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**Cover photo**: Mantid Fly, Photo by David Cappaert.
BEHAVIORS OF ADULT *AGRILUS PLANIPENNIS* (COLEOPTERA: BUPRESTIDAE)

Cesar R. Rodriguez-Saona1,2*, James R. Miller1, Therese M. Poland3, Tina M. Kuhn3, Gard W. Olis4, Tanya Turk4, and Daniel L. Ward5

ABSTRACT

A 2-year study was conducted in Canada (2003) and the United States (2005) to better understand searching and mating behaviors of adult *Agrilus planipennis* Fairmaire. In both field and laboratory, adults spent more time resting and walking than feeding or flying. The sex ratio in the field was biased towards males, which tended to hover around trees, likely looking for mates. There was more leaf feeding damage within a tree higher in the canopy than in the lower canopy early in the season, but this difference disappeared over time. In choice experiments, males attempted to mate with individuals of both sexes, but they landed more frequently on females than on males. A series of sexual behaviors was observed in the laboratory, including: exposure of the ovipositor/genitalia, sporadic jumping by males, attempted mating, and mating. Sexual behaviors were absent among 1-3 day-old beetles, but were observed regularly in 10-12 day-old beetles. Females were seen exposing their ovipositor, suggestive of pheromone-calling behavior. No courtship was observed prior to mating. Hovering, searching, and landing behaviors suggest that beetles most likely rely on visual cues during mate finding, although host-plant volatiles and/or pheromones might also be involved.

*Agrilus* (Coleoptera: Buprestidae) is one of the largest insect genera, with thousands of species (Jendek 2000 and references therein); however, the genus remains rather poorly understood. Reproductive and other behaviors have been studied in only a few economically important species. Carlson and Knight (1969) suggested that *Agrilus* may utilize host trees for mating encounters. Once on trees, beetles could find mates via visual (Gwynne and Rentz 1983), auditory/vibrational (Fenton 1942), tactile, or pheromonal signals, or combinations of these signals. Dunn and Potter (1988) demonstrated that male two-lined chestnut borers, *Agrilus bilineatus* (Weber), landed on cages containing unmated female beetles significantly more often than on cages lacking females; they were not attracted to oak logs or to males. This suggests that female *A. bilineatus* release pheromones or, less likely, produce auditory signals attractive to males. Whether other *Agrilus* species use non-contact signals in mate finding is unknown.

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive species in North America. Native to China, Japan, Korea, Mongolia, and eastern Russia (Yu 1992), this insect borer was first discovered in

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southeastern Michigan, USA, and neighboring Ontario, Canada, in 2002 (Haack et al., 2002, McCullough and Roberts 2002). In eastern North America, A. planipennis attacks and can kill all native species of ash (Fraxinus spp.). The land area of counties in which A. planipennis has been detected in the USA and Canada now exceeds 190,000 km², and it is estimated that more than 20 million ash trees have been killed in the core-infested area (EAB Info 2008).

Little is known about the behavior of A. planipennis, mainly due to its recent presence in North America and because this insect is rarely a pest in its native range. In China, A. planipennis adults are often active from 0600 to 1700 h, especially when weather is warm and sunny. Mating has been recorded between 0900-1500 h and lasts 20-90 min (Chinese Academy of Science 1986, Yu 1992). Adult beetles often rest in bark cracks or on foliage during rainy or very cloudy weather and usually remain on foliage at night. Adults typically fly in 8-12 m bursts (Yu 1992), but are capable of longer flights. They most often attack ash trees that are growing in the open or along the edge of forest stands (Chinese Academy of Sciences 1986), but entire stands can be killed during outbreaks (Yu 1992).

As in China, A. planipennis adults in North America preferentially attack trees in open areas compared to trees within closed-canopy stands (Poland et al. 2005). More beetles were captured on trap trees exposed to full sunlight than on trap trees that were mostly or totally shaded (McCullough et al. 2006). Lelito et al. (2007) found that males search trees during flight and descend rapidly onto pinned beetles of either sex and attempted to copulate, suggesting that visual cues are used in mate finding. Lance et al. (2007) observed that beetles spend most of their time resting on leaves and typically fly from leaf to leaf in the tops of trees.

While the overall behavior of A. planipennis in North America appears to be similar to that in China (Poland and McCullough 2006), many details of host and mate-finding and mating behaviors are not clear. Effective management of any insect pest often benefits from a comprehensive understanding of behavior. For instance, knowledge about the distribution of an insect within its host can aid in the development of effective tree surveys (Timms et al. 2006).

Because understanding of host and mate finding and mating behaviors is useful for developing techniques to detect populations, manipulate them, disrupt mating, or reduce attacks on host trees, our main objective was to study these behaviors for adult A. planipennis in the field and laboratory. Previous studies showed that adult beetles hover around ash tree trunks (Bauer et al. 2004, Lelito et al. 2007); thus, we postulated that male beetles hover around trees searching for females. Because adult emergence is greater from upper parts of tree trunks (Brown-Rytlewski and Wilson 2005), we postulated that feeding damage within a tree would be greater in the top of canopy at the time of beetle emergence. Specifically, we conducted field studies to determine the searching pattern in A. planipennis, the proportion of males and females hovering around trees, the distribution of damaged leaves within a tree, and its mate-searching behaviors. Additional laboratory experiments were conducted to quantify male and female behaviors under controlled conditions. We tested the hypotheses that A. planipennis performs gender-specific behaviors and that sexual behaviors appear later in the life of beetles.

MATERIALS AND METHODS

Searching behaviors

Beetle behaviors. This study was conducted to record adult A. planipennis behaviors on and off their host plants. The study was conducted in June and July 2005 at a commercial nursery (Quality Tree Transplanting Nursery, Whitmore Lake, MI), composed of young closely planted ash trees. The nursery was located in a 13-ha field surrounded by mature mixed hardwoods with a significant
component of ash infested by *A. planipennis*. Several tree species were planted in 3-6 ha blocks throughout the nursery. The study was conducted within a 3-ha planting of 6-7 year-old ash trees (~4m tall and ~10 cm diameter). Trees were planted 1.5 m apart in rows 75 m long with 7 m between rows. Varieties included 3 green ash (*Fraxinus pennsylvanica* Marsh.) cultivars (Marshall, Platmore, and Summit) (what is known as green ash in USA is known as red ash in Canada), and 2 white ash (*F. americana* L.) cultivars (Autumn Purple and Autumn Applause). All observations were made on wild beetles of unknown age and mating status, and were conducted between 0900-1400 h under sunny and warm (24-30°C) conditions. This time of day was selected because all beetle behaviors were observed being performed in the morning and midday in laboratory bioassays (see results). Observations were conducted throughout the planting on all ash varieties present.

Wild beetles (n = 33), found on ash leaves, were selected using a “first encountered” approach, and their activities, i.e., resting, walking, eating ash leaves, flying/hovering, and wing-opening (i.e., wings open and exposing the magenta-colored dorsal abdomen) were recorded for a maximum of 5 min or until they flew out of sight. This time period was sufficient to record the full repertoire of beetle activities; for instance, preliminary data showed that doubling this time period did not yield additional behaviors. Time spent performing each of the behaviors was recorded using a hand-held microcassette audio recorder. Observations were conducted on 24 June, 1 July, and 7 July 2005. The time of the first observation coincided with the peak of adult emergence.

Only beetles observed for more than 100 sec were used for data analyses (n = 28). A graphical descriptive summary for the percentage of time a beetle performed each of the behaviors is presented as a box and whiskers plot.

**Beetles flying / hovering around trees.** We examined the entire crown of more than 100 trees and collected all beetles on and off trees to determine the proportion of males and females flying and hovering around the trees. If males search for females, we expect the proportion of individuals caught flying/hovering to be biased towards males. The study was conducted at the Quality Tree Transplanting Nursery under the conditions described above. Hovering/flying beetles were caught using a long-handled sweep net. Collected beetles (n = 195) were placed in vials and their sex determined in the laboratory. Sex was determined under a microscope by sexually-dimorphic external characteristics, including the generally larger size of females (Cappaert et al. 2005), the presence of abundant sternal setae in males, and the narrow tapered abdomen for males compared to the rounded enlarged abdomen for females (Fig. 1). Collections occurred between 1000-1200 h and on four different dates (29 June, 1 July, 7 July, and 8 July 2005).

To compare males and females with regard to the proportion observed hovering, an exact logistic regression model was fit using the LOGISTIC procedure of the SAS System (ver. 9.13, SAS Institute Inc., Cary, NC). The model included gender and date as classification variables as well as their interaction. There was no significant interaction effect (Wald $\chi^2 = 0.528; df = 3; P = 0.9127$), so the effect of gender pooled across dates is presented. Proportions hovering and exact (Clopper-Pearson) 95% confidence intervals are presented.

**Distribution of feeding damage.** We quantified the proportion of damaged leaves on trees at three different locations within trees and times in the season. This study was conducted at the Quality Tree Transplanting Nursery under conditions described above. Using clippers, we retrieved branches across the upper, middle, and lower thirds of the tree canopy. A total of 65 different trees was used. For each tree, one branch was collected from each canopy position on 24 June (n = 25), 29 June (n = 20), and 8 July 2005 (n = 20). The number of damaged and undamaged leaves per branch was recorded. Leaf damage was caused almost exclusively by *A. planipennis*, since other herbivores were rarely seen on trees. Damaged leaves had about 10-20% area loss (estimated visually).
Fig. 1. Photograph of three emerald ash borer females (A), and three emerald ash borer males (B), depicting the overall larger body size for females, the glabrous prosternum of females and rugulose and rough texture of the male pro-sternum, and the enlarged and rounded abdomen of females compared to the narrow and straight-sided abdominal shape for males. Photograph of emerald ash borer male (left) and female (right) depicting the dense setae on the male pro-sternum (C). Scale is in mm. Photographs by Debbie Miller, USDA Forest Service.
We tested for main effects of relative canopy height and date as well as their interaction effect on the proportion of leaves damaged by *A. planipennis* feeding. The experiment was analyzed as a split-plot with date as the whole-plot factor and height as the sub-plot factor. Individual trees were the whole-plots and canopy strata within a tree were the sub-plots. The mixed effects ANOVA was fit using the MIXED procedure of the SAS System. Because the number of leaves sampled varied, the analysis was weighted with the number of leaves per experimental unit. Model adequacy was assessed using plots of standardized residuals. A significant interaction effect was further investigated by pairwise comparisons of means within each date using Tukey’s adjustment.

