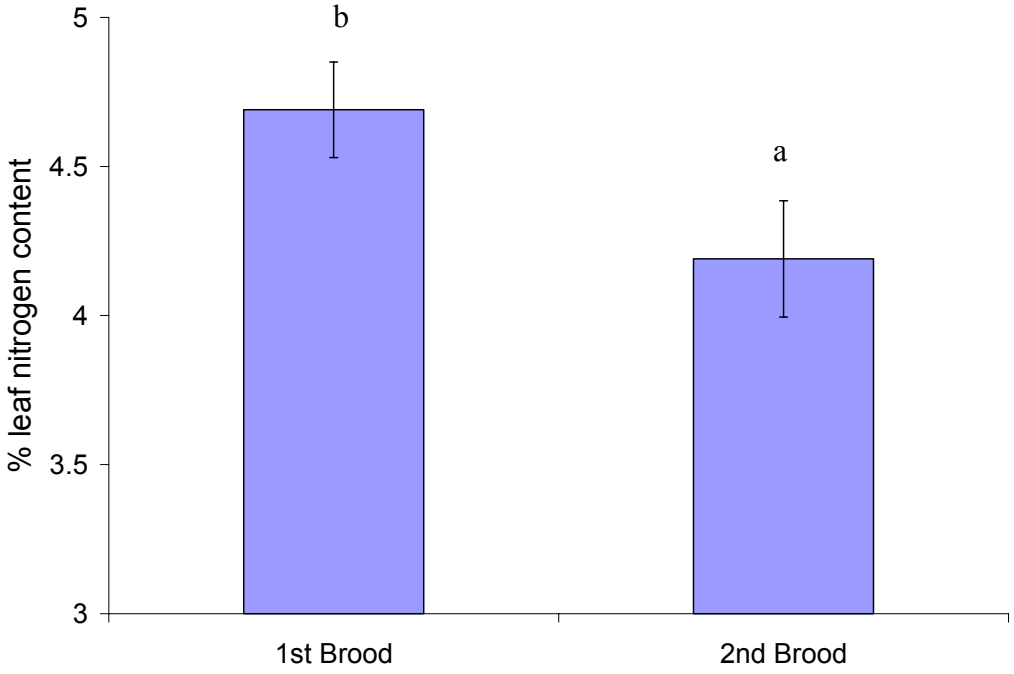

Figure 8. Lupine leaf nitrogen, as percent weight, versus Karner blue brood; excluding south slopes for the second brood. Error bars represent standard deviation. Letters indicate significantly different values ($p<0.0001$).

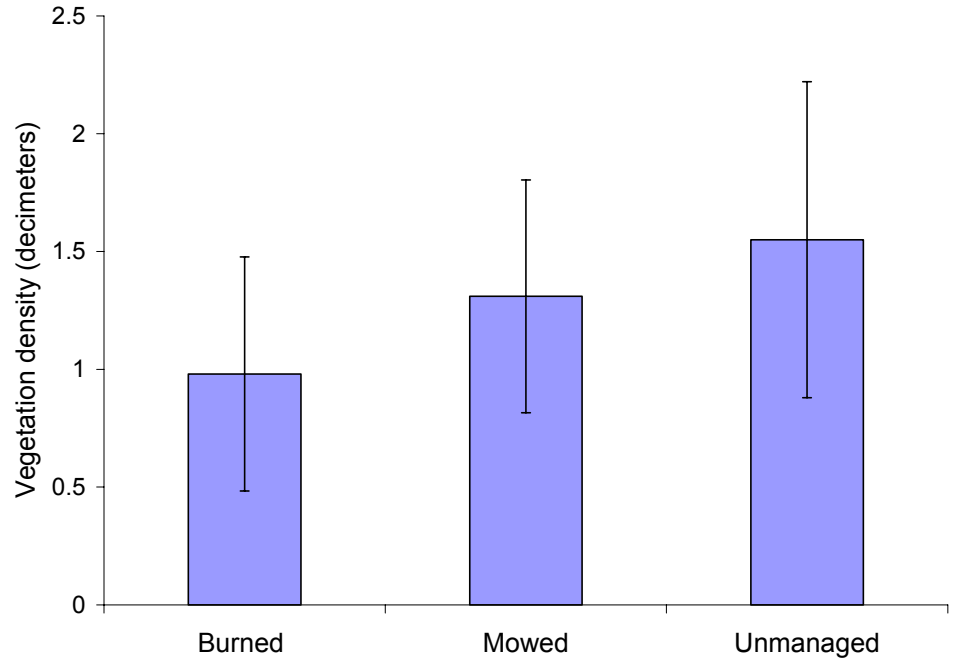

Figure 9. Vegetation density, in decimeters, versus management unit. Error bars represent standard deviation (p=0.34).

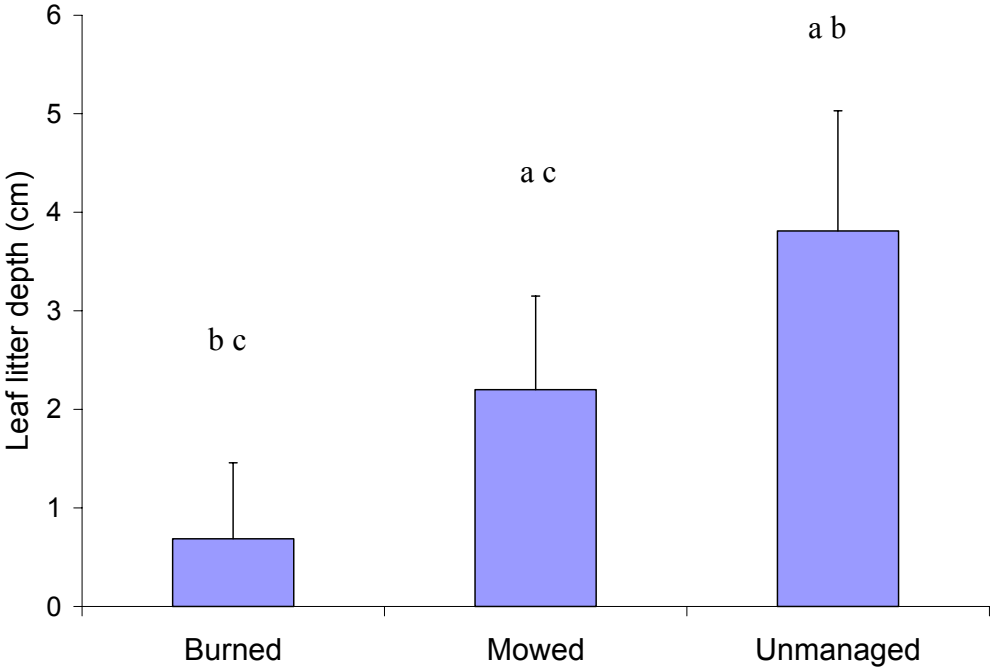

Figure 10. Leaf litter depth, in centimeters, by management units. Error bars represent standard deviation (n=48). Letters indicate significantly different values (p<0.0001).

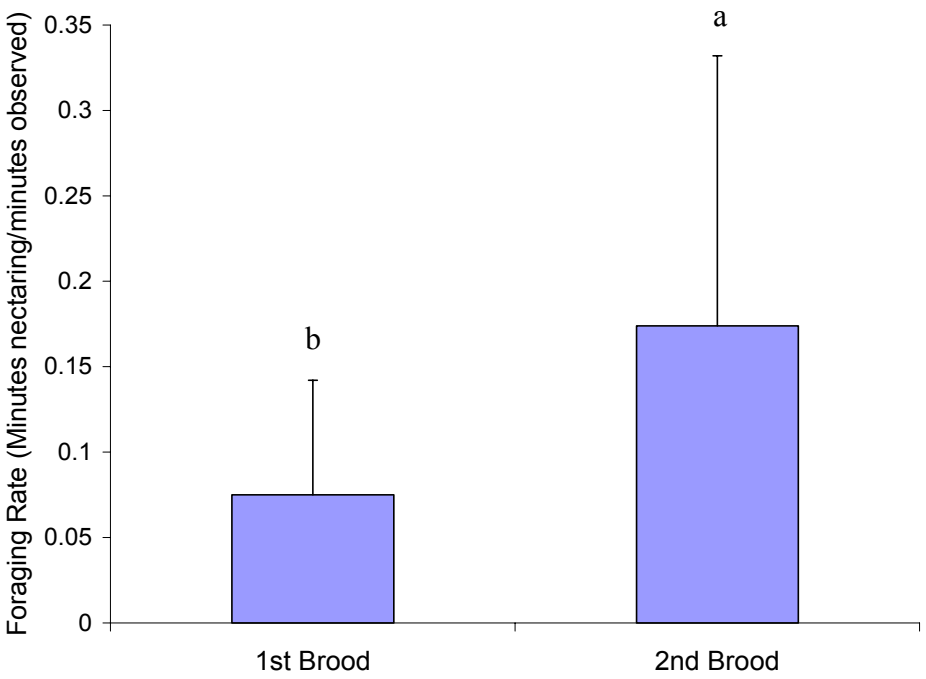

Figure 11. Foraging rate of female Karner blues, as minutes spent foraging as a proportion of minutes observed by brood. Error bars represent standard deviation (1st brood n=57; 805.5 minutes; 2nd brood n=116; 1,643 minutes). Letters indicate significantly different values. ($p<0.008$)

Using Abundance and Behavior as Tools for Assessing a Management Strategy for an Endangered Butterfly

ABSTRACT

The effects of management are well known for many vertebrate species, but species with small, cryptic life stages are often difficult to evaluate without long-term data or a large quantity of sample locations. The monitoring of a species' behavior has the advantage of examining immediate responses to rapidly changing environments, and these methods can provide robust sample sizes, which is often problematic for species of conservation concern. Both of these characteristics make behavioral observations an invaluable tool for evaluating management of rare and endangered species. In this study, we evaluated the consequences of a management strategy for the federally endangered Karner blue butterfly (Karner blue), *Lycaeides melissa samuelis*. The Karner blue is unusual because the species always has two broods per year, and the larvae are specialists on wild blue lupine, *Lupinus perennis.* Karner blues have recently been reintroduced into the globally rare Ohio oak savannas, and in order to sustain this community, prescribed burning and mowing have been employed. The current management regime divides each Karner blue site into three management units based on the number of lupine stems. A third of the site's lupine is burned, mowed, or left unmanaged each year. I used Karner blue surveys and behavioral observations of females to identify how Karner blues responded to these management treatments. My results showed no significant management treatment differences in male or female abundance during the first brood. In the second brood there were significantly more males and females in burned areas compared to the other two treatments. Female Karner blues oviposited significantly less in unmanaged treatments compared to burned and mowed treatments. These results demonstrate how monitoring the behavior of a rare species can assist in evaluating the effects of management in a rapidly changing environment. By using this

management strategy, burning unmanaged areas characterized by a high amount of leaf litter will have a minimal impact on the Karner blue population.

