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**LYCAEIDES MELISSA SAMUELIS (LEPIDOPTERA: LYCAENIDAE)
RESPONSE TO AN AGGREGATION OF LYTTA SAYI
(COLEOPTERA: MELOIDAE) ON LUPINUS PERENNIS (FABACEAE)**

Jodi A. I. Swanson^{1, 2} and Paula K. Kleintjes Neff¹

ABSTRACT

Lycaeides melissa samuelis Nabokov, frequently called the Karner blue butterfly, is a Federally endangered species found in savanna/barren type ecosystems of New England and the Great Lakes region of North America. We observed sporadic and localized feeding aggregations of *Lytta sayi* LeConte (Coleoptera: Meloidae) on *Lupinus perennis* L. (Fabaceae) occupied by *L. m. samuelis* during the summers of 2000-2004, in Eau Claire County, Wisconsin. In 2004, we quantified the phenology and behavior of an aggregation (> 900 beetles) within a 1,020 m² stand of lupine and measured its effect upon adult *L. m. samuelis* behavior. The *L. sayi* aggregation formed and dispersed within 11 days with three beetles observed on day one and a maximum of 951 beetles on day seven. By the eighth day of the aggregation, the beetles had consumed 100% of the lupine flowers, 2% of lupine seeds and no lupine leaves. In comparisons of *L. m. samuelis* activity before and during the beetle aggregation, *L. m. samuelis* males spent significantly less time perching on *Potentilla simplex* Michaux (Rosaceae) and more time flying during the beetle aggregation. *L. m. samuelis* females spent significantly less time under lupine leaves during the beetle aggregation. Distribution of *L. m. samuelis* larval feeding damage suggests adult females avoided ovipositing in areas containing large numbers of beetles.

The US Fish and Wildlife Service placed the *Lycaeides melissa samuelis* Nabokov on the Federal endangered species list in 1992 (Clough 1992). *L. m. samuelis* reside in savanna/barren type ecosystems of New England and the Great Lakes region of North America in association with their sole larval host plant, *Lupinus perennis* L. (Fabaceae) (Blessner 1993, Dirig 1994). Interruption of naturally occurring disturbance regimes (i.e., fire, drought, grazing) has contributed to the succession and fragmentation of more than 99% of the historic distribution of savannas and barrens in North America (Nuzzo 1986, Leach and Givnish 1999). This is considered the most influential factor responsible for *L. m. samuelis* population declines (Clough 1992).

The US Fish and Wildlife Service (2003) identified larvae of the painted lady butterfly *Vanessa cardui* (L.) (Lepidoptera: Nymphalidae) and beetles in the family Meloidae as lupine herbivores of concern, but little is known about their potential effects on *L. m. samuelis*. Research suggests competition does not contribute significantly to the shaping of insect communities (Hairston et al. 1960, Strong, Jr. 1983); however, due to the restrictive lifecycle of *L. m. samuelis* and diminishing suitable habitat, further investigation of potential competition from lupine herbivores was warranted.

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We and others (J. Anklam pers. comm) witnessed annual aggregations of the blister beetle *Lytta sayi* L., (Coleoptera: Meloidae) feeding on lupine from 2001-2007 at one *L. m. samuelis* occupied site in Eau Claire County, Wisconsin. Our objective was to investigate the biology and behavior of *L. sayi* on lupine at this site and whether its presence had an effect upon adult *L. m. samuelis* behavior.

METHODS

Study insects. *Lycaeides Melissa samuelis* complete two generations per year. Adults fly from late May to mid June (spring flight) and mid July to early August (summer flight). Flight lengths average 24-35 days and 25-60 days, respectively. Adult *L. m. samuelis* live an average of four to five days (Andow et al. 1994). Females oviposit on the leaves and stems of wild lupine and in leaf litter near the base of lupine (Lane 1999). Summer flight eggs overwinter and hatch the following spring (Haack 1993). *L. m. samuelis* larvae feed on the top or bottom mesophyll of *L. perennis* leaves, leaving the epidermis of the opposite side intact (Blesser 1993, Swengel 1995). This results in a characteristic windowpane appearance that is statistically correlated with larval abundance (Swengel 1995). Lane and Andow (2003) found *L. m. samuelis* larvae remain near the site of oviposition and often on a single lupine stem.