**Mating behaviors**

*Male orientation toward mates.* We investigated the role of visual stimuli in male orientation towards potential mates. This study was conducted on 14 and 15 July 2003 at the Ojibway Park Reserve (Windsor, Ontario) that has a mixture of park-like and natural habitats containing many mature green ash trees infested by *A. planipennis*. The study was conducted on ash trees located on the edge of the parking lot adjacent to the visitors’ center. We observed beetle behaviors on two trees, with one observer assigned to each tree. All observations were made on feral beetles of unknown age and mating status and were conducted between 1300-1600 h under sunny and warm (24-30°C) conditions. This time of day was selected because sexual behaviors increase at midday-afternoon (see lab results).

Five dead female *A. planipennis* “decoys”, mounted on insect pins, were affixed at a height of 1.5 m onto the trunks of two trees on which we observed the greatest beetle activity. We had three decoys on one tree (placed equidistantly on the tree, such that they were 120 angular degrees apart, and a beetle searching the trunk could only see one decoy at a time) and two decoys on the other tree (on opposite sides of the tree). All decoy beetles were dead, oriented vertically (head up), placed at the same height (1.5 m), and their bodies were touching the tree trunk. Inconspicuous black dots (pen-drawn, 0.5 cm in diameter) were marked on the trees 10 cm away from the decoys at the same height. The dots (control treatment) were applied to test whether flying beetles preferentially land on other beetles. The position of the female decoy relative to the black dot was switched every ~15 min, from left to right and back again, to avoid bias of location on tree on male attraction. We recorded the number of males landing on either the decoys or the control spots. Once a male beetle landed on a decoy, we removed and released it so it would not influence other searching males. In a variation of the above test, both male and female decoys were presented simultaneously 10 cm apart at a height of 1.5 m. The position of male decoys and female decoys was switched every ~15 min. The decoys remained in place during interactions with courting males.

Differences in male preference between female decoys and controls and between male and female decoys were compared using the exact binomial test of proportions (FREQ procedure of the SAS System). We tested whether the observed proportions was significantly different from 0.5. The exact 95% confidence intervals are also presented.

**Laboratory bioassay of behavioral ontogeny.** Adult *A. planipennis* beetles (*n* = 15 males and 15 females, sexed by body size and shape, Fig. 1) were collected from field-infested logs upon emergence on 13 June 2003, separated by sex, and refrigerated overnight. The next morning, these virgin beetles were placed individually in screened containers (made by attaching 2 cups at their open ends; total volume = 568 ml; height = 20 cm) containing green ash leaflets inserted into a water pic. Fresh leaflets were supplied to the beetles every three days. The experiment was conducted in the laboratory, with the temperature in the containers ranging from 23-32°C.
Behavior of each beetle was scanned and recorded in a consistent order, then repeated six times each hour. Behavioral scans started at 0600 h and continued until 1800 h. At each scan we scored: resting or inactivity, walking, grooming (consisting of crossing and/or stroking legs, or cleaning antennae with mouthparts), eating ash leaves, raising the abdomen between elytra and/or wing-opening, antennating rapidly, lowering the abdomen, excreting waste or flying, exposing ovipositor (females) or genitalia (males), sporadic jumping behavior of males, attempted mating, and mating. After days 1-3 of recording the behaviors in isolation, males and females were paired and observations were continued on days 4-6 and 10-12. On days 7-9, when observations were not made, the beetles were isolated to prevent mating. When a beetle died, the remaining beetle was paired with another survivor. Sex of all beetles was confirmed by dissection once observations ceased. Data from two pairs had to be discarded because they were same-sex pairings. We also recorded the number and duration of successful matings. A mating was considered successful if the male inserted its aedeagus into the female’s genitalia without female rejection. Mating duration was recorded from aedeagus insertion to withdrawal.

To analyze the behaviors, scans were grouped into 3 four-hour time periods: morning (0600-1000 h), midday (1000-1400 h), and afternoon (1400-1800 h) and into age blocks of three days each: young (1st, 2nd, and 3rd days of adulthood), middle-aged (4th, 5th, and 6th days of adulthood), and old (10th, 11th, and 12th days of adulthood). The “young” group includes beetles at a pre-mating age, the “middle-aged” group includes beetles at the age of mating (Cappaert et al. 2005), and the “old” group includes beetles at a post-mating age. The counts of observed behaviors were then analyzed using the LOGISTIC procedure of the SAS System to fit a generalized logits model. The generalized logits models multiple nominal discrete responses (the several behaviors) as the probability of their occurring conditioned on the explanatory variables. To reflect the response modeled and simplify interpretation the predicted probabilities were converted to the proportions of time engaged in each behavior. Mating pair was initially included in the model; however, it explained little variation and did not change the effects of the other regressors. Therefore, pairing was omitted from the model in order to include data from the first three dates of observation (before pairing was imposed) in the analysis. The final model included age and gender as classification variables and time of day as a continuous regressor variable.

RESULTS

Searching Behaviors

Beetle behaviors. Beetles spent more (~70%) time resting and walking on the leaves than in feeding or flying (Fig. 2). Beetles spent as much as 85%, but on average 35%, of their time resting (median = 33%). On average, beetles spent 32% of their time walking (median = 32%), and 70% of beetles were observed performing this behavior. Beetles spent only 15% of their time feeding (median = 0%), and about 60% of all beetles were observed feeding. Similarly, beetles spent 15% of their time flying (median = 12%). For the 64% of beetles observed flying, we recorded a total of 33 flying events; in 88% of these, the beetles landed on the same tree from which they took off, indicating that most beetles were moving within a single tree. Beetles spent the least time (average = 2.5%, median = 0%) opening their wings and exposing their abdomens (Fig. 3). In fact, only 4 of the 28 beetles (14%) were observed to perform this behavior.

Beetles flying/hovering around trees. The sex ratio of field-collected beetles was 2.8:1 male:female. More males (62% of all collected male beetles) were found flying/hovering around trees (exact 95% confidence interval (CI): 53% (lower limit), 70% (upper limit)), whereas only 28% of collected female beetles were hovering (exact 95% CI: 17%, 42%) (Wald $\chi^2 = 5.68$, df = 1, $P = 0.0171$).
Fig. 2. Percentage of time spent by adult *Agrilus planipennis* performing individual behaviors in the field. Each box represents the inter-quartile range for each of the behaviors and the whiskers represent the range of the data. The line crossing each box indicates the median (the median for feeding and wing opening was zero) and the dot indicates the mean. Observations were conducted between 0900-1400 h in June-July at the Quality Tree Transplanting Nursery in Whitmore Lake, Michigan. \( n = 28 \).

There was also a significant date effect (Wald \( \chi^2 = 13.48; \) df = 3; \( P = 0.0037 \)), indicating that the proportion of beetles hovering varied by date from 30% on 7 Jul to 67% on 8 Jul.

*Distribution of feeding damage.* Leaf damage by *A. planipennis* increased over time (\( F = 9.08; \) df = 2,62; \( P = 0.0003 \); Fig. 4); the proportion of leaves damaged was below 40% during the first two sampling dates, and increased to 47% by the last sampling date. Leaf damage varied with position within the tree (\( F = 6.41, \) df = 2,124, \( P = 0.0022 \)); initial damage was significantly higher in the middle and upper thirds of the trees than in the lower third (\( P \leq 0.05 \)) (Fig. 4). This difference disappeared over time (significant time-by-position interaction: \( F = 4.16, \) df = 4,124, \( P = 0.0034 \); Fig. 4).

*Mating behaviors*

*Male orientation toward mates.* In beetle decoy experiments, 12 male *A. planipennis* landed on dead female decoys while none landed on or near the controls (black dots), demonstrating a strong preference of males to orient to the dead beetles (\( P = 0.0005 \); exact 95% CI: 0.74, 1.00). Occasionally, male beetles tried to mate with the dead female decoy (Fig. 5). During the experiment with female decoys, an additional 14 male *A. planipennis* landed on live male and female beetles on the tree with the decoys. When both male and female decoys were presented simultaneously 10 cm apart, 27 out of 35 males landed on the female decoy while 8 landed on the male decoy (\( P = 0.0019; \) 95% CI: 0.60, 0.90).
Thus, males preferentially oriented to and landed on female decoys; however, it is evident that male decoys also presented attractive stimuli to searching males.

Laboratory bioassay of behavioral ontogeny. The results of our laboratory observations are summarized in Fig. 6. There was a significant three-way interaction effect (Wald $\chi^2 = 30.7$, df = 16, $P = 0.0145$), so the individual predicted probabilities for each of the behaviors at each age by gender by time of day combination were plotted and interpreted.

It was clear from the predicted probabilities that the proportion of time spent resting (R) decreased from morning to afternoon. The decrease in resting was offset by increases in other behaviors, primarily walking (W), grooming (G), and feeding (F), and later in life by sexual behaviors (SB). Grooming (G) was more frequently observed in females than in males and decreased in frequency
in both sexes with age. Feeding (F) occurred most frequently at midday and increased with age. Raising the abdomen between elytra and/or wing-opening (R/WO) were very infrequent in the morning and declined with age. Rapid antennation (RA) also decreased with age. Lowering the abdomen (LA) and excreting waste or flying (E/F) were the most infrequent behaviors performed under laboratory conditions, and mostly observed at midday and afternoons in both males and females.

Exposing the ovipositor/genitalia, sporadic jumping by males, attempted mating, and mating were completely absent from the behavioral repertoire of beetles 1-3 days old, but were observed regularly in beetles 10-12 days old. We believe that these behaviors are aspects of sexual behaviors (SB), in part because they appeared later in the lives of the insects. However, it is unclear how exposing the ovipositor and male jumping behavior relate to sexual activity. Their frequency was highest at midday.

We observed two successful matings by one male during our laboratory study. No courtship was noticed prior to mating. The successful male mated with the female with which he was paired on Day 10 for 56 min. He attempted to mate with the same female twice on Day 11, but she was unreceptive. That male was then placed with a different female of the same age, and within 1 h he tried to mate but was not successful. On Day 12, he mounted the female twice. On the first attempt, he mounted the female for 36 min; although the female did not actively reject him and the male had his aedeagus fully exposed, no mating occurred. One hour later, he mounted the female again, quickly inserted his aedeagus, and remained in copula for 83 min.

![Proportion (±SE) of damaged leaves per branch taken from the upper (top), middle (center), and lower (bottom) thirds of the ash tree canopy. Samples were conducted on three different and consecutive weeks between June-July at the Quality Tree Transplanting Nursery in Whitmore Lake, Michigan. Columns with different letters are significantly different ($P \leq 0.05$), while ns = not significant ($P > 0.05$) (Tukey tests). n = 65.](image-url)
Fig. 5. Male *A. planipennis* mating with a dead female decoy. Larger mean size of female *A. planipennis* is evident in this photo.
© G.W. Otis.
DISCUSSION

Altogether, the present study provides evidence for the following conclusions: adult *A. planipennis* spend the majority of time resting, beetles engage in more non-resting behaviors later in the day, early-season feeding is more abundant higher in the tree than towards the bottom, visual cues are important in helping males find females, and sexual behaviors are observed regularly in older beetles (10-12 day-old).