INTRODUCTION

The effects of management treatments are widely studied for birds (Blake 2005; Murray & Best 2003; Siegel & DeSante 2003), mammals (Sullivan et al. 1999; White & Garrot 2005), and plants (Coates et al. 2006; Menges et al. 2006), but data is often scarce for more cryptic taxa such as amphibians (Bury 2004), reptiles (Bury 2004), and invertebrates of conservation concern (Parr & Chown 2003). With these species, demography is often difficult or impossible to quantify due to inherent problems in estimating abundance, survivorship, and fecundity for various life stages. Either long-term data on abundance (Harpole & Haas 1999) or a large number of sample locations for metapopulation studies (Bergman & Kindvall 2004; Moilanen & Hanski 1998; WallisDeVries 2004) are needed in order to evaluate the effects of management for species such as salamanders and butterflies. This data is often unavailable or impossible to obtain for rare and endangered species. Additionally, only a few studies have experimentally shown the effects of management treatments for insect species of conservation concern (Schultz & Crone 1998). Yet, these species often inhabit early successional habitat in need of active management. Assessing management treatments by monitoring the behavior of a species has the advantage of providing an immediate, measurable response to rapidly changing environments, such as degrading habitats. These methods can also provide a robust sample size within a single year, which is often a problem for threatened or endangered species. Both of these characteristics can make behavioral observations an invaluable tool for evaluating management practices for species of conservation concern.

In my investigation of the Karner blue butterfly (Karner blue), *Lycaeides melissa samuelis,* I used behavior to assess the effects of management on the species. There are several advantages to using this methodology with butterflies: 1) other taxa (e.g. birds) have shown that presence is not always indicative of good habitat quality; ecological traps (Battin 2004) or source-sink dynamics may exist (Kawecki 2004); 2) a large sample size can be accumulated in a single year; 3) observations do not depend on finding eggs, larvae, or juveniles in complex vegetation; 4) behavioral responses should accurately reflect immediate responses to habitat quality.

The Karner blue is an early successional species, which specializes on the host-plant wild blue lupine (*Lupinus perennis)* in oak savannas or pine barrens in the Midwestern and Eastern USA. The Karner blue was extirpated from five U.S. states as well as Ontario, Canada before being listed as a federally endangered species in the USA in 1992 (U.S. Fish and Wildlife Service 1992). Extirpation of the Karner blue in Ohio took place by 1989, and subsequently, the species was reintroduced in 1998 by a cooperative effort between the The Nature Conservancy, Toledo Zoo, Ohio DNR, Michigan DNR, U.S. Fish and Wildlife Service, and other organizations. As of 2005, Karner blues occupied four sites at The Nature Conservancy's Kitty Todd Preserve in northwest Ohio. This Karner blue population has undergone slow upward growth since reintroduction, but the population is still relatively small. From 1998-2000 no management activity was performed in occupied Karner blue areas, and lupine seemed to decline as well (personal communication, Gary Haase, TNC). Since 2001, the U.S. Fish and Wildlife Service has issued permits to burn 1/3 and mow 1/3 of lupine stems (representative of lupine quantity) at each occupied site, leaving 1/3 of the lupine stems unmanaged. These management units have been rotated, so in this study, mowed management units had been burned 1-2 years

previously. Three unmanaged management units had been burned 4 years ago, and one unmanaged unit had not been burned in 7 years due to low fuel loads at this site (South Piels).

Primary Karner blue behaviors of interest are adult foraging and ovipositing. Butterflies use chemical cues, leaf shape, texture, and colors to help determine oviposition preferences, which may increase the survival of their offspring (Rutowski, 2003). However, it is unknown at what distance a butterfly may be able to detect host-plants or the quality of host-plants. This is important because management such as prescribed burning cannot be utilized without killing resident butterfly species (Swengal 1995), so the recognition of high quality habitat by potential recolonizers is critical for rapid recovery and long-term persistence of the species. Karner blue daily movement distances differ by brood and sex, but average from 70 to 457 meters (King 1998). The current management strategy always has burned management units at Kitty Todd within 120 meters of a potential source management unit (unburned), so Karner blues should be able to recolonize burned areas.

In this Chapter, I assessed habitat use and oviposition rates of Karner blues in three differing management treatments: burned, mowed, and unmanaged, in order to determine the effects of this management strategy on the Karner blue. I used vegetation surveys and measurements at the scale of management units to account for differences in area, stems, and available shade. I hypothesized that the nutrient quality of lupine (Chapter 1) would be higher in burned management units, and Karner blues would respond by being attracted to these burned units and showing a relatively high oviposition rate as well.

SPECIES OF INTEREST, STUDY AREA, AND MANAGEMENT

The Karner blue butterfly is a bivoltine species, with adults living an average of 3.5 days (Knutson et al. 1999). First brood larvae emerge mid-April, first brood adults emerge mid-May, second brood larvae emerge 5-10 days after oviposition, and second brood Karner blues fly during July. Second brood Karner blues oviposit eggs, which overwinter until the next April. Management occurs during this overwintering egg period. The Karner's host-plant, wild blue lupine, *Lupinus perennis* is a perennial plant, which primarily lives in partially shaded to open areas, nutrient poor soils, and early successional habitats (U.S. Fish and Wildlife Service 2003). Lupine can reproduce vegetatively, so it is often impossible to differentiate between lupine plants by stems alone (Grigore & Tramer 1996).

Karner blues currently occupy four sites in Ohio, and these are at The Nature Conservancy's Kitty Todd Preserve located in Lucas County, Ohio (41^0 37' N, $083^0$47' W). The Toledo Airport, 10 km from Kitty Todd Preserve, reports a total precipitation average of 840 mm per year, and mean temperature averages range from $-4.5C^0$ in January to $22\ C^0$ in July (NOAA 2006). Elevation ranges from 154-254 meters, and soils are generally well-drained and sandy. Vegetation includes an herbaceous layer and a woodland canopy generally covering between 30-80% of the area. Dominant woody vegetation includes black oak (*Quercus velutina),* northern pin oak (*Quercus ellipsoidalis),* and white oak (*Quercus alba)*. Common herbaceous species include New Jersey tea (*Ceanothus americanus)*, wild blue lupine (*Lupinus perennis)*, western sunflower (*Helianthus occidentalis)*, rough blazing star (*Liatris aspera),* butterfly milkweed (*Asclepias tuberosa),* flowering spurge (*Euphorbia corollata*), and wild yellow indigo (*Baptisia tinctoria*). Little bluestem (*Schizachyrium scoparium)* and big bluestem (*Andropogon gerardii)* are the primary grasses.

Each Karner blue site ranged from 0.39-2.15 ha in size (Figures 1-4), and tree canopy covered 56-61% of the area at three sites, and only 4% of the fourth site. In 2005, approximately a third of each occupied site's lupine was burned, mowed, or left unmanaged (Figures 1-4). In late November 2004, two sites' management units were burned (South Piels, Julia's Savanna) and at the end of March 2005, the other two sites' management units were burned (Bond, Oak Dune). In March, the appropriate areas were mowed to approximately 15.2 cm (6 inches) in height, and the clippings were left on the ground.

METHODS

Vegetation Surveys

Between November 2004 and April 2005, I flagged the border of each burned, mowed, and unmanaged management unit in order to facilitate identification of management units when surveying and performing observations. After the lupine began to bloom, on May 17th, I used a Trimble Pro XRS system GPS unit (Trimble, Sunnyvale, CA), with an accuracy <1m, to map lupine areas according to their management unit at all four occupied Karner blue sites. This defined each "patch" of lupine, and guided further vegetation surveys. Lupine plants covering <1 m^2 and greater than 10 meters from other lupine plants were excluded from these areas. GPS points were imported to ArcGIS 8.3 (ESRI, Redlands, CA), and polygons of lupine management units were created from the GPS points.

In May 2005, I used a $1m^2$ quadrat to estimate the amount of lupine at each site. Transects were created every 10 meters from the south end of each patch, and surveys were taken every 10 meters along these transects. For logistical reasons, larger patches were surveyed with transects spaced every 15 meters. The first survey on each transect was taken 5 m from the edge to ensure that smaller sites contained an adequate sample size. No samples were taken < 2

meters from another management unit in order to avoid edge effects, which may cause bias since very few transects would be taken along an edge.

At each survey location, I recorded the number of lupine stems and took a canopy cover photograph. Canopy cover was recorded using a Nikon Coolpix2000 digital camera. Digital photographs were taken with the assistance of a level attached to the camera to ensure a consistent picture. Pictures were transferred to Adobe PhotoShop 7.0 (Adobe Systems Incorporated, San Jose, CA) and converted to black and white photographs. The darkest portion of the sky was then selected, and everything darker than the sky was considered canopy cover. The histogram function determined exact values of these darker portions. This procedure is further outlined by Klingenbock (2000). The percent of management unit area shaded was calculated by summing the number of samples with >15% canopy cover and dividing by the total number of samples taken. Lupine density was estimated by counting each stem sprouting from the ground. Since individual lupine plants can often not be distinguished in the field (Grigore & Tramer 1996), and robustness of plants vary greatly, this provided the most accurate count of available lupine. Samples per management unit ranged from 14 to 71 with a total of 386 samples taken from the four sites. This variability was unavoidable due to differences in management unit areas, and extremely variable numbers of lupine stems in some cases, including many zeros.