The distribution of *L. m. samuelis* in central Wisconsin follows a band slightly wider than the tension zone (Blesser 1993) which is the boundary between northern and southern plant types (Curtis 1959).

Blister beetles go through hypermetamorphosis (more than one larval form) with a parasitic larval stage and phytophagous adult stage. Species of the genus *Lytta* complete one generation per year. *L. sayi* adults emerge in late spring and are active until mid-late summer (Selander 1960). Females create burrows in the soil for oviposition (Selander 1960, J. S. pers. obs.). First stage larvae actively seek out nests of bees (species unknown) where they feed through summer and overwinter as a non-feeding grub (Selander 1960). Selander (1960) lists the following hosts of adult *L. sayi*: *Prunus* (peach, cherry, plum), *Pyrus* (pear), *Rosa* (Rosaceae); *Kolkwitzia*, elder, and *Viburnum lentago* (Caprifoliaceae); *Robinia pseudo-acacia* and beans (Leguminosae); butternut (Juglandaceae); and wheat (Gramineae). There are anecdotal accounts of massive defoliation by *L. sayi* but this damage has not been scientifically quantified (Selander 1960). There are three discrete populations of *L. sayi* in the United States: New England (Connecticut, Massachusetts, Pennsylvania, New Jersey, New York and Vermont); north central United States (Illinois, Iowa, Minnesota and Wisconsin); and Wyoming (Selander 1960). Selander's distribution for *L. sayi*, which is the most recent published record, restricts its Wisconsin distribution to southern Wisconsin, however, recent sightings extend this distribution up to the tension zone of central Wisconsin. These recent sightings show an overlap between the ranges of *L. sayi* and *L. m. samuelis*.

Study area and design. We conducted our study May-August 2004 on private property in the Environmental Quality Incentive Program in Fall Creek, Wisconsin. We chose the site based on past sightings of *L. sayi* and an existing *L. m. samuelis* population (J. Anklam, pers. comm). The study area occurred between a native prairie restoration and a forest consisting of: white pine, *Pinus strobus* L. (Pinaceae); jack pine *P. banksiana* Lamb. (Pinaceae); and red oak, *Quercus rubus* L. (Fagaceae). Lupine occupied an area approximately 10 m × 125 m along the forested edge (Fig. 1). We established one transect through this area within a 10 m wide band of lupine. Each side of the transect was further divided into twenty-five, 5 m² quadrats. We numbered the quadrats 1-25 and designated them as north (n) or south (s) of the transect, e.g., 4s or 15n. We visually estimated percent cover of flowering lupine per quadrat. The same researcher (JS) made this estimation before the beetles arrived, during the beetle