In the field, we observed that *A. planipennis* adults spend about 35% of their time resting, 32% walking, 15% feeding, and 15% flying (Fig. 2). Lance et al. (2007) also reported these four behaviors to be the most common in *A. planipennis* in the field; however, they observed beetles spending more time resting (70%) and less time walking (10%). This discrepancy between studies is likely due to differences in the time of day of the observations and variable environmental field conditions. Our laboratory study shows that beetles can spend between 30 to 75% of their time resting depending on the time of day (Fig. 6), increasing their activity as the day progressed. Field conditions can also influence *A. planipennis* behaviors. In fact, adults are active mostly in warm and sunny conditions (Chinese Academy of Science 1986, Yu 1992). Wing-opening behavior was observed infrequently both in the field and laboratory.

Most beetles observed hovering around trees were males. Although Bauer et al. (2004) also observed adult *A. planipennis* hovering around ash tree trunks, the sex of beetles was not determined. This hovering behavior consisted of a slow upward flight, casting laterally back and forth along the ash tree trunk, suggestive of mate searching. Beetles often landed on the same tree or one close to the one from which they took off, indicating that they tend to fly around a single tree or a few trees in close proximity when searching for mates.

The sex ratio of all field-collected adult *A. planipennis*, found both on and off trees, in our study was biased towards males. This differs from a 1:1 sex ratio for *A. planipennis* adults reared from naturally-infested logs (Lyons et al. 2004). Interestingly, Lyons and Jones (2005) reported more females captured on TangleTrap™-coated sticky traps. For *A. bilineatus*, Cote and Allen (1980) also found a 1:1 adult sex ratio for adults reared from infested logs. However, the sex ratio of beetles drawn to sticky traps placed on trees or on traps containing oak bolts was biased towards females, indicating a greater attraction of females to potential hosts (Cote and Allen 1980). Our male bias may result from females being less conspicuous or less active, making them more difficult to find under field conditions. Alternatively, if female *A. planipennis* become unreceptive after mating and either reduce their activity or disperse to lay eggs, the apparent sex ratio will become male-biased. Taylor et al. (2004) found mated females capable of flying longer, farther, and faster than males or unmated females. Another factor that may contribute to a male-biased sex ratio early in the season is the fact that males emerge slightly earlier than females (Lyons and Jones 2005).

Leaf-feeding damage within trees was greater higher in the canopy than in the lower parts early in the season (Fig. 4). This pattern of leaf damage is consistent with beetle emergence and activity patterns within trees. Brown-Rytlewski and Wilson (2005) found greater adult *A. planipennis* emergence from upper parts of tree trunks. Similarly, Lance et al. (2007) found that flight activity and landing rates were concentrated in the tops of trees. By the end of June the difference in leaf damage within a tree disappeared, indicating an even distribution of leaf damage, and likely adult beetles, within infested trees. Rodriguez-Saona et al. (2006) found that female *A. planipennis* were attracted to leaf volatiles in behavioral assays and that the antennae of both sexes respond to several of these volatiles. Males may use plant volatiles as short-distance cues to locate females or as indicators of the presence of conspecifics. More research is needed to fully understand the role of host-plant volatiles on *A. planipennis* behavior.
Fig. 6. Proportion of time (± 95% confidence intervals) spent by female (A-C) and male (D-F) *Agrilus planipennis* performing individual behaviors in the laboratory. Behaviors include resting (R), walking (W), grooming (G), eating ash leaves (F), raising the abdomen between the elytra and/or wing-opening (wings open and exposing the abdomen) (R/WO), rapid antennation (RA), lowering the abdomen (LA), excreting waste or flying (E/F), and sexual behaviors (SB), which include exposing ovipositor (females) or genitalia (males), sporadic jumping behavior of males, attempting mating, and mating. Recordings were made during the first three days after emergence (A and D), days 4-6 (B and E), and days 10-12 (C and F). Behaviors were grouped into three periods: morning (0600-1000 h), midday (1000-1400 h), and afternoon (1400-1800 h). n = 15 males and 15 females.
Furthermore, the middle and top canopy might emit a higher amount of “attractive” volatiles than the lower canopy at the time of high beetle emergence due to increased leaf damage in those parts of the tree. Our results suggest that traps to monitor early infestations of *A. planipennis* populations should be placed high in trees.

In addition to host-plant derived cues, males might use more reliable cues to find mates, such as those associated with conspecifics. Male beetles oriented identically to both live and dead beetles of either sex, indicating that mate-searching by males appears to be based mainly on visual cues from conspecifics. Males oriented toward beetles they could see and failed to orient toward beetles that were hidden from view by the tree trunk (G.W. Otis, pers. obser.), also suggesting the predominant use of visual stimuli. Upon encountering beetles on tree trunks, searching males landed quickly on them, with no apparent courtship by beetles of either sex (G.W. Otis, pers. obser.). Similarly, Lelito et al. (2007) found that males landed on decoys pinned to leaves of ash trees and quickly attempted mating. The preference of males for female decoys in our study may reflect the greater mean size of females (McCullough and Roberts 2002, Lyons and Jones 2005). Lelito et al. (2007) found no preference of males for female beetles.

*Agrilus planipennis* females begin to mate 5-7 days after emergence (Cappaert et al. 2005). In our laboratory study, shifts in frequency of behavioral activities were likely related to achieving sexual maturity at 4-6 days of age. Older unmated females exhibited sexual behaviors, suggesting that they may switch to more active mate-atraction behaviors if not mated soon after becoming sexually mature. In the laboratory, 10-12 day-old females frequently extruded and slowly rotated their genitalia in a circular motion. Females were also seen exposing their ovipositor, at times in a pulsating motion, suggestive of pheromone-calling behavior exhibited by many other insect species (Tamaki 1985). For example, in the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, calling involves extension and ventral curving of the telescopic terminal abdominal segments; these structures sometimes pulsate rhythmically (Hammack 1995). Female *A. bilineatus* likely emit pheromones; male beetles land on cages containing unmated female beetles in significantly greater numbers than on cages that lacked female beetles (Dunn and Potter 1988). Recently, Bartelt et al. (2007) identified the antennally active macrocyclic lactone, (3Z)-dodecen-12-olide, from *A. planipennis*; this compound was emitted predominantly by females.

A 13-day-old virgin female *A. planipennis* exhibited a behavioral display that greatly enhanced her visibility to human observers (referred to here as wing-opening; Fig. 3). The behavioral sequence appeared to be a fixed action pattern, in which the female first rotated her body, then pumped the head and thorax, and ended with an open wing display in which the magenta-colored abdomen was exposed. The same behavioral components were noted in 10-12 day-old unmated females during the laboratory study. This same behavior was observed in the field. Whether this behavior is a mating display remains unknown.

From our field and laboratory observations we propose a dual mating strategy for *A. planipennis*. Our field observations suggest that the primary mate-finding strategy of *A. planipennis* involves active visual search by males for females on tree trunks. Thus, under high beetle density, it is likely that females will mate soon after reaching sexual maturity. Additionally, our laboratory observations suggest the presence of mating behaviors in *A. planipennis* that develop as the beetles age. We suggest that, if females fail to mate within a few days, they might utilize two alternative strategies to attract mates: active pheromone-calling and a behavioral display sequence that increases their visibility to searching males. These alternative mate-finding strategies are more likely to be used in low-density situations in which females are rarely encountered by males. An analogous dual mating strategy has been documented in the spruce budworm moth (*Choristoneura fumiferana* (Clemens)), with active male
search for females during the daytime at high densities and female pheromone attraction of males at night at low moth densities (Kipp et al. 1995).

The information presented in this study has practical implications for monitoring A. planipennis populations. Current efforts focus on the development of traps baited with host-plant volatiles (Poland et al. 2005). This study identified features in the beetle’s mating behavior that could help in the improvement of traps. For instance, pheromone-traps or traps using visual stimuli attractive to male beetles could be used alone or in combination to host-plant volatiles in areas of low beetle densities, which are regularly encountered at the leading edge of the expanding distribution of A. planipennis in North America. Although Bartelt et al. (2007) identified an antennally active compound from A. planipennis, predominantly emitted by females, pheromone traps have yet to be developed for field monitoring of this beetle. Visual cues are important in A. planipennis mate-finding (this study; Lelito et al. 2007). One such cue currently tested for monitoring this beetle is color. For example, Otis et al. (2005) and Francese et al. (2005) found purple traps to be more attractive to beetles than black or yellow. Whether this color preference is associated to the wing-opening behavior described here remains unknown. The fact that greater feeding damage was observed higher in the tree canopy, indicates that traps should be placed at that height.

ACKNOWLEDGMENTS

The authors thank Erin Clark for assistance in the field and Bruce Judkins for permission to use field sites at the Quality Tree Transplanting Nursery. Helpful comments on an early draft were provided by David Cappaert, Deepa Pureswaran, and two anonymous reviewers. We acknowledge funding from The Tree Fund and from the Great Lakes Forestry Centre of the Canadian Forest Service to G.W. Otis, and from the USDA Forest Service Special Technology Development Program (Project No. NA-2003-02) to T.M. Poland.

LITERATURE CITED


**ABSTRACT**

*Trimerotropis huroniana* Wlk. is a “Threatened” species in Michigan and Wisconsin with a distribution limited to open dune systems in the northern Great Lakes region of North America. Pitfall traps were utilized in the Grand Sable Dunes of Pictured Rocks National Lakeshore, MI, along with an herbaceous plant survey, to identify the relationship of *T. huroniana* with native dune plant species, *Ammophila breviligulata* Fern. (American beachgrass, Poaceae), *Artemisia campestris* L. (field sagewort, Asteraceae), and the exotic invasive plant *Centaurea biebersteinii* DC. [=*Centaurea maculosa*, spotted knapweed, Lamarck] (Asteraceae). The absence of *C. biebersteinii* resulted in an increased likelihood of capturing *T. huroniana*. This was most likely due to the increased likelihood of encountering *A. campestris* in areas without *C. biebersteinii*. The occurrence of *A. breviligulata* was independent of *C. biebersteinii* presence. A significant positive linear relationship occurred between the percent cover of *A. campestris* and the traps that captured *T. huroniana*. There was no significant relationship between *A. breviligulata* percent cover and the traps that captured *T. huroniana*. The occurrence and distribution of *T. huroniana* is closely related to the presence and abundance of *A. campestris*. Habitat conservation and improvement for *T. huroniana* should include increases in *A. campestris* populations through the removal of *C. biebersteinii*.