Surveys and Behavior Observations

On days without precipitation and a temperature above 17 C^0 (62.5^0 F) in the shade, butterfly surveys can be performed with accuracy (Pollard & Yates 1993). I used modified Pollard-Yates transects (Pollard & Yates 1993; Thomas 1983) to obtain an index of Karner blues in each management unit for the first and second brood in 2005. One to three trained observers performed Karner blue counts by using a zig-zagging transect throughout all areas of lupine

within a particular management unit (see Figure 5), and the number of females and males within 3 meters of the observer were recorded. The transect lines paralleled each other at a distance of approximately 3.5 meters, which minimized double counting. The sex was recorded for each observed butterfly in addition to the initial management unit and canopy cover. Canopy cover estimates were determined by visual sight after practice with a digital camera by the principle investigator. Canopy cover was later classified as either open (≤15% canopy cover) or shaded (>15% canopy cover), which is modified from Lane's three classes (1999), and indicates general changes in lupine abundance with canopy cover.

When a female Karner blue was observed, I stopped and performed a 15-minute behavior observation. At the beginning of the second brood, 16 observations were performed for only 10 minutes since a larger population of butterflies was anticipated during the second brood. When numbers remained low, I switched back to 15-minute observation periods in order to be consistent with the first brood and optimize the number ovipositions I could observe. These ten minute observations accounted for only 16 of 121 observations for the second brood. During behavior observations, I recorded "foraging" or "not foraging" at one-minute intervals. If a Karner blue foraged at any time during a minute of observation, the minute was counted as foraging. This accounted for butterflies foraging, and then briefly searching for more flowers, before continuing to forage.

Ovipositions occurred when a Karner blue crawled down a plant stem, usually lupine, flexed its abdomen, and deposited an egg. I used an eTrex Legend GPS Unit (Garmin International, Inc., Olathe, KS) to record oviposition locations, and the following variables were recorded after observations: total time of observation, number of ovipositions in each management unit, canopy cover of oviposition, height of oviposition, whether the oviposition

was within 3 meters of another management unit, aspect (first brood only), and substrate of oviposition. A single location was recorded if eggs were within $1m^2$ of each other. If a butterfly was demonstrating obvious oviposition behavior when 15 minutes expired, the observer was allowed to continue the observation for a maximum of 3 minutes.

HOBO Temperature Loggers

Two HOBO temperature data loggers (Onset Computer Corporation, Bourne, MA) were placed in an open, sunny area and a well-shaded area at the South Piels site. Temperatures were recorded every 40-minutes and were correlated with survey and oviposition dates.

Analysis

SAS 8.01 (SAS Institute 2000) was utilized for all data analysis, and means are reported with ± standard deviation. I used SAS Generalized Linear Models with a Poisson distribution for the survey analysis. The distribution of foraging rate was underdispersed, so I corrected the model using the SAS quasi-likelihood function to adjust the scale parameter based on the Pearson χ^2. The oviposition data was slightly overdispersed (by a factor of three), therefore, I used a negative binomial distribution, which uses an extra parameter to adjust to the variance in the data set.

Independent variables for survey data was measured at the management unit scale (e.g. area, percent area shaded). Likelihood ratio type III tests determined if each main effect should be included in the final model via a backwards selection process. When a variable was discarded from the model or the model was finalized, I reported the chi-square and p-values for each variable and its relevant set of comparisons (e.g. Burned vs. Mowed).

Behavior analysis was performed to determine if female Karner blues were in a particular management unit for oviposition or foraging behavior. First, all observations of less than 7.5

minutes were discarded, since this was the minimum observation time in which I recorded an oviposition. For oviposition analysis, I assumed that the initial survey management unit was an indicator of where each observation took place. Given that Grundel (1998b) reported that only 8.4% of females moved >10 meters during 10-minute observations, this is a reasonable assumption. On occasions when a butterfly laid an egg in a different management unit than where the initial observation took place, I divided this into two separate observations. This also included four occurrences when a Karner blue oviposited in two adjacent management units in a single observation. In the negative binomial model, I used site and brood as covariates and initial management unit as a main effect to explain total ovipositions.

Since flower species, abundance, and Karner blue foraging time differed by brood, I analyzed the foraging rate separately for each brood. I used site as a covariate with initial management unit as a main effect. Foraging was not recorded per management unit in the field, so it was assumed that foraging was done in the initial survey management unit. Prior to foraging analysis, I eliminated any observations when the butterfly was known to oviposit, and thus, be present in a multiple management units.

In order to estimate the total number of eggs oviposited per female, I used the results of Lane's (1999) lab experiments, which concluded 24.6^0C (76^0F) was the minimum temperature necessary for female Karner blue movement. From the HOBO temperature loggers, I averaged the number of hours available for oviposition behavior for each brood and multiplied by the brood's overall oviposition rate. Knutson et al. (Knutson et al. 1999) reported no significant difference between first and second brood residence time (minimum survival estimate), and the Karner blue residence time was reported to be an average of 3.5 days (Knutson et al. 1999).

Since this number does not include dispersing individuals, I arbitrarily used a four day lifespan to estimate lifetime fecundity for each brood.

RESULTS

Surveys and Behavior

I recorded 146 males and 58 females for the first brood and 130 males and 124 females in the second brood. I used area of management unit and percent of management unit area shaded instead of stems and shaded stems, since the areas explained more variation in Karner blue abundance than lupine stem counts, and these numbers were highly correlated ($n=12$, $r=0.70$, $p<0.015$). I used sites and area as covariates, and percent of area shaded and management unit as main effects in the model.

The results, separated by brood and sex, are displayed in Tables 1-4. Area was significant in 3 of 4 Poisson regressions, and this was always a positive association with Karner blue abundance; only the second brood females did not have a significant association with area. Likewise, the only cohort responding to the percent of area shaded was the first brood of females, and this was an affinity to management units with less canopy cover.

First brood males (Table 3, $df=2$, $\chi^2=3.2$, $p=0.20$) and females (Table 1, $df=2$, $\chi^2=3.4$, $p=0.18$) had no significant differences in their use of the three management units. During the second brood (Table 2), females were more abundant in burned units compared to unmanaged ($df=2$, $\chi^2= 17.54$, $p<0.0001$) and mowed units ($df=2$, $\chi^2= 25.54$, $p<0.0001$). There was no significant difference between mowed and unmanaged management units ($df=2$, $\chi^2= 0.15$, $p=0.70$). Second brood males (Table 4) were more abundant in burned ($df=2$, $\chi^2=18.72$, $p<0.0001$) and mowed units ($df=2$, $\chi^2=6.86$, $p<0.009$) compared to unmanaged areas. Karner

blue males were also more abundant in mowed units compared to unmanaged (df=2, χ^2=8.72, p<0.003).

Forty-six ovipositions were observed for the first brood and 84 were observed during the second brood (Figures 1-4). Overall, Karner blues had a higher oviposition rate in burned (df=2, χ^2=5.43, p<0.015) and mowed (df=2, χ^2=5.58, p<0.016) management units compared to unmanaged units (Table 5; Figure 6). There was no significant difference in oviposition rate between the burned and mowed management units (χ^2=0.02, p=0.90) (Table 6). At the individual scale, I observed two ovipositions by one butterfly on a shaded lupine. All other ovipositions were <16% canopy cover. Overall, 34/130 ovipositions, or 26%, were within two meters of another management unit. Foraging rate for the first brood did not differ by site (df=3, χ^2=2.03, p=0.57) or management unit (df=2, χ^2=0.72, p=0.70) (Figure 7). In the second brood, site (df=3, χ^2= 3.83, p=0.28) and management unit (df=2, χ^2 =2.12, p=0.35) also did not significantly differ in foraging rate (Figure 7). Foraging rate did not change with temperature (df=1, χ^2=0.05, p=0.82) when brood (df=1, χ^2 =1.25, p=0.26) and sites (df=3, χ^2=8.20, p=0.15) were covariates in the model.

Life History

The frequency of oviposition per observation (1 or 0) differed between Karner blue broods. The first brood oviposited more frequently than the second brood (2-sample t-test for proportions, 46% vs. 22%, Z=3.26, p<0.002). However, when Karner blues did oviposit (excluding all 0's), the mean number of ovipositions per location (all eggs within 1m^2 were counted as the same location) for the second brood was greater than the first brood (Wilcoxon 2-sample test, n=71, mean=2.89 ± 0.11 vs. mean=1.05 ±0.77, p<0.0001).

From the HOBO temperature loggers, I estimated the number of hours available for oviposition behavior was 10.7 hours/day for the first brood and 11.3 hours/day for the second brood. Multiplying by the observed ovipositions/minute for each brood, I estimated each female oviposited 34.9 eggs/day for the first brood and 34 eggs/day for the second brood. Using the 4 day lifespan of Karner blues, this resulted in 139.6 eggs/female for the first brood and 136 eggs/female for the second brood.

Thirty one oviposition heights were recorded during the first brood, and 80 were recorded during the second brood. Any failure to estimate height was random due to observer error. Oviposition heights ranged from 0-10 cm (0-4 inches) for the first brood and 2.5-13 cm (0.5-5 inches) for the second brood. The means differed significantly (t-test, df=109, t-statistic=-2.6, $p<0.015$) indicating oviposition heights were higher during the second brood adult stage. During the first brood of Karner blues, ovipositions were only on lupine plant stems. For the second brood, we observed Karner blues laying eggs on lupine (79.8%), grasses (16.7%), dewberry (1.2%), early goldenrod (1.2%), and on the ground (1.2%).