Forested edge

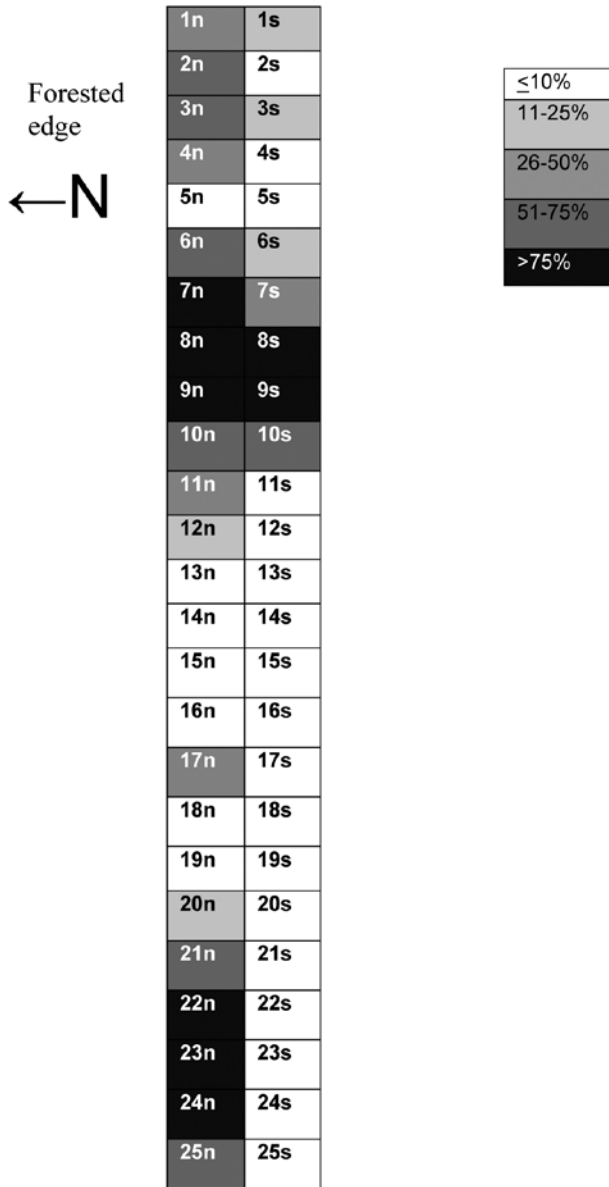


Fig. 1. Design layout of sampling quadrats in lupine occupied area of the Schofield study site, Fall Creek, WI. Shading represents percent cover of *L. perennis* in each 5 × 5-m quadrat. Quadrats are numbered consecutively 1-25 n (north) or s (south). The east and west regions of the site include quadrats 1-12 and 17-25, respectively.

aggregation and after the beetles dispersed. We counted the number of stems with flowers from 40 randomly chosen clumps of lupine. We also estimated percent cover of *Potentilla simplex* Michaux (Rosaceae) in late May, as it was the most abundant nectar source on the site.

We monitored adult *L. m. samuelis* of the spring flight in conditions outlined by the Wisconsin Department of Natural Resources (2000); partially sunny to sunny skies, temperatures above 15.5°C and winds less than 33 km/h (WI DNR 2000). We estimated the *L. m. samuelis* adult population size by walking a slow, steady pace along the transect and searching for butterflies within a 5 m arc of the observer. We recorded the sex of each butterfly and the number of the quadrat it occupied. We monitored *L. m. samuelis* adult behavior during ten-minute observation periods. We chose the number of observation periods to be proportional (2:1) to the number and sex of butterflies counted on the transect. We attempted to maintain a 2 m buffer between observer and butterfly to minimize disturbance. We started these observations by walking the transect until a butterfly was observed. We then followed the individual butterfly for 10 min and recorded the proportion of total observation time they spent flying or perching. We also recorded plant species chosen for perching, location on the plant, substrate (*P. simplex* flowers, *L. perennis* flowers or leaves, orange hawkweed *Hieracium aurantiacum* L. (Asteraceae), clover *Trifolium* spp. (Fabaceae), blackberry *Rubus fruticosus* L. (Rosaceae), grasses, soil), and the quadrat of occurrence. At the end of the ten minute period, we returned to the transect and continued in the same direction as previously traveled until another butterfly was encountered and another observation period began. At the end of each larval period, we counted the number of lupine leaves with *L. m. samuelis* larval feeding damage on each of the 40 designated clumps.

We monitored lupine daily for the presence of *L. sayi*. Once the aggregation appeared, we conducted absolute counts of adult beetles 1-3 times per day when walking the established transect through the lupine patch. We recorded the number of beetles per stem, mating status (mating or not mating) and the quadrat of occurrence.

We conducted presence/absence surveys of both *L. m. samuelis* and *L. sayi* at this site again in 2005, 2006 and 2007.

Data analysis. We used a two-way ANOVA to compare the interaction of (sex × time) the mean proportion of observation time, male and female *L. m. samuelis* (sex) spent perching or flying, before and after (time) the appearance of *L. sayi* on lupine. We also used a two-way ANOVA to compare the mean proportion of observation time the sexes (sex) spent perching before and during (time) the appearance of *L. sayi* on lupine and their potential interaction (sex × time period) on each substrate. Between subjects effects were tested for each substrate. All analyses were performed with ©SPSS (2003) and data were transformed as needed (i.e., arcsin transformation for proportions) to meet the assumptions of ANOVA.