**INTRODUCTION**


*Ammophila breviligulata* Fern., *Artemisia campestris* L., and *Calamovilfa longifolia* (Hook.) Scribn. (prairie sandreed, Poaceae) are three native dune plant species identified as the most likely food plants for *T. huroniana* (Rabe 1999, Scholtens et al. 2005). Scholtens et al. (2005) suggested that the presence of *T. huroniana* was not related to the presence of native plant species. The landscape scale of their survey efforts in an attempt to delineate population distribution within the known range of this locust species may not have been adequate to determine finer scale correlations. Also, Scholtens et al. (2005) performed a qualitative assessment of the plant communities within dunes.

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where \( T. \text{huroniana} \) occurs. Such a survey technique may not have provided the detail necessary to identify relationships between a rarely occurring organism and its food resources. A localized comparison of \( T. \text{huroniana} \) occurrences with the important dune food plants may provide a clearer understanding of mechanisms influencing the distribution of \( T. \text{huroniana} \).

\textit{Centaurea biebersteinii} DC is an exotic plant species introduced from Europe into North America in the late 1800s (Watson and Renney 1974). Since its introduction, \( C. \text{biebersteinii} \) has become established throughout North America and has locally decreased native plant diversity, altered arthropod distributions, reduced wild and domestic ungulate productivity, and indirectly increased runoff and sedimentation rates (Lacey et al. 1989, Thompson 1996, Kedzie-Webb et al. 2001, Olson and Wallander 2001, Marshall et al. 2008). The introduction of \( C. \text{biebersteinii} \) to sensitive dune plant communities may alter the occurrences of the “Threatened” \( T. \text{huroniana} \), which already experiences limitations to population size and distribution.

The objective of this study was to test the hypotheses that \( T. \text{huroniana} \) occurrence was independent of the presence of \( C. \text{biebersteinii} \), \( A. \text{campestris} \), and \( A. \text{breviligulata} \).

\textbf{METHODS AND MATERIALS}

Areas with and without \( C. \text{biebersteinii} \) were utilized within the Grand Sable Dunes of Pictured Rocks National Lakeshore in the Upper Peninsula of Michigan (46°39'38"N, 86°1'54"W). \( C. \text{biebersteinii} \), along with other major vegetation cover types, was mapped within the Grand Sable Dunes during the summer of 2000 (B. Leutscher, personal communication). The majority of the Grand Sable Dunes are covered by herbaceous dune plant communities, with natural dune stabilization occurring as \textit{Pinus banksiana} Lamb. (Jack pine, Pinaceae) and Northern Hardwood forests invade.

The three largest delineated areas of \( C. \text{biebersteinii} \) (10.7, 6.3, 4.8 ha), which had been established for at least five years (B. Leutscher, personal communication), were selected for this study. A transect (500-600 m) was established along the long axis of each area of \( C. \text{biebersteinii} \). In areas of native dune plant communities without \( C. \text{biebersteinii} \) adjacent to each \( C. \text{biebersteinii} \) area, transects of comparable length were established. Along each transect in the survey area, two arrays of five pitfall traps (8.5 cm diameter, 12.5 cm height) were installed on a linear 5-meter spacing following the transect approximately 200-250 m apart (10 traps per transect). Approximately 75 ml of 50 percent propylene glycol (Preston LowTox® Antifreeze) was used in each trap as a killing agent and preservative. Pitfall traps were open for one week and then closed for approximately three weeks to reduce the likelihood of population depressions due to trapping. At the time of closing, traps were emptied and upon re-opening, new propylene glycol was added to each trap. A total of five trapping cycles were carried out from 2 May 2003 to 28 August 2003, however for analysis, only the final two trapping cycles from 23-30 July and 21-28 August (3 transects × 2 trap groups × 5 traps × 2 trapping cycles = 60 traps/treatment with and without \( C. \text{biebersteinii} \)) were used. These cycles were the only with \( T. \text{huroniana} \) captures due to the late season activity of adults (Rabe 1999).

A plant survey was conducted within five 1-m\(^2\) quadrats along each transect within 5 m of each trap (3 transects × 2 trap groups × 5 quadrats = 30 quadrats/treatment with and without \( C. \text{biebersteinii} \)) identifying percent cover of \( C. \text{biebersteinii} \), \( A. \text{campestris} \), and \( A. \text{breviligulata} \). Mean percent cover for each taxon was calculated for individual transects. A chi-squared analysis was used to test the hypothesis that traps capturing \( T. \text{huroniana} \) were independent of \( C. \text{biebersteinii} \) presence, as well as to test the hypothesis that the presence of \( A. \text{campestris} \) and \( A. \text{breviligulata} \) were independent of \( C. \text{biebersteinii} \) presence.
Simple linear regression was used to test for the relationship between the percent cover of *A. campestris*, as well as *A. breviligulata*, and the traps that captured *T. huroniana*.

**RESULTS AND DISCUSSION**

Traps that captured *T. huroniana* were not independent of the presence of *C. biebersteinii* (Table 1). Traps installed in areas without *C. biebersteinii* were more likely to capture *T. huroniana* than traps in areas with *C. biebersteinii*. This relationship may be due to the increased likelihood of encountering *A. campestris* in quadrats without *C. biebersteinii* (Table 2). Along with *A. campestris*, two dune grasses occurred in the Grand Sable Dunes, however, *C. longifolia* was rare and *A. breviligulata* was the dominant grass species. Usually these two grass species singularly dominate, as in the Grand Sable Dunes, or co-dominate suitable *T. huroniana* habitat and are also known plants fed on by this locust (Scholtens et al. 2005), however, the presence of *A. breviligulata* was independent of the presence of *C. biebersteinii* ($\chi^2 = 0.33$, df = 1, $P = 0.567$). The number of traps that captured *T. huroniana* was not related to the percent cover of *A. breviligulata* ($F = 0.25$, df = 1,4, $P = 0.644$, $R^2 = 0.059$).

As *A. campestris* percent cover increased, the number of traps along each transect that captured *T. huroniana* also increased (Fig. 1). This relationship corroborates the suggestions made by Rabe (1999) and Scholtens et al. (2005) that *A. campestris* is one of the important plant species in the distribution of *T. huroniana*. As a native dune plant species and an important component of *T. huroniana* habitat, changes in *A. campestris* distribution and occurrence would be expected to alter *T. huroniana* distribution and occurrence.

*Trimerotropis huroniana* habitat conservation may be enhanced by increasing the dune coverage of *A. campestris* by reducing the coverage of *C. biebersteinii*. The occurrence of *A. breviligulata* was independent of *C. biebersteinii* presence and suggests that this dune grass may not be the most influential

Table 1. Traps capturing *Trimerotropis huroniana* in areas with and without *C. biebersteinii* in the Grand Sable Dunes, Pictured Rocks National Lakeshore, MI.

<table>
<thead>
<tr>
<th>Trimerotropis huroniana</th>
<th>Captured</th>
<th>Not Captured</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. biebersteinii</em></td>
<td>Present</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

$\chi^2 = 4.23$, df = 1, $P = 0.039$

Table 2. Number of quadrats sampled encountering *Artemisia campestris* and *C. biebersteinii* in the Grand Sable Dunes, Pictured Rocks National Lakeshore, MI.

<table>
<thead>
<tr>
<th>Artemisia campestris</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. biebersteinii</em></td>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
</tr>
</tbody>
</table>

$\chi^2 = 13.01$, df = 1, $P < 0.001$
factor in determining the occurrence of *T. huroniana* in the Grand Sable Dunes, however, increasing the coverage and distribution of this dune grass would also be beneficial to *T. huroniana*.

Efforts within the Grand Sable Dunes, Pictured Rocks National Lakeshore, to control *C. biebersteinii* by hand pulling have been carried out by the National Park Service but the availability of funding has limited the size and recurrence of such operations (B. Leutscher, personal communication). A more viable option may be classical biological control. While early biological control agents selected for *C. biebersteinii* control have been plagued with limited efficiency, parasitoid activity, and predation, more recent control agents have demonstrated effective reductions in *C. biebersteinii* density and biomass (Myers 2000, Long et al. 2003, Marshall et al. 2005, Corn et al. 2006, Story et al. 2006). Based on the results of this study, reducing the populations of *C. biebersteinii* in the dune habitat of *T. huroniana* would increase populations of *A. campestris* to the benefit of this “Threatened” locust.

**ACKNOWLEDGMENTS**

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LITERATURE CITED


ABSTRACT

*Dianthidium simile* (Cresson) is a small ground-nesting megachilid bee restricted to sandy areas in Michigan, often bordering lakeshores. Females dig their nests in sand, at the base of dried clumps of grass. Nests are small clusters of cells, formed from conifer resin and sand grains, with each 1-1.5 cm in length by 8.5 mm in diameter. Collection records and field observations indicate a flight period from late June to early September. This is the first report on the behavior of this species.

Here I report on the behavior and nesting habits of *Dianthidium simile* (Cresson), a colorful, fairly small but robust member of the Megachilidae (Hymenoptera) that nests fossorially, most often in sandy lacustrine regions. It is a member of the tribe Anthidiini, a diverse group of megachilids that use a variety of materials to line their nests, ranging from plant resins to trichomes (Michener 2000). Members of the genus *Dianthidium* are known to use resins and small stones and debris to construct the brood cells in the ground, in trap nests, and on above-ground substrates. *D. simile* ranges from the Great Lakes region to Maine, and south to Georgia (Krombein, et al. 1979). Fischer (1951) reported rearing two specimens from a partly rotted log, but nothing else has been published about its biology. Although Romankova (2004) included it in the list of the anthidiines of Ontario, no new biological information was presented for it.

The Megachilidae are known for their diverse array of nesting sites, nesting materials, and behavior, even within a genus. Members of the genus *Dianthidium* display a range of nesting preferences: multi-celled nests on small shrubs; vertical faces of gravel pits; and edges of dunes amongst grass rhizomes. All of the published behavioral observations have one commonality: cells made of resin with a matrix of sand grains and plant debris. Several western North American species of *Dianthidium* have been studied, and the plasticity of nesting behavior within this genus of small megachilids is evident from those studies. *Dianthidium ulkei* (Cresson) was briefly studied by Hicks (1933) near Boulder, Colorado. He reported that the bees constructed short tunnels in natural cavities in soil. Nests of one or two cells were constructed with pebbles and plant debris in a resin matrix. Frolich and Parker (1985) studied the nesting and mating behavior of *D. ulkei* in a greenhouse, and were able to induce females to nest in wood cavities. The nests were lined with a resin, pebble, and soil matrix. Krombein (1967) described the nests of a number of southwestern species from trap nests, as well as a nest of the Floridian species *D. floridiense* Schwarz. An Arizona nest of *Dianthidium pudicum pudicum* (Cresson) was studied briefly by Clement (1974), and in that instance the nest was found in the fork of small branches of a small tree (*Larrea tridentate* (DC.) Coville, creosote bush). The aerial, external nest was triangular in shape (held in the “V” between 2 branches), and contained 10 cells. The nest was comprised of resin.