DISCUSSION

I was able to successfully quantify a behavioral response of Karner blues to management treatments, and infer differences in habitat quality from these results. During the second brood, male and female abundance shifted to the burned management units, and overall, Karner blues avoided ovipositing in unmanaged areas, often characterized by high amounts of leaf litter. Additionally, adult foraging did not significantly change due to management treatments.

There are a multitude of oviposition studies, which indicate butterflies use cues such as host-plant nitrogen (Ellis 2003; Myers 1985; Prudic et al. 2005), size of host-plants or leaves (Ellis 2003), ants (Fraser et al. 2002; Pierce & Elgar 1985), and host-plant scent (Feeny et al.

1989) to determine oviposition preferences. Previously (Chapter 1), I found that lupine nutritional quality did not differ between management units. However, recent population viability analyses have confirmed that specific fire regimes positively affect survival and fecundity of plant species in fire-prone ecosystems (Menges & Quintana Ascencio 2004; Menges et al. 2006). Butterfly studies have yet to incorporate such knowledge, even though it is logical that some butterflies, which overwinter as eggs or larvae, could use cues of host-plant survival to determine oviposition preferences. In fact, many plant studies have documented that visible plant characteristics, such as plant size, indicate future survival and fecundity (Gurevitch et al. 2002). In this study, lupine stems did decrease in high leaf litter areas (Chapter 1), and aboveground biomass of lupine has been shown to increase after burning (Grigore & Tramer 1996). Three unmanaged units in this study were not burned for approximately 4 years, and generally accumulated a high amount of leaf litter (>3.5 cm). The other unmanaged unit, South Piels, had not been burned in 7 years, since the area is open and only a small amount of litter (and fuels) accumulated. South Piels (Figure 4) was the only site where Karner blues oviposited in an unmanaged management unit.

The behavior of the Karner blue in this study supports previous research on Karner blues and other butterfly species. Maxwell (1998) found second brood Karner blue larvae were more abundant in small burned areas compared to control treatments. Research on the Fender's blue butterfly, *Icaricia icarioides fenderi*, by Schultz and Crone (1998) found prescribed burning significantly increased the butterfly's per capita oviposition rate. Although host-plant nutrients, foraging rate, and movement were not directly examined, a behavior mediated response, as I have found with the Karner blue, could be responsible.

Previous Karner blue studies in Minnesota/Wisconsin (Lane & Andow 2003; Maxwell 1998) and Indiana (Grundel et al. 1998b) have supported the notion that Karner blues oviposited preferentially on lupine shaded by canopy cover, and management has followed these recommendations. Counts of eggs to infer oviposition preferences in the field are problematic for two reasons. First, eggs are often difficult to find (Karner blue eggs are <0.8mm in diameter). Second, we do not know if butterfly movement corresponds to host-plant quantity or is random in a given area. This creates a question of whether to use individual plants or area as part of the dependent variable (eggs/m^2 or eggs/plant) in oviposition studies, and results do differ based on this assumption (Lane & Andow 2003). Karner blue larvae have been found to be significantly more abundant in shaded areas (Maxwell 1998), but an experimental study provides evidence that higher survivorship could be the reason (Lane & Andow 2003). In my study, Karner blue abundance did not significantly increase with more canopy cover on a management unit scale, and few individuals were seen under canopy cover.

I quantified an immediate behavioral response of the Karner blue to a quickly degrading habitat, and this behavioral response appears to be critical for implementing adaptive management for the species. My survey data shows recolonization of burned areas within 120 meters of a source of Karner blues is rapid, and supports the idea of inspecting daily movement distances before determining areas for temporarily detrimental management treatments. The preference for burned management units in this study may indicate hierarchal decision-making by the species. The burned management units had a general trend towards lower vegetation density and less canopy cover compared to other treatments, although these were not significantly different. Based on the lower lupine nutritional quality associated with these characteristics (Chapter 1), I would have expected a decreased use of burned units. Therefore,

high leaf litter depth or smaller plant size may provide primary cues to Karner blues to avoid ovipositing on plants in such areas, while nutritional quality may be a secondary preference.

In the Midwestern, USA there should be natural selection for Karner blues that leave savanna areas where succession is proceeding towards an oak forest, since the oak savanna region in Ohio was historically a mosaic of oak forests, oak savanna, and prairie (Brewer & Vankat 2004). Karner blues that recolonize early successional savanna, such as recently burned areas, should provide high quality habitat for their offspring. Similar evidence for the benefits of disturbance has been gathered in Wisconsin, where lupine stems and Karner blue larval signs per stem were greater in disturbed areas such as vehicle ruts (Smith et al. 2002). Disturbance could also lead to changes in the abundance of ants, which are mutualistic with Karner blue larvae. Hence, future studies of the Karner blue should focus on the factors that affect lupine survival and ant abundance.

Life History

Pickens (in prep) found that Karner blue populations in New York were more density-dependent during the first brood compared to the second brood, and the oviposition strategy presented here supports those conclusions. If lupine is a limiting factor, it should be more limited for the first brood adults because it appears optimal for Karner blues to spread their ovipositions on many lupine plants. There are two distinct possibilities for the observed difference in oviposition strategy between broods.

The Karner blues may not be able to predict which plants are vulnerable to droughts in June-July, so the eggs are spread as much as possible during the first brood adult stage. This would involve a trade-off of expending more energy and risking predation in order to spread

ovipositions compared to efficiently placing 3-4 eggs on a single plant as was done during the second brood adult stage.

A second potential reason for a differing oviposition strategy is that the survival of eggs through the nine month overwintering stage could be less than the survival of eggs for the summer, which hatch 5-10 days after oviposition. Three or four feeding larvae could compete for lupine foliage. But if only 1 of 4 eggs survive through the winter to hatch, there will be no problem with larval competition. Each of these theories is plausible and a combination of these two theories could have lead to the observed behavior of the species. Future studies could assist in determining if this behavioral strategy is genetic or a response to environmental conditions.

A study in Indiana found Karner blues oviposited on lupine 24.1% of the time during the second brood and all eggs were on lupine for the first brood (Grundel et al. 1998b). The substrates recorded in this study were primarily lupine for both broods (first brood- 100%, second brood- 79.8% of ovipositions), and this could be indicative of different vegetative structures or changes in selection pressures in geographically separate regions of the Karner blue range. Our data showed Karner blues deposited their eggs at a greater height during the second brood compared to the first. Oviposition heights could be lower during the first brood to provide vegetative shade to eggs and larvae since they hatch in the hot, dry conditions of June-July. The range of heights used for oviposition during the second brood, ≤ 13 cm (≤ 5 inches), supports management by mowing at the height of 15-20 cm (6-8 inches), which is already implemented throughout the Karner blue range.

Total Eggs

The total number of eggs produced by females in the field (e.g. 139.6 or 136 per female) is comparable to the number of eggs produced by females brought into captivity, which has been

noted at a maximum of about 200 eggs, but is usually much less (unpublished reports, Toledo Zoo). The increased foraging time for the second brood adults (see Chapter 1) did not result in more ovipositions per butterfly for the second brood. This further supports the theory of a nutrient source trade-off between life stages. When lupine nitrogen is low in the second brood larval stage (Chapter 1), adult foraging must increase to sustain similar egg production. Additionally, Karner blue egg size or composition may differ between the two broods. Butterfly research has yet to been conducted on such research questions.

Stems vs. Area

The U.S. Fish and Wildlife Service currently issues permits for annually burning 1/3, mowing 1/3, and leaving 1/3 of the lupine stems unmanaged at each occupied Karner blue site in Ohio. This is probably a response to general correlations of Karner blue and lupine abundance on the landscape. However, if movement is random within each lupine site, an increase in management unit area will result in increased habitat use. Although we found lupine stems and lupine area to be correlated, area explained the abundance of Karner blues better than lupine stems. For future consideration, I recommend management to be based on the area of lupine and on-the-ground observations of Karner blue habitat, which will also allow beneficial management to proceed.

Sun, Shade, and Butterfly Stress

Out of 373 total surveys, 91 (24.3%) were made with the temperature in the shade $<24.5C^0$ (76^0 F), and only 21 of these were in the second brood. Based on these results, it is unlikely that shaded habitat use was limited by low temperatures. Individually, no females were initially observed in shaded areas during the first brood and only 8 (6 burned area of Oak Dune, 1 Julia's

Savanna burned, 1 Julia's Savanna unmanaged) of 124 second brood females were observed in the shade during the second brood. Only 1 out of 246 males were observed in a shaded area. This supports a previous study (Grundel et al. 1998b), which had shown female Karner blues used areas with canopy cover more often than males.

Conclusion

In this relatively small, isolated insect population, monitoring the behavior of the Karner blue led to robust sample sizes and substantial conclusions that can be directly applied to management for the species. I have found my hypothesis of Karner blues preferring burned areas to be supported, but the preference for these areas cannot be attributed to a higher nutritional quality of lupine (Chapter 1). Instead, a high leaf litter accumulation appears to deter ovipositions from female Karner blues. Many Karner blues found in unmanaged areas looked like fresh, newly emerged butterflies and they frequently did not oviposit. This suggests that unmanaged areas acted as a refuge for overwintering Karner blues, but recent deterioration of the habitat deterred future reproduction in unmanaged areas. The behavioral response of the Karner blue was immediate, and was able to explain the increased abundance in burned management units during the second brood. Since the presence of a species does not always indicate high quality habitat, and demographics are often impossible to measure, the behavior of a species can be a valuable, rapid indicator of how management or habitat degradation affect species.

MANAGEMENT IMPLICATIONS

It is clear that intensive monitoring is critical for managing the reintroduction of the Karner blue in Ohio, since the ecology of this species is relatively unknown for the region. There has been no particular burn frequency recommended for Karner blue habitats. In Wisconsin, Maxwell (1998) suggests prescribed burning should not be implemented in Karner

blue areas until canopy cover results in a severally diminished amount of lupine, since lupine with canopy cover was claimed to be valuable. Fire intervals in the Albany Pine Bush in New York was estimated to be between 6 to 18 years (Givinish et al. 1988) before European settlement, and a Wisconsin savanna remnant had an average fire interval of 3.7 years according to tree ring analysis (Wolf 2004). For *L. perennis* populations, Grigore (Grigore & Tramer 1996) suggests intervals of greater than two years to allow for successful germination.