RESULTS

Lupine began vegetative growth the second week of April and began flowering approximately two weeks later. Lupine patches developed from two centers of concentration designated as east and west regions (Fig. 1). During *L. m. samuelis* first flight (3-17 June) and the *L. sayi* aggregation (6-15 June), lupine was in full bloom with apical seed development. Nectar sources available during *L. m. samuelis* first flight were lupine, *P. simplex*, clovers, blackberry and orange hawkweed.

We observed the first butterflies on 3 June 2004, and the last on 17 June 2004. Total numbers of butterflies per survey ranged from 1-6 with a mean of 3.3 (± 1.2 SD) per survey over the 15-day first flight period. We obtained 56

independent 10-min observation periods of individual butterflies, 14 of each sex before and during the presence of the *L. sayi* aggregation (Table 1).

The proportion of time spent perching and flying significantly differed by sex ($F = 91.36$, $df = 1$, $P < 0.01$) and time period \times sex ($F = 4.99$, $df = 1$, $P < 0.05$). Males spent more time flying before (46.7%) and during (68.2%) the beetle aggregation than did females, 9.3% and 7.1%, respectively. Females spent significantly more time perching before (90.7%) and during (92.9%) the beetle aggregation than did males, 53.3% and 31.8%, respectively. Both sexes spent the majority of perching time in the east region of the study area during the entire flight period (Table 1).

Butterflies perched on a variety of substrates, which were analyzed in the following categories: *P. simplex* flowers, *L. perennis* flowers or leaves, grasses, soil, other flowers and other forb leaves. Other flowers and other forb leaves, were used less than 1% of total observation time. The proportion of time butterflies spent perching on all substrates differed significantly by sex ($F = 239$, $df = 7$, $P < 0.05$) but not by time period or sex \times time period. The use of lupine differed significantly between the sexes ($F = 7.70$, $df = 1$, $P < 0.01$). Males spent the greatest amount of perching time on *P. simplex* flowers (44.3%) before the *L. sayi* aggregation and on lupine leaves (31.2%) during the aggregation (Table 2). Females spent the greatest amount of perching time on lupine leaves before (65.4%) and during (48%) the aggregation. Both sexes significantly reduced their time on *P. simplex* flowers ($F = 4.9$, $df = 1$, $P < 0.05$) during the presence of the beetles and increased their use of other flowers ($F = 8.001$, $df = 1$, $P < 0.01$). Although both sexes reduced their perching time on lupine leaves during the presence of the beetles, it was not significant.

The *L. sayi* aggregation began with three beetles on 6 June and increased to 951 beetles by 12 June. Numbers diminished to zero by 16 June (Fig. 2). The mean (\pm SD) number of beetles per lupine stem was 2.0 (\pm 0.58) within a range of 1-18. Mating individuals composed 32.1% of the population size early in the aggregation (9 June). This percentage declined during a period of heavy rains (9-11 June) followed by a rapid rise in the population on 12 June (Fig. 3). By 13 June, beetles had consumed all lupine flowers and began to disperse and the proportion of mating individuals was 24.2%. The majority of the aggregation occurred in the East region (7s and 8s) for most of the aggregation although on the peak day the population was dispersed across the site (Table 3). The mean (\pm SD) percent cover of lupine per quadrat before the beetles arrived was 31.8 (\pm 1.3) % (Table 3) and declined to 7.8 (\pm 0.5) % by 10 June. Before the beetles appeared, the mean (\pm SD) number of stems with flowers per clump was 16.2 (\pm 8.5) which declined to 2.5 (\pm 3.4) by 10 June and to zero by June 13. On 13 June the beetles began feeding on lupine seeds and consumed approximately 2% of the seeds before dispersing off the site.

First brood *L. m. samuelis* larvae feeding signs were found on 26 lupine leaves on 15% of the designated clumps. 38.4% of this feeding occurred south of the transect (i.e. less shade). Second brood feeding signs were found on 63 leaves on 35% of the clumps with 14.3% of these signs south of the transect (Table 3).