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small pebbles, and plant debris. Fischer's (1951) report on Dianthidium concinnum (Cresson) also revealed that the 10-celled nest was constructed externally on a branch of a small elm in Kansas. The nests were comprised of the same materials as above. Fischer (1951) made some additional observations on D. sayi, Cockerell (now D. curvatum sayi Cockerell [Krombein et al. 1979]) which nested in the soil in a vertical bank in Kansas. The cells were also constructed of soil and plant resin. Michener and Michener (1999) studied an aggregation of the same species for several years in a vertical sandy bank adjacent to the Oldman River in Alberta, Canada.

**OBSERVATIONS**

In Michigan, D. simile has been collected predominantly from lacustrine sandy areas bordering the Great Lakes, but there are a few records towards the center of the state (Fig. 1). Adults have been collected from 30 June to 12 September, with most records from late July to early August. Specimen labels indicate that they visit the following flowers: Coreopsis lanceolata L., Rudbeckia hirta L., and Polanisia graveolens Raf.

I collected adults, excavated several nests, and made the following observations at two sites in Michigan: in the Lower Peninsula at Ludington State Park (LSP), Mason Co., in a stretch of vegetated back dunes well away from Lake Michigan from 1745 -1845 hr on 7 August 1990, and in the Upper Peninsula (22°C) at 1400 hr, on 29 July 1994 in Mackinac Co. in back dunes along Lake Michigan at Big Knob State Forest Campground (BSF) area. Both sites were similar in that areas of bare sand were intermingled with grasses and other herbaceous plants, and were at the edges of old dunes that bordered ecotones leading to conifer-dominated woods. These sites were all more or less horizontal, with a slight slope leading away from the nesting area.

**LSP:** I collected 8 females at the site, and at least 10 D. simile were seen nesting at the base of dead clumps of grass, in an area 30 × 15 cm. Cells were intermingled with dead rhizomes, often just below the surface. Bees were flying in with provisions every 2-3 minutes. I dug up a few clumps of cells, but not the entire aggregation. Cells were 1.0 – 1.5 cm long × 0.8 – 0.9 cm in diameter. Thirteen cells were in one cluster, all facing up, nearly perpendicular to the substrate (Fig. 2). Another nest (Fig. 3) had 7 cells arranged around a grass stem, also pointing upwards. These nests were placed in sealed containers but were never subjected to cool temperatures. During January-February 1991, 4 females and 3 males emerged.

**BSF:** Nests were located in a back dunes-beach area closest to the edge of woods in stable grassy/shrubby area. I estimated that there were 20-24 nest entrances in one square meter. I also observed several females coming and going from the nest site. All nest entrances were located at the base of old clumps of dried grass, all facing south, and partially obscured by dead grass leaves. In one 30 × 30 cm area, I counted 7 nest entrances. All cells were made of coniferous resin embedded with sand grains. I collected 6 females there.

Most clumps of cells were at the very basal rhizome portion of the grasses, just below the surface, varying from 1-5 cm deep. Some nests that appeared to be older were 3 cm deep. All were very close to the main stems of grass. None of the cells were grouped like those from LSP. The nests that I found had no more than 3 or 4 cells grouped together. All cells were made of coniferous resin embedded with sand grains and small bits of plant debris.

Six clumps of cells were placed in small snap-top containers, and sometime over the winter, 4 males and 3 females emerged from the cells. No parasitoids emerged from the collected cells. The pungent aroma of honey and pine resin was quite noticeable after the nests were placed into small containers.
Figure 1. Map of Michigan, with dots representing collection localities of *Dianthidium simile* from specimens in the Univ. of Michigan Museum of Zoology and Michigan State Univ. Cook Arthropod Research Collection. The two study sites are designated as follows: BSF = Big Knob State Forest Campground, and LSP = Ludington State Park.
Figure 2. Excavated nest of *Dianthidium simile*, Mason Co., MI. The scale is in mm.

Figure 3. Excavated nest of *Dianthidium simile*, Mason Co., MI. The scale is in mm.
DISCUSSION

*Dianthidium simile* is an interesting inhabitant of the dune ecosystem, and it would be desirable to know how much variation exists in nest site selection across its range. Romankova (2004) listed many Ontario sites that also appear to be located near water and a similar flight period (July-August). In Michigan, most of the localities are around the margins of the state, with a few in the interior (Fig. 1). Based on observations, I would predict all of the sites to be in sandy substrates.

Nests from BSF differed from those found at LSP. For example, the LSP nests had most of the cells oriented in the same direction and the clumps of cells were more connected forming a mass of cells in a single plane, whereas the BSF cells were more random in orientation and fewer than 5 cells in a cluster was typical (Fig. 4). Of course, there was a mixture of old (previous years) and new cells in the BSF matrix of cells amidst the grass rhizomes, whereas the LSP cells all appeared recently constructed.

Although there were some differences in the maximum number of cells and orientation between nests in Mason and Mackinac Counties, that may only be a reflection on the ability of a bee to dig amongst grass rhizomes and construct adjacent cells. Otherwise, the two sites are consistent in terms of substrate, cell size, materials used in lining the cells, and location of the nests. Based upon the two sites, it appears that a given nesting area supports many individuals nesting in close proximity, as reported for the closely related *D. curvatum sayi* (Michener and Michener 1999).

I am skeptical of Fischer’s (1951) report of rearing two *D. simile* from a partially-rotted log. Without further attribution as to the site and specimen verification, it’s a bit anomalous compared to what I have seen for *D. simile* in Michigan, however, if the rotted log came from a sandy area, the record would certainly be more credible.

![Figure 4. Excavated cells from nests of *Dianthidium simile*, Mackinac Co., MI. The scale is in mm.](image-url)
ACKNOWLEDGMENTS

I thank Virginia Scott (now at the University of Colorado Museum) for her assistance with records from the Michigan State University. Mike Arduser of the Missouri Dept. of Conservation kindly supplied me with some updated references and greatly improved the manuscript.

All specimens and nest materials collected by me are deposited in the collection of the Insect Division at the University of Michigan Museum of Zoology.

LITERATURE CITED


DO GENERALIST TIGER SWALLOWTAIL BUTTERFLY FEMALES SELECT DARK GREEN LEAVES OVER YELLOWISH – OR REDDISH-GREEN LEAVES FOR OVIPosition?

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Abstract

In late August and September, using leaves from the same branches, the polyphagous North American swallowtail butterfly species Papilio glaucus L. (Lepidoptera: Papilionidae) is shown to select mature dark green leaves of their host plants white ash, Fraxinus americana L. (Oleaceae) and tulip tree, Liriodendron tulipifera L. (Magnoliaceae) rather than the pale green or yellowish-green mature leaves in laboratory oviposition arenas. In early August, similar results were observed for black cherry, Prunus serotina Ehrh. (Rosaceae). Dark green leaves were preferred over light green and yellowish green leaves. These green leaves of black cherry were the most nutritious leaves for larval growth indicating a clear correlation between adult preference and larval performance on this plant. However, tulip tree leaves in the summer did not elicit different oviposition responses between green and light green leaves. A field evaluation of oviposition preferences for young expanding reddish leaves of red bay, Persea borbonia (L.) Spreng (Lauraceae) versus slightly older expanded green leaves of the same branch also suggested avoidance of “young” red leaves in Florida by Papilio troilus L. and Papilio palamedes Drury during the spring season (March-April).

Many intrinsic factors (e.g., female age, time since last oviposition, egg load, time since last mating; Singer 1983, Miller and Strickler 1984, Bossart and Scriber 1999) and extrinsic factors (plant volatiles, leaf color, texture, leaf shape, contact chemosensory cues, and species of host plant) influence the choice of host plant by ovipositing females (Courtney and Kibota 1990; Thompson and Pellmyr 1991; Carter et al. 1999; Frankfater and Scriber 1999, 2003; Renwick 2002; Mercader and Scriber 2007). Additional ovipositional-determining factors not directly related to the plant may include avoidance of natural enemies (Redman and Scriber 2000, Murphy 2004), microclimate temperature and/or humidity preferences (Grossmueller and Lederhouse 1985), and seasonal thermal constraints on voltinism which can select for the most nutritious hosts (Nylin 1988, Scriber and Lederhouse 1992). Lack of availability or low abundance of some host species can also result in local host plant preferences (Rausher 1978, Fitt 1986, Scriber 1986, Scriber et al. 2006).

The selection of host plants by polyphagous species is governed both by factors affecting the rank order of preference and also by the “specificity” (Courtney and Kibota 1990, Mercader and Scriber 2005, 2007). It has been seen that Papilio glaucus L. generally selects (specializes on) the host plant species that support fast larval growth in thermally-constrained areas (thus allowing the possibility of an extra generation), but use a wider array of potential hosts in thermally-relaxed areas (i.e., where enough Degree-days accumulate seasonally to complete development of the extra generation on all host plant species, even those of rather low suitability/nutritional quality; Scriber and Lederhouse 1992). This voltinism-suitability hypothesis suggests that preference performance

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relationships may exert strong selection pressures, where allelochemical toxin barriers are not involved, interacting with leaf nutritional quality (bottom up regulation) and natural enemies (top-down regulation).

We know that different species of tree leaves differ in their ability to support growth of lepidopteran species (Scriber and Slansky, Jr. 1981, Slansky, Jr. and Scriber 1985, Mattson and Scriber 1987). The seasonal variation in leaf suitability (Scriber 1984) has less frequently been evaluated for its impact on host selection (Finke and Scriber 1988). The neural limitations hypothesis of host selection (Bernays 2001) predicts that polyphagous species incur costs in the form of prolonged decision making time and increased error rates in host selection relative to more specialized insects. Therefore, the ability of polyphagous insects to detect differences in host plant quality is expected to be lower, as has been previously noted in other butterfly species (Janz and Nylin 1997). Given the likely constraints on information processing in the highly polyphagous *P. glaucus*, we used 2-choice oviposition arenas to determine if *P. glaucus* was capable of selecting between leaves that were light green or reddish-green versus leaves (fully expanded, but) with dark green color, as would be encountered late in the growing season. It is known that for black cherry trees different colors reflect different leaf water and nitrogen content which declines seasonally in green leaves (from 5.1% Nitrogen and 80% water) to 1.5 - 2.0% N and 65% water in yellowish green leaves, and less nitrogen (<1%) and water (<55%) in reddish green, yellow-brown leaves (Scriber 1977). The correlations of color (possibly with nutritional value) and oviposition preference of *Papilio* females for green leaves over yellowish-green mature leaves in the fall and potential toxins/deterrents for younger expanding reddish spring leaves are addressed here.