Although the tree density of an area plays a large role in the accumulation in leaf litter, generally, 4 years of leaf litter accumulation appears to deter oviposition behavior of Karner blues. Peterson and Reich (Peterson & Reich 2001) found a fire return interval of ≤ 3 years eliminated oak saplings, while 8 year intervals promoted "thickets of saplings." The Nature Conservancy has recently initiated management allowing some oaks to grow to 1-2 meters in height (about 3 years old), and, subsequently, the rare Edward's hairstreak (*Satyrium edwardsii)*, has extended its range well beyond its previously documented extent noted by Shuey (1986). *S. edwardsii* is a specialist on black oak or scrub oak leaves (Struttmann 2006), and probably has benefited from this management. A small amount of saplings could also assist in providing high quality, shaded lupine to Karner blues as well.

Heterogeneous vegetation structure should be promoted by prescribed burning with fire return intervals of approximately 4 years to prevent leaf litter accumulation. This coincides well with Shuey's recommendation of burning on a 5-year interval for the Kitty Todd preserve (Shuey 1986). Careful consideration should be given to planning management units because burning or not managing completely shaded or completely open areas should be avoided. Temporarily detrimental management, such as burning, should accommodate the recolonization rates for at-risk species. The Karner blue probably represents the dispersal ability of the other three

Lepidoptera species of concern at Kitty Todd (Frosted elfin- *Incisalia irus*, Persius duskywing- *Erynnis persius*, and Edward's hairstreak- *Satyrium edwardsii*) since they are all similar sizes, and Karner blues recolonized burned areas within 120 meters of source populations easily.

In conclusion, in order to sustain Karner blue populations and the pre-European fauna and flora of Midwestern oak savanna, high leaf litter areas (>3.5 cm) should be burned. In Midwestern oak savanna, the Karner blue represents one of the most sensitive species to disruptions in natural disturbance processes, so if we can preserve this species, we will also be restoring natural processes to the oak savanna. This should be beneficial to the 100+ state threatened and endangered species that also occupy this ecosystem.

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TABLES AND FIGURES

Table 1. Generalized linear model using the Poisson distribution for **first brood female Karner blue abundance** and management unit variables. Chi-square and p-values are given for the likelihood ratio type III tests, and, when applicable, comparisons within a variable.
* = variable included in final model; other variables are reported when they exited model

Variable	Chi-square	p	Association
Site*	5.2	0.16	none
Area of MU*	8.2	<0.005	positive
% of MU shaded*	18.3	<0.0001	negative
Management Unit	3.4	0.18	none

Table 2. Generalized linear model using the Poisson distribution for **second brood female Karner blue abundance** and management unit variables. Chi-square and p-values are given for the likelihood ratio type III tests, and, when applicable, comparisons within a variable are in italics.
* = variable included in final model; other variables are reported when they exited model

Variable	Chi-square	p	Association
Site*	24.9	<0.0001	N/A
Area of MU*	0.97	0.32	none
% of MU shaded*	1	0.31	none
Management Unit*	37.5	<0.0001	
Burned vs. Unmanaged	17.54	<0.0001	more abundant in burn
Burned vs. Mowed	25.54	<0.0001	more abundant in burn
Mowed vs. Unmanaged	0.15	0.70	none

Table 3. Generalized linear model using the Poisson distribution for **first brood male Karner blue abundance** and management unit variables. Chi-square and p-values are given for the likelihood ratio type III tests, and, when applicable, comparisons within a variable.
* = variable included in final model; other variables are reported when they exited model

Variable	Chi-square	P	Association
Site*	125.1	<0.0001	N/A
Area of MU*	31.0	<0.0001	positive
% of MU shaded*	1.0	0.31	none
Management Unit	3.2	0.20	none

Table 4. Generalized linear model using the Poisson distribution for **second brood male Karner blue abundance** and management unit variables. Chi-square and p-values are given for the likelihood ratio type III tests, and, when applicable, comparisons within a variable are in italics.
* = variable included in final model; other variables are reported when they exited model

Variable	Chi-square	P	Association
Site*	52.2	<0.0001	N/A
Area of MU*	5.0	<0.03	positive
% of MU shaded*	1.26	0.26	none
Management Unit*	20.4	<0.0001	
Burned vs. Unmanaged	18.72	<0.0001	more abundant in burn
Burned vs. Mowed	6.86	<0.009	more abundant in burn
Mowed vs. Unmanaged	8.72	<0.004	more abundant in mowed

Table 5. Generalized linear model using the negative binomial distribution for the **first and second brood** oviposition rate by management unit. Chi-square and p-values are given for the likelihood ratio type III tests, and, when applicable, comparisons within a variable are in italics. * = Variable included in final model; other variables are reported when they exited model
N/A= not relevant to analysis and not reported

Variable	Chi-square	p	Association
Site*	1.23	0.74	N/A
Brood*	0.41	0.52	none
Management Unit*	6.86	<0.035	
Burned vs. Unmanaged	5.43	<0.015	higher oviposition rate in burned
Burned vs. Mowed	0.02	0.90	none
Mowed vs. Unmanaged	5.58	<0.016	higher oviposition rate in mowed

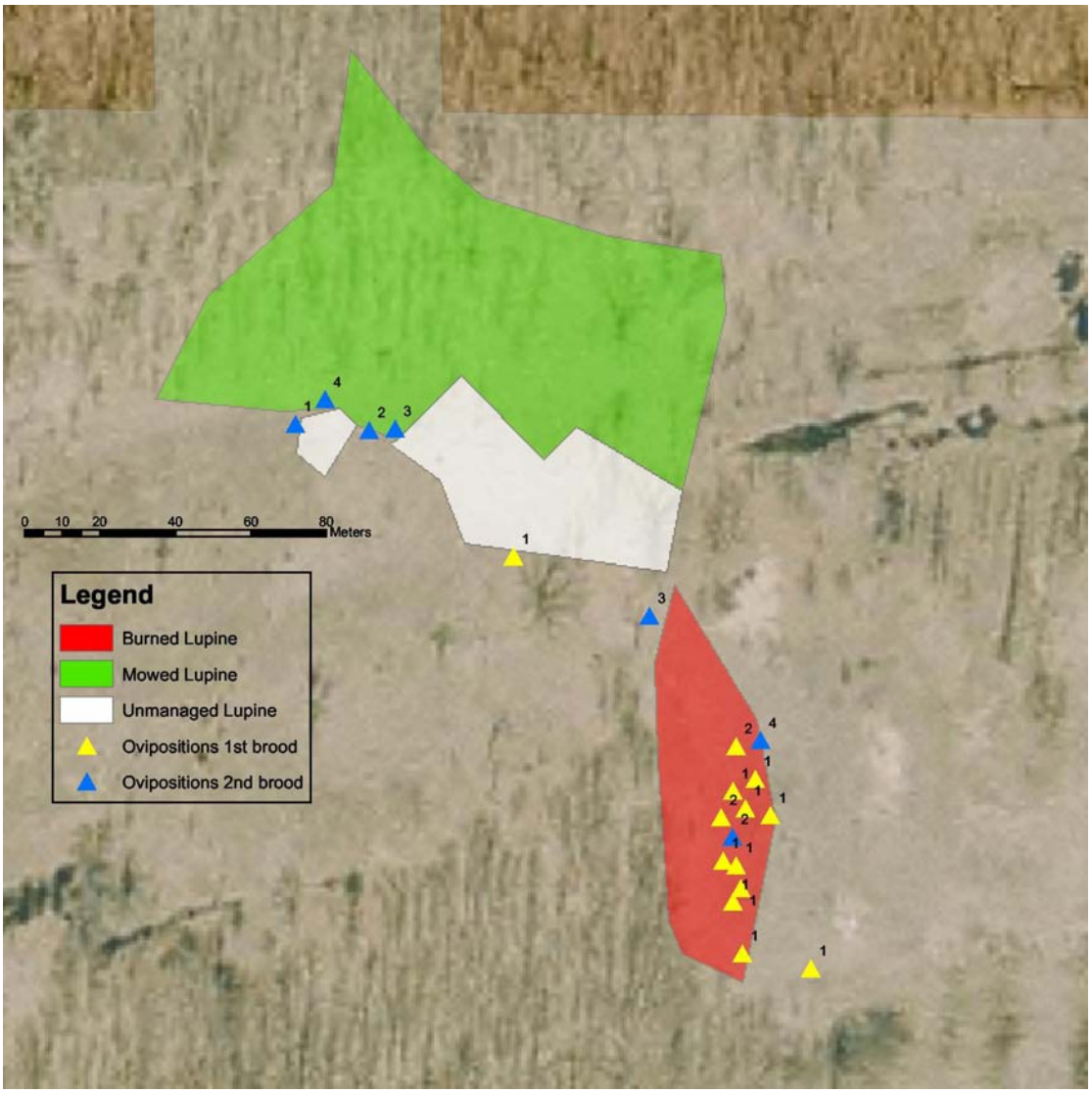

Figure 1. Map of ovipositions for each brood, indicated by triangles, by management unit at Julia's Savanna, Kitty Todd Preserve, Ohio. Numbers near each triangle indicate the number of eggs laid.