Table 1. Distribution (%) of perched male and female *L. m. samuelis* in the east vs. west regions of the lupine occupied area before and during the formation of the *L. sayi* aggregation, 2-17 June, 2004.

	Females (n=14)		Males (n=14)	
	East	West	East	West
Before	92.5	7.4	87.1	12.9
During	87.6	12.3	85.2	14.7

Table 2. Mean proportion of total perching time *L. m. samuelis* spent on specified substrates before and during the establishment of *L. sayi* aggregation (3-17 June, 2004). Substrates with less than 10 observations of *L. m. samuelis* were pooled into a single category (OTHER).

Sex	Period	% Time		¹ <i>P. Simplex</i>		¹ <i>L. perennis</i>		Other		Grass		
		Perching	Flying	Flower	Leaf	Flower	Leaf ^A	Flower ^B	Leaf			
Female	Before	90.7	9.3	21.9	0.0	1.0	65.4	14.9	0.0	0.6	3.6	5.4
	During	92.9	7.1	11.0	2.0	1.3	48.0	7.6	16.6	5.3	7.7	16.5
Male	Before	53.3	46.7	44.3B	1.3	1.2	24.2	0.0	0.0	0.3	23.0	4.0
	During	31.8	68.2	18.2B	0.7	0.0	31.2	0.0	8.7	7.6	14.0	8.51

¹Multi-way ANOVA $P < 0.05$.

^A between sexes.

^B between periods per respective substrate.

Table 3. Observational data recorded per 5-m² sampling quadrat (n=50) of the designated study area.

Q	¹ Mean % of <i>L. sayi</i> population		² %Cover <i>P. simplex</i>		³ %Cover <i>L. perennis</i> 6 June		⁴ Cover <i>L. perennis</i> 11 June (n=26)		⁵ Larval damage sites before <i>L. sayi</i> (n=63)		⁶ Larval damage sites during <i>L. sayi</i>	
	N	S	N	S	N	S	N	S	N	S	N	S
1	0	0.8	0	0	40	25	5	10	0	0	0	1.6
2	0	4.9	0	0	75	5	10	0	0	0	0	0
3	0	4.9	0	0	75	15	5	0	0	0	9.5	4.8
4	3.2	0	0	0	40	0	5	0	0	0	1.6	7.9
5	0	0	0	0	5	0	0	0	0	0	0	0
6	0.5	0.5	0	25	60	20	15	5	0	38.4	0	0
7	3.0	39.4	0	50	90	40	25	5	0	0	3.2	0

Table 3. Continued.

Q	¹ Mean % of <i>L. sayi</i> population		² %Cover <i>P. simplex</i>		³ %Cover <i>L. perennis</i> 6 June		<i>perennis</i> 11 June (n=26)		⁴ % Cover <i>L. sayi</i> damage sites before <i>L. sayi</i> (n=63)		⁵ % Larval damage sites during <i>L. sayi</i>	
	N	S	N	S	N	S	N	S	N	S	N	S
8	1.9	20.7	0	5	85	90	25	60	0	0	4.8	0
9	0.7	0	0	0	90	80	20	60	0	0	19	0
10	0.3	0	0	0	75	60	15	40	7.7	0	20.6	0
11	0	0	0	0	50	0	10	0	19.2	0	0	0
12	0	0	0	0	20	0	10	0	15.4	0	6.3	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	5	0	0	0	0	0	0	0
17	0	0	0	0	40	0	0	0	0	0	3.2	0
18	0	0	0	0	5	0	0	0	0	0	0	0
19	0	0	0	0	5	0	0	0	0	0	0	0
20	0	0	15	0	20	0	0	0	0	0	11.1	0
21	0	0	15	0	75	15	10	5	0	0	0	0
22	0	0	5	0	95	5	10	0	0	0	0	0
23	0	0	10	0	90	10	10	0	0	0	0	0
24	0	0	20	0	85	10	10	0	19.2	0	6.3	0
25	10	0	20	0	75	5	10	0	0	0	0	0

Q = quadrat number; N S = north or south of transect.