**MATERIALS AND METHODS**

Most *P. glaucus* oviposition assays were conducted using wild females of *P. glaucus* collected in Clinton Co. and Allegan Co. in southern Michigan. Due to small sample sizes black cherry assays were supplemented using females obtained from Clarke Co. Georgia and sent to our lab by Express Mail.

Oviposition preferences using leaves from the same branches were conducted in the Fall (September, when most leaves were changing color) using white ash (*Fraxinus americana* L.; n = 5 females) and tulip tree (*Liriodendron tulipifera* L.; n = 9 females). In mid-August, again using leaves from the same branches light green and dark green black cherry leaves (*Prunus serotina* Ehrh.; n = 10 females) and green versus light green tulip tree leaves (n = 8 females) were assessed. Leaf petioles were inserted into water-filled vials with rubber caps, and the leaves were draped along the inside wall of clear, round, large plastic dishes on rotating platforms in front of a bank of incandescent lights (Fig. 1; Scriber 1993). Adults were fed using a 15% honey water solution and eggs were counted and removed daily. Only females laying 9 or more eggs were included in the analyses. The proportion of eggs laid on each leaf was compared within each paired comparison using Bonferroni corrected Paired Wilcoxon Tests using the R Statistical Package V 2.4 (R Development Core Team 2006) with a 0.05 alpha level to test if there were differences in preference between leaves of different ages.

A similar ovipositional study was conducted in late summer, using light green and dark green leaves of black cherry and tulip tree. The eggs from two laboratory reared families that originated from individuals collected in Clarke Co. Georgia were held at 27°C and newly hatched neonate larvae were transferred using camel hair brushes to host plant leaves. Larvae from the two families were reared at 24°C (18:6 h L:D photoperiod) on green (n = 14 from one family and n = 26 from the other family) and light green or yellowish-green (n = 14 and n = 18, respectively) cherry leaves, similar to those used in oviposition assays and weighed ten days after emergence. We tested the effect of leaf age
(green or yellowish-green black cherry leaves) on larval growth with a Nested ANOVA (leaf age nested within larval family) using the R statistical package V 2.4 (R Development Core Team, 2006).

In addition to these lab experiments, we evaluated the differences in field oviposition preferences between “new” expanding reddish leaves compared to more fully-expanded slightly older green leaves of red bay for *Papilio palamedes* Drury and *Papilio troilus* L. during the spring (late March - early April) in Levy Co. and Highlands Co. Florida.

**RESULTS**

In studies done in September, when the leaves of most trees were beginning to change color, females of *P. glaucus* presented with a choice of light green (or yellowish-green leaves) and dark green leaves from the same branch of white ash trees, showed a clear preference for the green leaves (Figure 1). Nine females that laid 9 or more eggs all placed the majority (84.6%) of these on the green versus yellowish-green leaves (Fig 2A). The same result was obtained for 5 females offered tulip tree leaves (Figure 2B); few were placed on the light green or yellowish-green leaves and most (95.5%) were placed on the green (from the same branches).

In the second study (conducted in August), using black cherry and tulip tree. Females placed in assays with green and yellowish-green black cherry leaves (n = 10) selected green leaves about three times as often as yellowish-green leaves (72.8 ± 5.5 vs. 27.2 ± 5.5, mean ± SE, Figure 2C). However, these summer assays with tulip tree leaves showed mixed results with no strong preference for dark green versus light green tulip leaves (Figure 2D and 3B).

The growth rates of resulting neonate larvae on these green and light green leaves verified that suitability for larval growth was better on the dark green leaves than on the yellowish-green leaves of black cherry (Fig. 3A). The means ± SE for family #20196 and 20192 for larval weight were significantly higher on the green (97.5 ± 6.8 mg) versus the yellowish-green leaves (64.6 ± 5.1 mg) at 10 days ($F = 199.59$, df = 1, 1, $P = 0.04$).

In Highlands County, Florida the newly expanding red bay (*Persea borbonia* (L.) Spreng.) leaves were red or reddish-green, and a careful search of more than 1500 such red bay leaves from 21 different trees in March 2007, yielded no eggs or larvae of *Papilio*. However, very often on the adjacent one or two more expanded green leaves larvae were found feeding and resting in leaf rolls. Sixteen neonate and early instar larvae and one egg were found on green leaves (of more than 2500 searched) of these same 21 trees. Apparently the ovipositing *P. troilus* and *P. palamedes* avoid these red leaves of red bay (Fig. 4). Over a three year period in Levy Co. Florida, eggs of *P. glaucus* were found on young (still expanding) green ash (*Fraxinus pennsylvanica* Marshall) leaves (> 15 eggs or neonates on more than 2000 leaves), but not on any young reddish-green leaves of the same branch (Fig 5).

**DISCUSSION**

We have shown here that female oviposition preferences of *P. glaucus* are for greener leaves rather than light green, yellowish-green, leaves of black cherry and white ash (Figs 1 & 2). *P. glaucus* females also prefer green over yellowish-green tulip tree leaves in the fall, but in the summer they do not always prefer green over light green leaves from the same branch (Fig 2). This might indicate that cues between these August tulip tree leaves are insufficiently different to be distinguished or of similar chemistry. It seems that this generalist butterfly discerns host quality through contact chemoreception since volatiles would be confused/confounded in these multi-choice lab arenas.
Fig. 1. The experimental oviposition arena showing eggs on the dark green but not on the yellowish-green ash leaves. The inset shows the stacks of arenas on the turntable in front of the light bank.
Fig. 2. A). Median proportion of eggs laid by *P. glaucus* butterflies using green (G) and yellowish-green (YG) leaves of white ash (WA) in the fall. B). Median proportion of eggs laid by *P. glaucus* butterflies using green (G) and yellowish-green (YG) leaves of tulip tree (TT) in the fall. C). Median proportion of eggs laid by *P. glaucus* butterflies using green (G) and light green (LG) leaves of tulip tree. D). Median proportion of eggs by *P. glaucus* butterflies using green (G) and light green (LG) leaves of black cherry (BC). In each panel, bars around the medians represent the inter-quartile ranges. Pairwise differences between proportions of eggs laid on each leaf were analyzed separately for each leaf species using Paired Wilcoxon tests. Leaves within each panel with the same letter did not have a significant difference at a Bonferroni corrected $\alpha < 0.05$. 
Fig. 3. A). Black cherry showing fully expanded (new) and fully expanded (over-mature) leaves. B). Tulip tree leaves showing the indeterminant growth, with continuous production of new leaves.
Fig. 4. Red bay leaves which had *Papilio* eggs deposited and larvae feeding on the green, but not on the red leaves (March 24-27, 2007; Highlands Co. Florida). Both *P. palamedes* and *P. troilus* form leaf rolls during all instars on red bay (see inset).
Fig. 5. White ash leaves with red expanding tip leaves ( Levy Co., Florida; March 2007). Upper right insert shows the new expanded green leaves where eggs are placed and where larvae feed.
White ash and black cherry tree leaves have been documented to show general seasonal decline in the amounts of leaf water and nitrogen seasonally, although tulip tree continuously generates new leaves all season (Scriber and Slansky, Jr. 1981, Scriber 1984). Accelerated differences in the fall can occur with abscission layer formation. In fact, the same branch (of black cherry, for example) can have all stages of leaf quality from dark green (3.2% - 2.8% N and 76% - 65% water), to light green (2.8 - 2.2% N), yellowish-green (2.1 - 1.8% N) reddish-green (1.5% N) and yellow-brown (1.2 – 0.8% N, and <55% water (Scriber 1977, and unpublished). While we did not evaluate the nutritional differences between differently colored tulip tree and ash leaves here, we did determine that the dark green black cherry leaves support a 3-fold faster growth rate than the yellow-green leaves.

Since neonate larvae of these tree-feeding lepidoptera need to start feeding near the location on a particular tree chosen by the mother, it has been assumed that strong selection for highly nutritional oviposition substrates might occur (Zalucki et al. 2002). Even in polyphagous Papilio species, the selection of the “wrong” young leaves (i.e., on a toxic, or nearly toxic unsuitable host) could be a fatal “mistake” (Straatman 1962, Wiklund 1975, Berenbaum 1981, Larsson and Ekbom 1995, Renwick 2002, Scriber 2002a, Graves and Shapiro 2003). Consequently, the general lack of correlation of adult oviposition preference and larval growth performance in many, if not most, herbivorous insects has been somewhat of an enigma (Thompson 1988, 1998; Mayhew 2001; Bossart 2003). The reasons for a general lack of genetic linkage of preference and performance remains largely unknown (Bossart and Scriber 1999, Berenbaum and Feeny 2008). However, it is known that abiotic factors can interact with nutritional quality to result in strong preference-performance correlations in Papilio in accord with the “voltinism-suitability hypothesis” (Nylin 1988; Scriber and Lederhouse 1992; Scriber 1996, 2002b, 2005). In this scenario, thermal constraints on seasonal degree-day accumulations may (on a poor host) result in fewer generations than might be possible on a host species that supports rapid growth. The selection of the “best” leaves available on a non-toxic host species (Feeny 1995, Scriber et al. 2007) is generally assumed to be always favored, in order to minimize time exposed to natural enemies (predators, parasites, and disease; Slansky, Jr. 1983, Bernays 1998, Scriber 2004) or freeze susceptibility (Fordyce and Shapiro 2003, Tesar and Scriber 2003). However, fast growth is not always possible (e.g., tree leaves are generally poorer than herbaceous plants and roots are notoriously poor in nutritional quality, resulting in slow growth of root feeders, etc. (Slansky, Jr. and Scriber 1985).