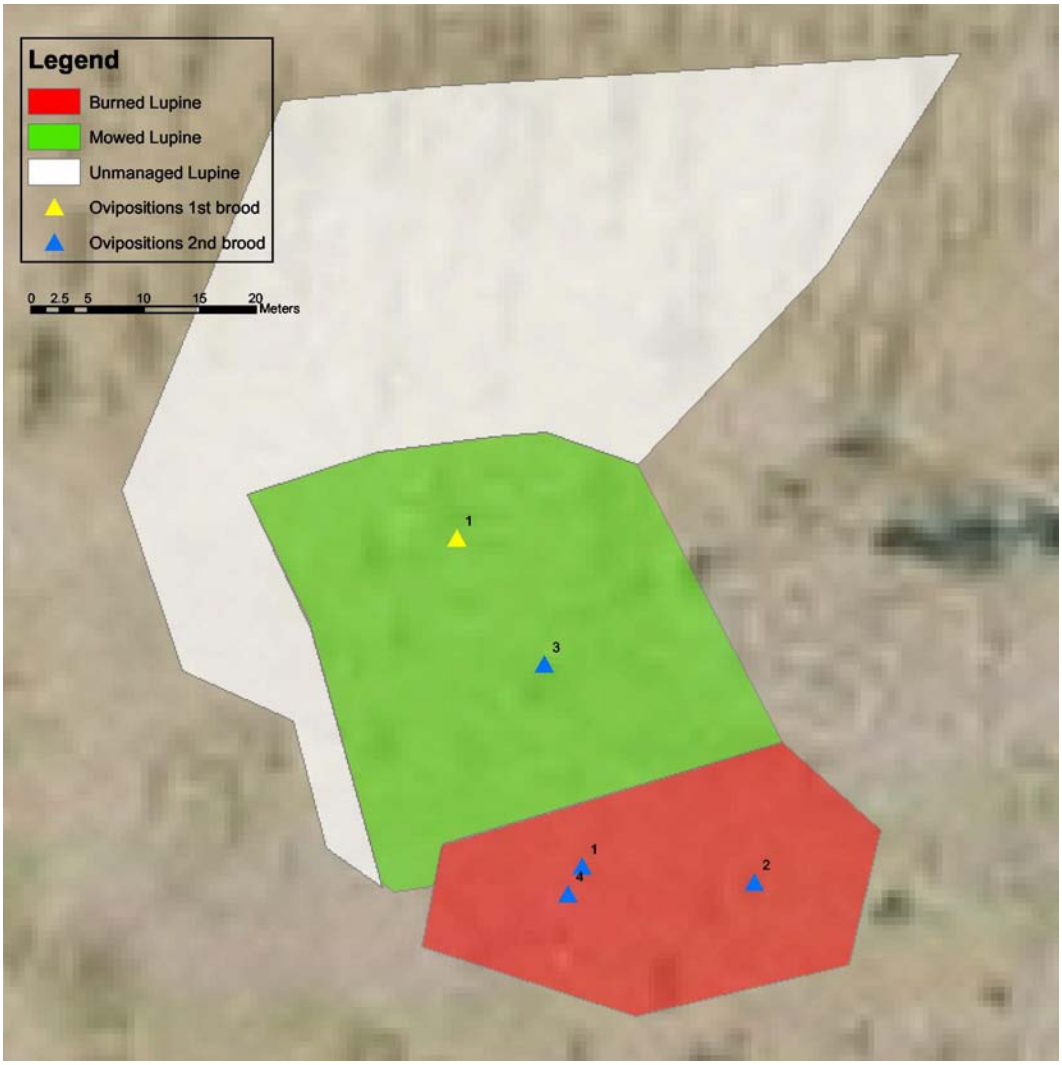

Figure 2. Map of ovipositions for each brood, indicated by triangles, by management unit at Bond, Kitty Todd Preserve, Ohio. Numbers near each triangle indicate the number of eggs laid.

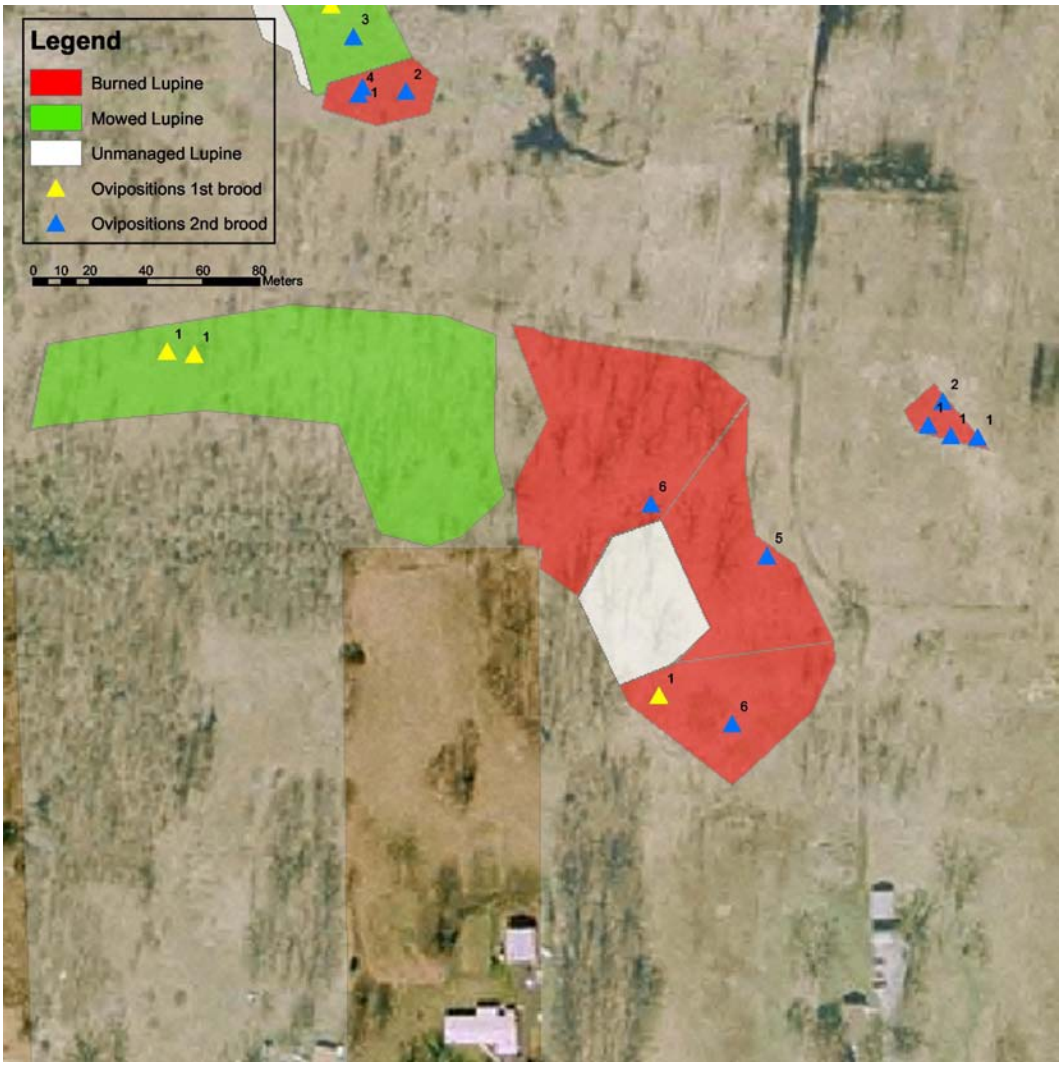

Figure 3. Map of ovipositions for each brood, indicated by triangles, by management unit at Oak Dune, Kitty Todd Preserve, Ohio. Numbers near each triangle indicate the number of eggs laid.

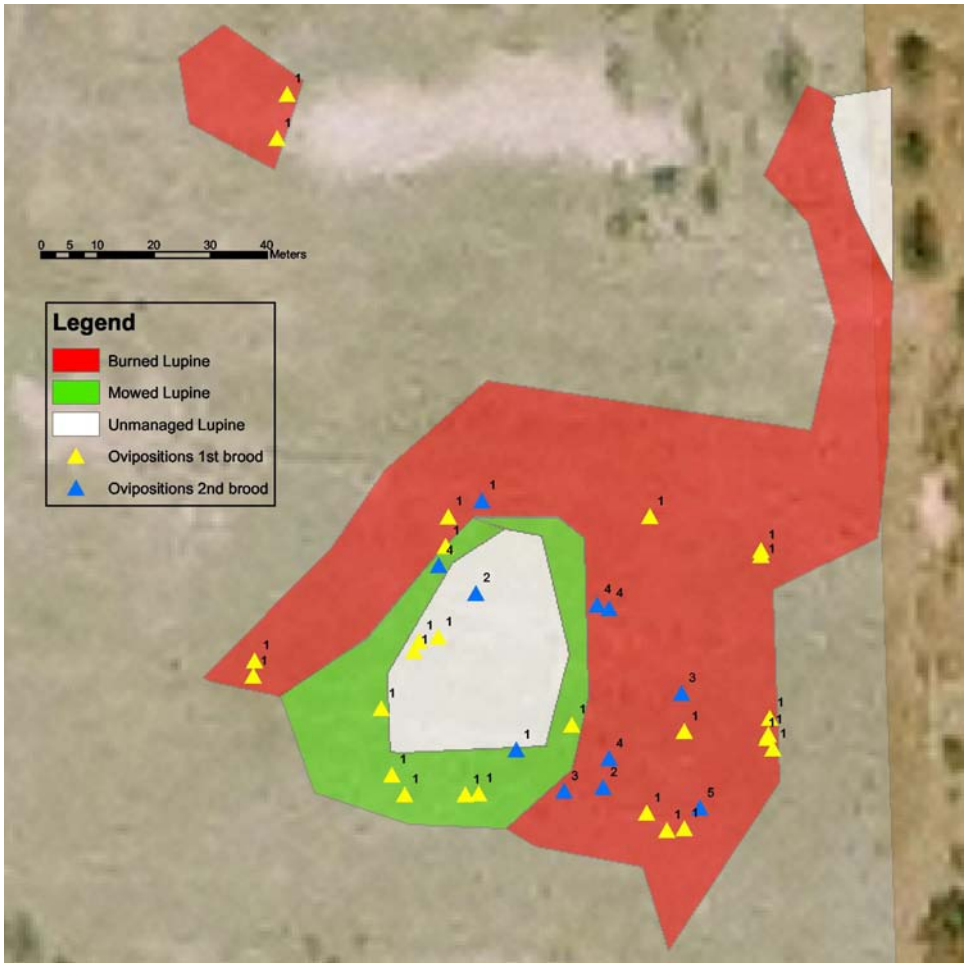

Figure 4. Map of ovipositions for each brood, indicated by triangles, by management unit at South Piels, Kitty Todd Preserve, Ohio. Numbers near each triangle indicate the number of eggs laid.