¹*L. sayi* (mean percentage of total counts);

²*P. simplex* (visual estimate of percent cover before *L. sayi* aggregation (6 June, 2004);

³*L. perennis* (visual estimate of percent cover before *L. sayi* aggregation (June 6) and the day before peak population counts (June 11);

^{5,6} Larval damage (percent of total *L. m. samuelis* larval feeding signs observed on *L. perennis* clumps resulting from spring (pre *L. sayi*) and summer (post *L. sayi*) broods).

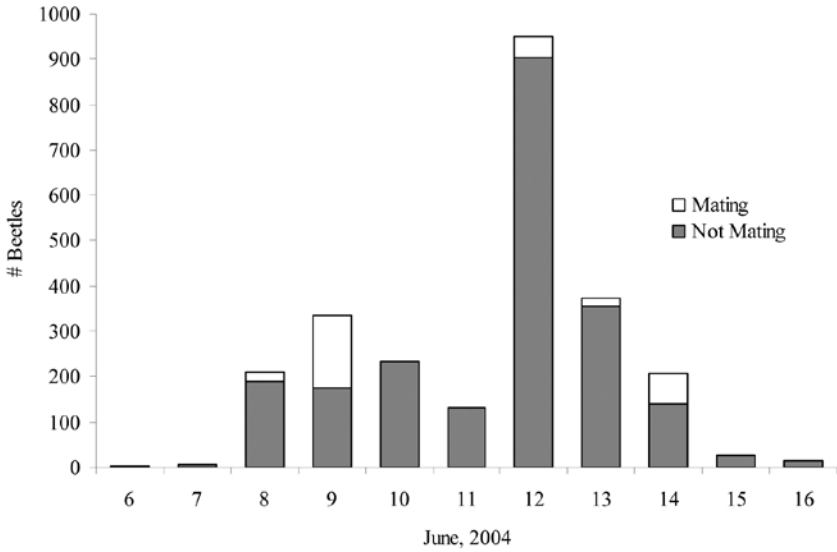


Figure 2. Total number of *Lytta sayi* adults observed during highest daily counts conducted every day of their aggregation, 2-17 June, 2004. Number of beetles categorized by mating or not mating.

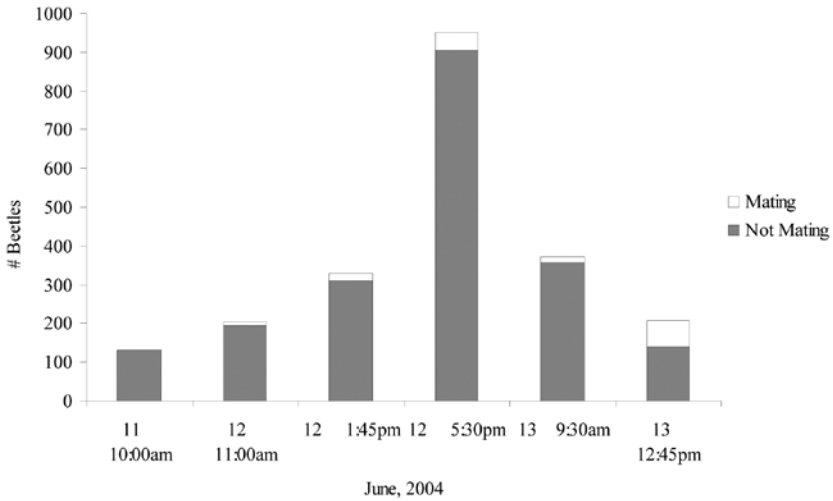


Figure 3. Total number of *Lytta sayi* adults observed during each of six surveys conducted over three days during peak aggregation, 11-13 June, 2004. Number of beetles categorized by mating or not mating.

Subsequent presence/absence surveys revealed few *L. m. samuelis* in 2005 and 2006 and none in 2007. *L. sayi* returned with similar results all three years.