From both the ecological and evolutionary perspectives, it remains unclear if rapid growth rates are always selected for (Slansky, Jr. 1993, Benrey and Denno 1997). In addition, adaptations for “compensatory feeding” may be invoked when diets are unbalanced in carbohydrates, protein, energy, or minerals (Scriber 1984, Slansky, Jr. and Scriber 1985, Mattson and Scriber 1987, Simpson and Simpson 1990, Fageria and Scriber 2001, Slansky, Jr. and Wheeler 1992, Trier and Mattson 2003 “diet-induced thermogenesis”). This feeding compensation may be via diet “self-selection” (Waldbauer and Friedman 1991) or “organismal stoichiometry” (Raubenheimer and Simpson 2004). However, larvae can not always compensate for eggs placed on older, over-mature leaves since higher risks as well as slower growth may be involved for the neonate larva, harder to consume and digest leaves, with higher vulnerability to enemies (Ayres and Scriber 1994, Zalucki et al. 2002). These slower growth rates were observed here with P. glaucus on older light green or yellowish-green versus newer dark green leaves of black cherry.

Field assessments of green versus younger expanding reddish leaves of red bay in Florida suggest that the Lauraceae-specialized P. palamedes and P. troilus butterflies avoid “reddish” leaves (Fig. 4). Our extensive searches of 21 trees resulted in no eggs or neonates feeding on red leaves (more than 1500),
but we found 16 neonate larvae and one egg on green leaves (>2500 searched). Similarly, in Florida, *P. glaucus* on green ash prefers green leaves and appears to avoid reddish-green leaves in the spring (JMS personal observation, and Fig. 5).

It is likely that these *Papilio* spp. may be using cues other than gustation to detect host plant quality. It is known, that unlike most invertebrates (Lee et al. 1987), *Papilio* species including *P. glaucus* (Briscoe 2000) have long wavelength red receptors that enable them to see and select green leaves (Kelber 1999). While most invertebrates lack red receptors (Menzel and Backhaus 1991), and peak reflectance from leaf anthocyanins lies in this region of about 630 nm (Lee et al. 1987), notable exceptions with a long wavelength receptor tuned to 610 nm include the *Papilionidae* (Arikawa and Ōchiyama 1996). One species, *Papilio aegeus* Donovan, uses this red receptor to avoid red leaves (Kelber 1999) and, perhaps *P. glaucus* can do the same (Briscoe 2000).

It has been suggested that some young leaves gain a protection from insect herbivores by a “delayed-greening” strategy for newly flushed leaves (which are reddish; Dominy et al. 2002). This “delayed greening” appeared to be true for ash leaves in Levy Co., Florida in 2007 (Fig. 5). This “delayed greening” has been suggested by Coley and Aide (1989) to be due to a high anthocyanin content, which, aside from being red, has fungicidal properties. However, this “delayed greening” may also protect leaves by keeping them devoid of nutritive value until they reach full size (Figs. 4 and 5, Dominy et al. 2002).

Regardless of the ecological/evolutionary advantages of selecting younger nutritious host leaves by *P. glaucus*, the physiological/chemical mechanisms permitting them to distinguish the best leaves remains unknown. Leaf color may play some unknown role in these *Papilio* as in the related *Battus philenor* L. (*Papilionidae*), which can learn leaf color and shape (Rausher 1978, Weiss and Papaj 2003, Miller and Strickler 1984). It is known that other *Papilio* species have red receptors to see green (Kelber 1999), as is the case for *P. glaucus* (Briscoe 2000), however, we do not really know if these specific color stimuli were used by the *P. palamedes* and *P. troilus* (or even *P. glaucus*) in these studies. Our ovipositional assays primarily detect differences in contact chemoreception and therefore the responses observed in our assays suggest that females of the polyphagous *P. glaucus* can detect and utilize leaf surface cues correlated with color. In these and related *Papilio*, the final and most important signal seems to come from chemosensory cues to the female tarsi as they taste the leaves before ovipositing (Feeny 1995; Nishida 1995; Frankfater and Scriber 1999, 2003).

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**LITERATURE CITED**


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ABSTRACT

Seasonal prevalence of fungal pathogens infecting soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), was assessed from 2004 to 2006 in two Michigan soybean production areas. In 2005 and 2006 field-collected soybean aphids were incubated, and fungal infection was detected at both sites early in August 2005 during soybean pod development and high soybean aphid densities. Significantly higher proportions of winged aphid morphs were infected (20 and 90% infection at the two sites) than wingless aphid morphs (1 and 3% infection). All cases of mycosis examined involved one pathogen species, *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Entomophthorales: Entomophthoraceae). In 2004 and 2005, we surveyed for pathogens of the soybean aphid in soybean as well as pathogens in other aphid species feeding on other crop plants (alfalfa, clover, corn, and wheat) by inspecting for sporulating aphid cadavers every 2 to 3 wk during the soybean growing season. Aphid cadavers were most abundant in alfalfa, especially in August; were less common in clover, corn, and soybean; and were not found in wheat. *Pandora neoaphidis* was associated with cadavers of *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) in alfalfa and clover during the same period when soybean aphid infection was detected. Overall, mortality of soybean aphid and other aphid species due to fungal infection was confirmed in Michigan. The results also implicate infected winged soybean aphid morphs as potential agents for fungal dispersal, and *A. pisum* in alfalfa and clover as a source of fungal propagules for soybean aphid.

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a pest of soybean from Asia, recently established in the soybean production regions of North America (Ragsdale et al. 2004). Heavy infestations are associated with soybean yield loss (Ragsdale et al. 2007). Insecticide use, which was previously uncommon in soybean, became widespread for control of this aphid (Myers et al. 2005). In Michigan, outbreaks of soybean aphid were reported in 2000, 2001, 2003, and 2005 (DiFonzo and Hines 2002, T. N., pers. obs.).

Aphid pathogens, especially certain entomophthoralean fungi, may play a role in soybean aphid suppression (Wu et al. 2004, Nielsen and Hajek 2005). In China, *Neozygites (=Entomophthora) fresenii* (Nowakowski) Batko (Entomophthorales: Entomophthoraceae) is one of the most common pathogens infecting soybean aphid, and its prevalence was positively correlated with humidity and aphid density (Wu et al. 2004). In North America, fungal disease was found in up to 84% of aphids sampled in New York State in 2003 and 2004 (Nielsen and Hajek 2005), and 3 to 70% of aphids sampled in Minnesota between 2002 and 2006 (K. Koch, personal communication). *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Entomophthorales: Entomophthoraceae) was the most abundant pathogen detected in both studies.

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Obligate aphid pathogenic fungi from the order Entomophthorales are widely distributed across temperate regions and infect a range of aphid species inhabiting various vegetation (Latgé and Papierok 1988, Nielsen 2002, Shah et al. 2004). Natural epizootics of aphid diseases often occur under favorable biotic and abiotic conditions (Latgé and Papierok 1988, Shah et al. 2004, Zhang et al. 2006). Many crop plants besides soybean that are cultivated in Michigan’s soybean production areas harbor aphids, including corn and wheat that are often rotated with soybean, and occasionally alfalfa (Blackman and Eastop 2000, USDA-NASS 2007).

In this study in Michigan, we quantified seasonal prevalence of fungal pathogens infecting soybean aphid, which allowed for regional comparisons with earlier studies in New York (Nielsen and Hajek 2005) and Minnesota (K. Koch, personal communication). In addition, we quantified abundance of sporulating aphid cadavers on other crop plants common in the soybean production region of lower Michigan, allowing for consideration of potential sources of inoculum near soybean fields.

MATERIALS AND METHODS

Study sites. Sampling of aphids and their pathogens were conducted at two Michigan locations: Kellogg Biological Station, Long-Term Ecological Research site, Hickory Corners (42°24'N, 85°23'W) and Entomology Farm, East Lansing (42°41'N, 84°29'W). Sites were managed by Michigan State University and were separated by 80 km. Soybean was sampled for soybean aphid at the Hickory Corners site in 2004 and at both sites in 2005 and 2006. Alfalfa, corn, wheat, and clover were also sampled for other aphids at the Hickory Corners site in 2004 and 2005. All crops sampled at both sites were cultivated under (rain-fed) condition without irrigation. We sampled four replicated plots of each crop through the soybean growing season in Michigan (June through September). The size of different crop plots at Hickory Corners were either 9 by 27 m (soybean [2004, 2005], wheat [2004, 2005], corn [2004, 2005], and clover [2004]) or 1 ha (soybean [2006] and alfalfa [2004, 2005]). All plots were set in the same experimental area devoted to crop rotational experiments, replications were randomized, and no insecticide or fungicide was used. The plot sizes of soybean at the Entomology Farm were 17 × 33 m.

Soybean aphid density. Soybean aphid populations were sampled four to eight times during the soybean growing season from 2004 to 2006 at each site. Soybean growth stages were noted using the scale of Fehr and Caviness (1977). The sampling periods were spread across a) mid-June, during early vegetative growth (V2-V3) when aphids may first migrate to soybean; b) mid-July, during flowering (R1-R2) when aphids may be multiplying on soybean; c) early August, during soybean pod fill (R3-R5) when aphids may be reaching peak densities; and d) late August, during plant senescence (R6) when aphids may be declining in density. Field aphid density was estimated by visually inspecting a random sample of 25 plants per plot (a total of 100 plants per site). On each plant, up to 50 aphids were counted, after which the number of aphids was estimated using a series of count ranges: 51-100, 101-500, 501-1,000, and 1,001-5,000. The high-end range was based on our field observations and past studies (DiFonzo and Hines 2002) that indicated aphid densities varied widely once populations surpassed several hundreds per plant but did not exceed 5,000 per plant during this period.

Latent fungal infections in field-collected soybean aphid. In 2005 and 2006, prevalence of aphid pathogenic fungi infecting soybean aphid at each site on each sampling date was estimated by incubating field-collected live aphids in the laboratory. Aphid-infested leaves were collected from at least 20 soybean plants, taken randomly across the four plots. The aphids were kept cool while being transported to the laboratory. Up to 100 aphids were selected for incubation
from the entire leaf collection representing each site. The selection consisted of all individuals of the winged morph (alate adults), which were always found in small numbers or absent, and all or a portion of the wingless morphs (apterous adults and nymphs that were third instar or older). The selected aphids were dislodged from the leaves and incubated on fresh soybean leaflets encaged in 1-ounce plastic portion cups lined with 2% water agar (Nielsen and Hajek 2005). Aphids, in groups of five individuals per cage, were incubated at 21°C with a photoperiod of 16:8 (L:D) h. Because different rates of fungal infection between aphid morphs have been noted (Nielsen and Hajek 2005), winged morphs were monitored for infection separately from wingless morphs. Aphid mortality was checked daily for 4 d, and aphid cadavers were removed from the cages and positioned in a 5-mm gap between two microscope slides (Nielsen and Hajek 2005). The slides were kept under high humidity at 15°C overnight to promote sporulation of fungal pathogens from the aphid cadavers. Discharged conidia that adhered to the slides were mounted in a drop of 90% lactic acid, and the morphology of conidia was examined under the compound light microscope for identification of aphid pathogens (Balazy 1993, Humber 1997, Keller 1991).