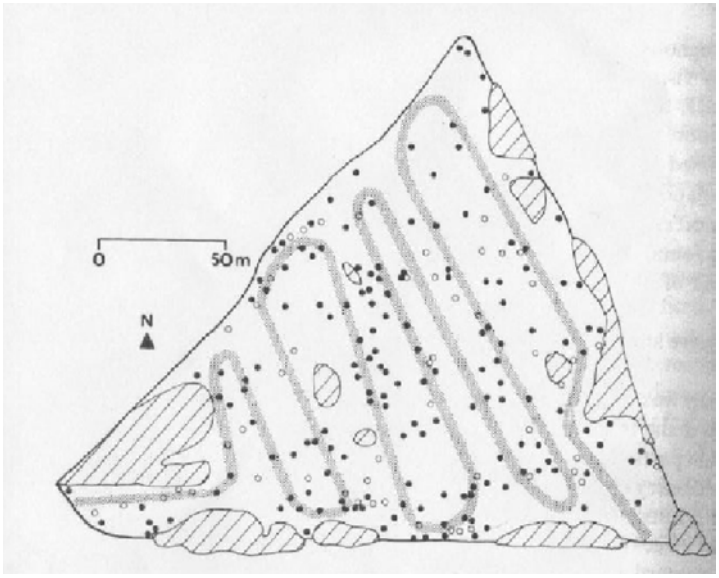

Figure 5. Example of survey methodology used to estimate number of Karner blue butterflies present based on modified Pollard-Yates transects (Pollard & Yates 1993; Thomas 1983). Figure from Thomas (1983).

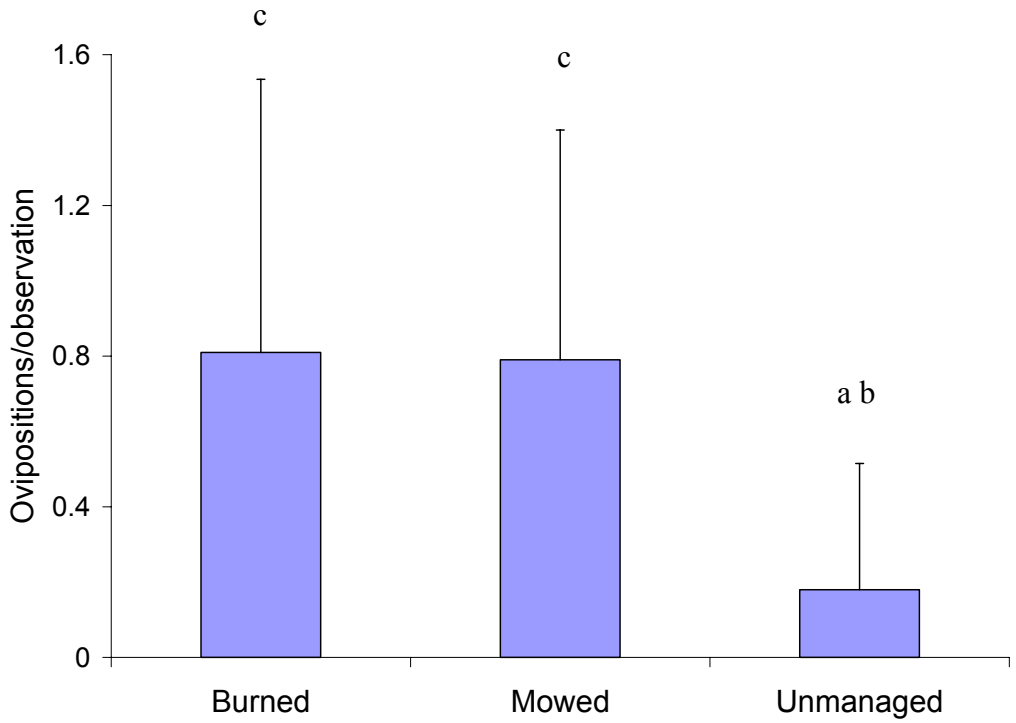

Figure 6. Oviposition rate (ovipositions per observation) by management unit.
Error bars represent standard deviation; Letters indicate significantly different values ($p<0.016$).

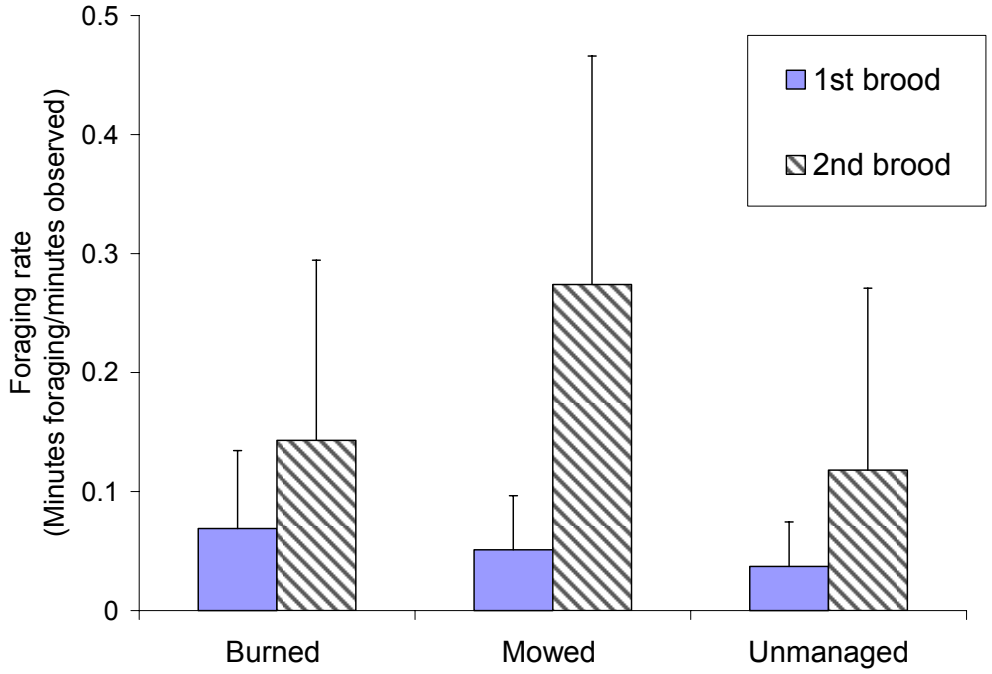

Figure 7. Female foraging rate for each Karner blue brood by management unit (Burned, Mowed, and Unmanaged). Error bars represent the standard deviation.

Miscellaneous notes and observations

Karner Blue Predation

At approximately 13:00 on July 3rd, 2005, I was surveying Karner blues when I observed two Karner blues fighting each other. They were both coming in and out of contact with each other and performing chases. In order to ensure the identity of both butterflies as male, I continued to observe them. As the Karner blues were flying approximately 1.5 meters from the ground, one male began to fly away from the encounter. At this point, I observed this male Karner blue getting quickly taken out of the air. I examined the immediate area and found a robber fly (Family Asilidae), perched on bare sand with a male Karner blue folded into the robber fly's mandibles.

Behavior

During hot and dry weather periods of the first brood adult Karner blue stage, females were observed alighting on lupine leaves and moving in a tight circle while batting their antennae against the lupine leaves. The butterfly would then decide to either crawl down the stem in order to oviposit or move to another plant. This circling behavior was much more prevalent late in the first brood even though the temperature was consistently hotter during the second brood. This does not support the notion that the circling behavior is only heat related (Lane 1999), but suggests the possibility that Karner blues might be examining lupine leaf quality.

APPENDIX

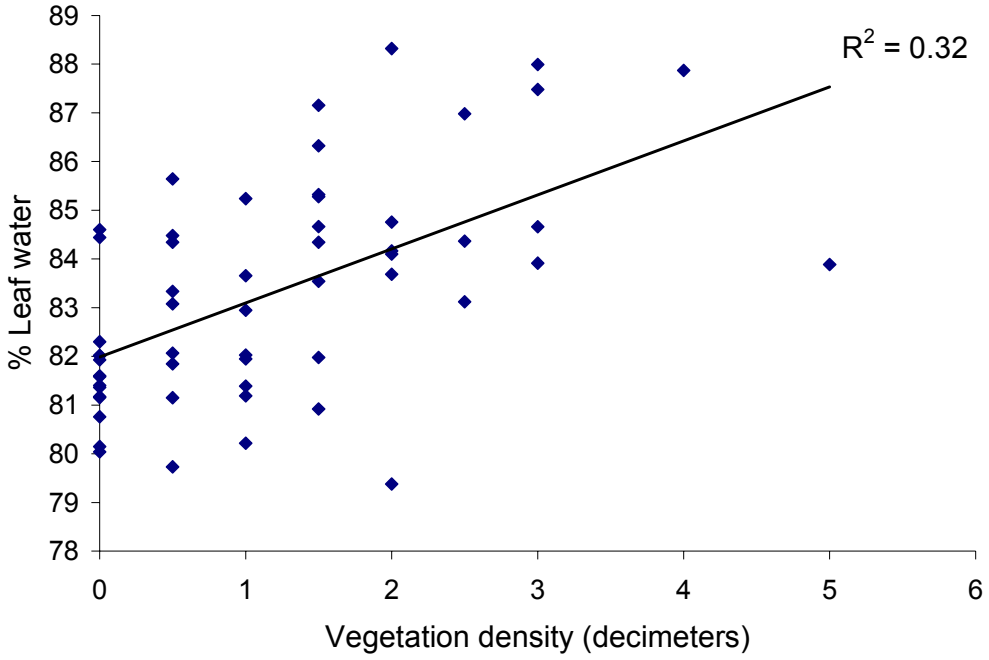

Figure 1. Lupine leaf water content, as percent weight, by vegetation density in decimeters for Karner blue second brood.