DISCUSSION

Our results suggest the presence of *L. sayi* on lupine had an effect upon the flying and nectaring activity of males and the oviposition behavior of females. Although the beetles did not cause a significant reduction in the time butterflies spent perching on lupine, they did cause butterflies to move away from areas of lupine occupied by high numbers of beetles. Oviposition site selection by adult butterflies is one of the most important factors influencing larval fitness as it determines the quality of host plant available to the larvae (Rausher 1979, Grundel et al. 1998). Females preferentially use lupine in open canopied areas for oviposition and this lupine is best suited for larval survival (Lane and Andow 2003). This study showed a reduction in oviposition in open canopied areas from first to second brood (38.4% and 14.3% respectively). Additionally, there was an absence of feeding signs from the summer brood on lupine in 6s, 7s and 8s which contained the highest density of *P. simplex* and the highest percentage of first brood feeding signs. Adult females that laid these eggs were flying during the beetle aggregation. We suspect that females choose shaded lupine which is less suited for larval survival in order to avoid lupine occupied by *L. sayi*.

According to the resource concentration hypothesis, specialist herbivores remain in areas of dense host plant cover (Root 1973). Our data support this hypothesis for *L. m. samuelis* spent the majority of total perching time in the eastern region of lupine concentration. This was the larger of two centers of lupine concentration and had the highest percent cover of *P. simplex*. This area, however, also contained the majority of the *L. sayi* population which leads us to conclude that the disturbance from *L. sayi* was not enough to overcome the butterfly's tendency to remain in a concentrated area of larval host plants.

Researchers agree that *L. m. samuelis* adults are not dependent on lupine for nectar and will use a variety of plant species. Our study indicated that *L. m. samuelis* spent little time on lupine flowers and the data support earlier studies that rank *P. simplex* as one of the most frequently used nectar plants of spring flight adults (Bleser 1994, Grundel et al. 2000, Swengel and Swengel 2000).

We did not observe *L. sayi* feeding on any substrate aside from lupine flowers and seeds. This includes flowers of *P. simplex*, even though it is in the family Rosaceae, a food plant family for *L. sayi* (Selander 1960). Of importance was that *L. sayi* did not feed on the leaves of lupine and therefore were not in direct competition with *L. m. samuelis* larvae for food.

Lytta sayi adults were docile and not easily disturbed by observers. They remained feeding on the same flower(s) during surveys. We believe this, coupled with the ease of sighting due to the large size of the beetles (13-25 mm) (Selander 1960, J. S. pers. obs.), reduced the chance that we missed or made multiple counts of a beetle. There are no previous quantitative studies on the behavior of *L. sayi*, however their aggregation formation, mating behavior and the ability to consume copious amounts of vegetation in a short period of time are consistent with the feeding behavior of other meloid species (Selander 1960, Church and Gerber 1977, Snead and Alcock 1985, Evans 1990, Chandel et al. 1996, Nead et al. 1996). Although we captured a noteworthy phenomenon of > 900 *L. sayi* aggregating upon and deflowering an entire field of lupine, the minimal size of both the study site and the *L. m. samuelis* population limited the conclusiveness of our results. In addition, the study units (quadrats) were not independent and the number of *L. m. samuelis* surveys was limited by a period of heavy rain mid-way through the beetle aggregation. Even with these caveats in mind, we conclude that the presence of *L. sayi* potentially disturbs

adult *L. m. samuelis*. Minimum viable population studies have shown that *L. m. samuelis* populations with spring broods of < 250 individuals should not be considered viable for conservation purposes and those with < 100 individuals have little chance of survival (Schweitzer 1994). Given the small size of this population, we cannot say that *L. sayi* is responsible for the extirpation of this *L. m. samuelis* population, however, a second site (within 10 miles), which had sporadic observations of *L. sayi* on lupine in the past (1999), also no longer sustains a population of *L. m. samuelis*. Albeit, all-terrain- vehicle (ATV) activity degraded the site and contributed to the decline of lupine.

Upon future identification of concurrent *L. m. samuelis* and *L. sayi* populations, further studies should be conducted on the potential impact of the beetle presence particularly on a robust *L. m. samuelis* population. Furthermore, if there is a continued expansion of *L. sayi* into *L. m. samuelis* territory, more intensive studies of *L. sayi* biology (i.e., other food sources in the region and parasitism behavior, including host species) would be warranted.

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