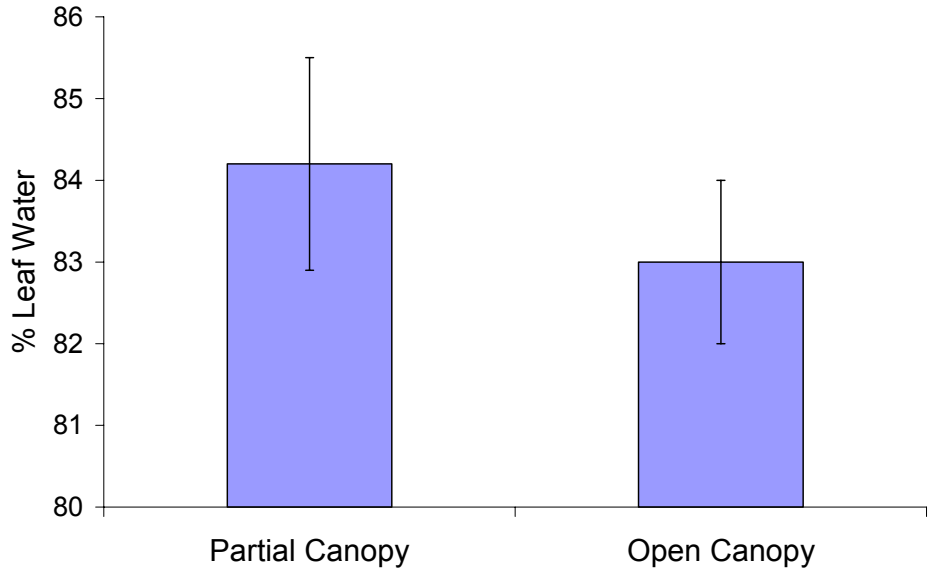

Figure 2. Lupine leaf water content, as percent weight, by canopy cover class for Karner blue second brood.

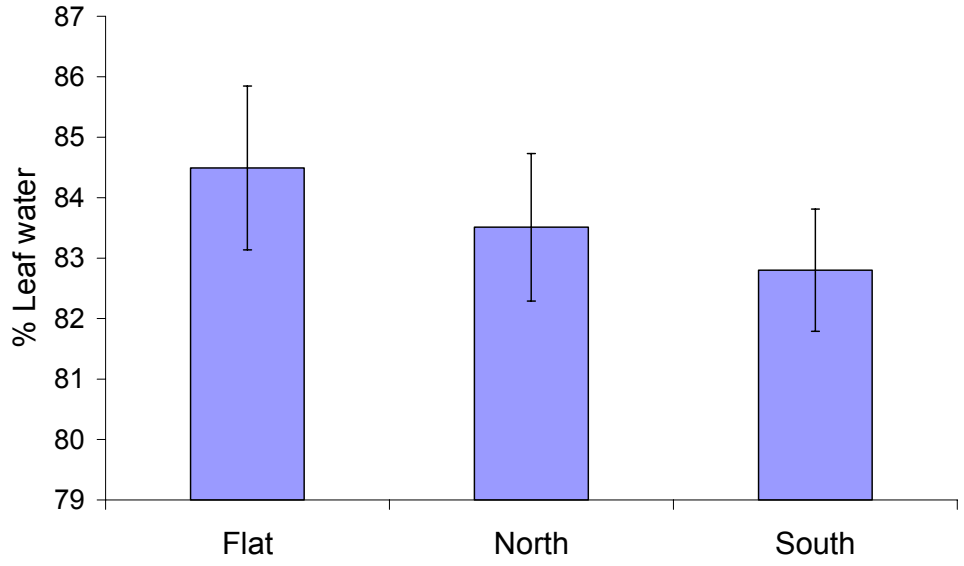

Figure 3. Lupine leaf water content, as percent weight, by aspect for Karner blue second brood.

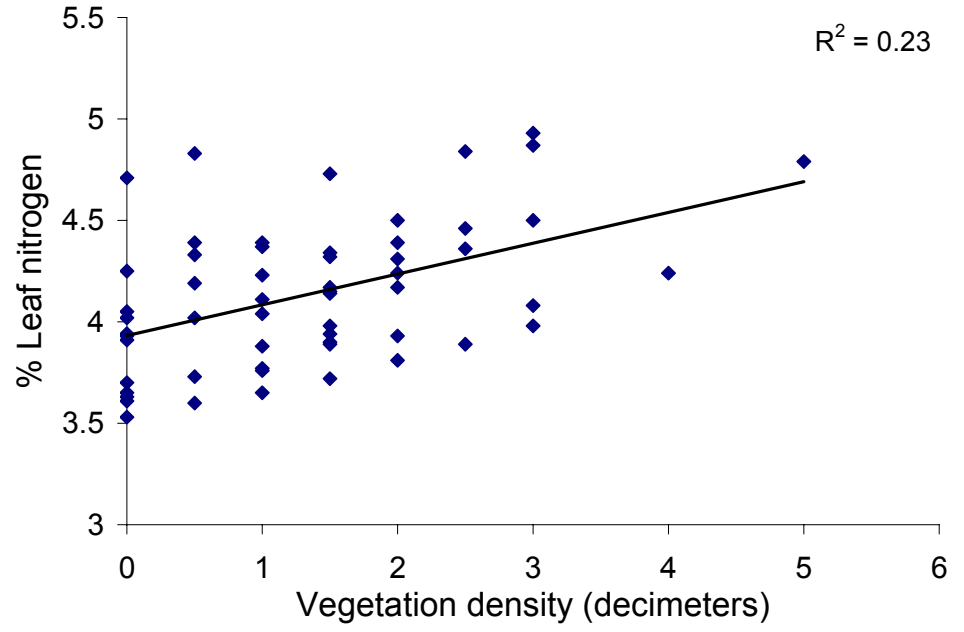

Figure 4. Lupine leaf nitrogen, as percent weight, by vegetation density in decimeters for Karner blue second brood.

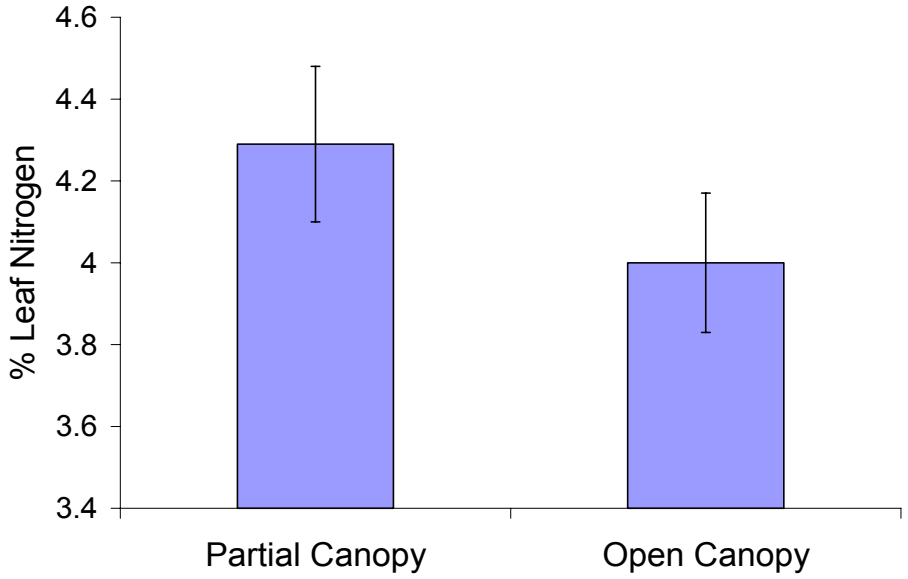

Figure 5. Lupine leaf nitrogen, as percent weight, by canopy cover class for Karner blue second brood.

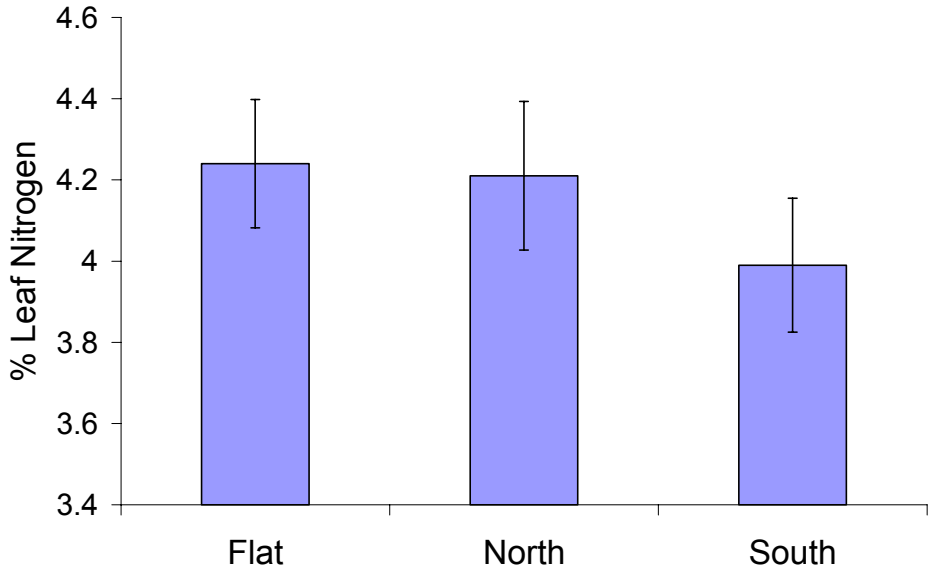

Figure 6. Lupine leaf nitrogen, as percent weight, by aspect for Karner blue second brood.