Mean aphid density per plant was calculated for each sampling date and site using the midpoint of the count ranges and soybean plots as replicates (n = 4). Prevalence of fungal infection was estimated for each sampling date and study site as a percentage of aphids from which sporulation by aphid pathogens was observed. The calculation was carried out separately for winged and wingless aphid morphs using aphid collections across the entire study site. Aphids collected from different plots were pooled together to estimate infection rates because of patchy aphid distribution in the soybean field and scarce collection of winged aphids. The chi-square test for independence (Gomez and Gomez 1984) was used to compare percent infection between the two aphid morphs.

**Active fungal infection of aphids in soybean and other crops.** At the Hickory Corners site in 2004 and 2005, soybean, alfalfa, corn, wheat, and clover were sampled for aphids and sporulating aphid cadavers. Soybean, alfalfa, and corn were sampled from mid-June through early September. Wheat was sampled from mid-June through mid-July until harvest, and clover, which was an under-story crop in wheat plots, was sampled from late July through early September 2004. Clover was not available in 2005 due to frost kill in the spring. For each crop, a random sample of 25 plants was inspected per plot (a total of 100 plants for each crop type per sampling date). The entire plant above the ground was inspected, and counts of aphids and aphid cadavers were recorded for each aphid species. In 2005 (but not in 2004), aphid cadavers found were brought back to the laboratory for identification of any pathogen involved. Fungal sporulation from aphid cadavers was promoted under high humidity, and aphid pathogens were identified using the same methods used for fungal pathogens of soybean aphid (Nielsen and Hajek 2005). Two sampling methods were used to quantify infection in soybean aphid (rearing pathogens from field-collected aphids and cadaver inspection) to maximize ability to detect infection. Infection in all other aphid species was sampled using cadaver inspection to check for similarity of infection patterns in other crop-infesting aphids common to soybean production areas.

Mean densities of aphids and aphid cadavers per plant were calculated for each crop type, sampling date, and aphid species using plots as replicates (n = 4). Numbers of aphid cadavers were compared among crops by year of sampling, using analysis of variance (PROC GLM, SAS Institute 2004). The independent variables included in the model were crop type, sampling date, plot, and the interaction between crop type and sampling date. Crop type was considered as a fixed variable, and sampling date and plot were considered random variables. The cadaver counts (number of aphid cadavers per plant [X]) were transformed into a logarithmic scale (log_{10} [100X + 1/6]) to satisfy the assumption of normality for analysis of variance
When the crop type or sampling date effect was significant, Tukey’s multiple comparisons test ($\alpha = 0.05$) was used to separate means of cadaver abundance.

**RESULTS AND DISCUSSION**

**Latent fungal infections in field-collected soybean aphid.** Soybean aphid occurred at relatively high densities in 2005 (Fig. 1) and relatively low densities in 2006 (data not presented graphically, peak aphid densities per plant were 3.6 in Hickory Corners and 0.5 in East Lansing) at both soybean sites. Fungal pathogens infecting soybean aphid were detected on 1 August 2005 at both study sites during soybean pod development (Fig. 1). All cases of infection detected involved one pathogen, *P. neoaphidis*, which was also the dominant pathogen of soybean aphid detected in New York and Minnesota (Nielsen and Hajek 2005; K. Koch, personal communication). Additional aphid pathogens were detected in the New York study, which was likely due to higher sampling intensity, but *P. neoaphidis* was by far the dominant species. Our findings were also similar to those of Nielsen and Hajek (2005) in that the fungal infection was associated with high aphid densities late in the growing season (Fig. 1). In 2006 when aphid densities were very low at both study sites throughout the season, no infection was detected.

When fungal infection was detected on 1 August 2005, significantly greater proportions of alate adults were infected than the wingless morphs at both study sites ($N = 100$ aphids per site): 20% alate adults and 1% wingless morphs were infected at the Hickory Corners site ($\chi^2 = 67.40$, df = 1, $P < 0.01$; Fig. 1a), and 90% alate adults and 3% wingless morphs were infected at the East Lansing site ($\chi^2 = 203.13$, df = 1, $P < 0.01$; Fig. 1b). We acknowledge that sample sizes were limited for quantifying infection rates among the alate adults compared with wingless aphids. The uneven sample sizes between the two aphid morphs reflected the fact that alate adults were always rare relative to wingless morphs during this study (Fig. 1). Regardless of the uneven sample sizes, our results (significantly higher proportions of alate adults were infected compared with wingless aphids) were consistent at both of our study sites and with the study in New York (Nielsen and Hajek 2005). These results suggest that movement of winged aphids infected by *P. neoaphidis* may play a role in the onset of disease (Zhang et al. 2006).

**Active fungal infection of aphids in soybean and other crops.** At the Hickory Corners site, sporulating aphid cadavers were found in soybean, alfalfa, clover, corn, but not wheat during the soybean growing season (Fig. 2c,d) (only dates when cadavers were detected are shown; other sampling dates were 14 June and 15 July in 2004 and 14 June, 12 July, 25 July, and 31 August in 2005). Of six aphid species found infesting these five crops during the two year study (Fig. 2a,b), fungal sporulation was most often observed on cadavers of *Acyrthosiphon pisum* (Harris) (69 cadavers, 57 on alfalfa and 12 on clover), followed by cadavers of *Therioaphis trifolii* (Monell) (19 cadavers, 18 on alfalfa and one on clover), *Rhopalosiphum maidis* (Fitch) on corn (7 cadavers), * Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) on corn (1 cadaver), and soybean aphid (1 cadaver). (Fig. 2 c,d). Two aphids, *Rhopalosiphum padi* (L.) on corn and wheat, and *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) on wheat, were detected but fungus-killed individuals were never found. The interaction between crop type and sampling date was significant in 2004 ($F = 8.47$; df = 13, 66; $P < 0.0001$) and 2005 ($F = 6.09$; df = 10, 54; $P < 0.0001$). In 2004, the numbers of cadavers found on alfalfa were greater than on clover and corn on 16 August, but there were no differences for other dates (Fig. 2c). In 2005, cadavers were only found on alfalfa on 27 June and 15 August (Fig. 2d). There were differences in plot sizes among crops (i.e., large plots for alfalfa and smaller plots for other crops), which could possibly contribute to differences in cadaver densities among crops. But greater numbers of aphid cadavers often
Figure 1. Mean soybean aphid densities (±SE) (lines) and percent infection found in winged (white bars) and wingless (black bars) aphid morphs monitored at two Michigan soybean sites in (a) Hickory Corners and (b) East Lansing during 2005. Percent infection was estimated on four dates. In 2006 (not shown), aphid densities were very low at both study sites, and no infection was detected. Percent infection was measured by incubating field-collected aphids (numbers of winged and wingless aphids, respectively, assessed are listed in brackets on top of graph). An asterisk over bars within a date indicates a significant difference in percent infection between winged and wingless aphids ($\chi^2$ test, $\alpha = 0.05$). Codes below the dates are soybean growth stages (Fehr and Caviness 1977). V, vegetative stages; R, reproductive stages.
Figure 2. Abundance of aphids (a, b) and sporulating aphid cadavers (c, d) among soybean, alfalfa, clover, corn, and wheat monitored in Hickory Corners, Michigan, in 2004 and 2005. Sampling dates on which cadavers were found on at least one crop are shown. Patterns of bar graphs indicate species composition of aphids (a, b) and aphid cadavers (c, d) in soybean (soy), alfalfa (alf), clover (clv), corn (crn), and wheat (wht). Different letters within a date in (c) and (d) indicate significant differences in cadaver abundance by Tukey’s test ($\alpha = 0.05$).
found in alfalfa appeared to be attributed to higher disease incidences associated with aphid species found in alfalfa (*A. pisum* and *T. trifolii*) and not to the larger plot sizes of alfalfa.

In 2005 when disease was identified from aphid cadavers, *P. neoaphidis* was associated with *A. pisum* on alfalfa, and *Zoophthora* sp. was associated with *T. trifolii* on alfalfa. Shah et al. (2004) found that *A. pisum* was highly susceptible to *P. neoaphidis*, and soybean aphid is also a suitable host of *P. neoaphidis* based on observations in this and other studies (Nielsen and Hajek 2005; K. Koch, personal communication). *Zoophthora occidentalis* (Thaxter) Batko (Entomophthorales: Entomophthoraceae) was infrequently detected from soybean aphid in New York (Nielsen and Hajek 2005) but was not observed in Minnesota (K. Koch, personal communication) or during the present study.

A few cadavers of *R. maidis* and *M. euphorbiae* were found on corn in 2004 (associated pathogens not identified) (Fig. 2c). In Idaho, *P. neoaphidis* and *Conidiobolus* spp. have been reported from these aphids (Feng et al. 1990). We did not detect diseased aphids on wheat. Although two aphid species (*R. padi* and *S. avenae*) were found infesting wheat, aphid densities were low (≤ 1.1 aphids per plant) from mid-June to mid-July when plants were mature and drying. Relatively low susceptibility to fungal infection has been previously reported for *R. padi* to *P. neoaphidis* (Shah et al. 2004).

There was a consistent under-reporting of fungal incidence by counting mycotized cadavers encountered in the field versus by the collection of living aphids that were incubated in the laboratory to allow development of any pathogens they carried. For instance, at the Hickory Corners site, infection was detected only by incubating field-collected aphids (Fig. 1a) and not by inspecting aphid cadavers (Fig. 2d). Similarly, Nielsen and Hajek (2005) observed consistently higher infection rates by incubating live aphids than collecting cadavers. In other systems involving different aphid species and crops, relative sensitivities of these sampling techniques varied widely (Nielsen and Hajek 2005). It seems prudent to utilize both methods when first assessing fungal pathogen infections of insect pests.

In conclusion, we observed fungal infection in soybean aphid populations (Fig. 1) with *P. neoaphidis* to be the most dominant aphid pathogen, but soybean aphid cadavers were rarely seen (Fig. 2c). Infections (as measured by percent infection of field-collected and laboratory-incubated aphids) were associated with high aphid densities late in the soybean growing season, and were primarily detected in the winged aphid morph. The same pathogen was the dominant species in other studies reported from the midwestern and eastern US (Nielsen and Hajek 2005; K. Koch, personal communication). The seasonal patterns observed in this and other studies implicate that fungi have the potential to disrupt annual life cycles of soybean aphid by infecting late season aphid populations, especially migratory populations. On the other hand, fungi may have limited potential for within-season control of soybean aphid. In our study, mycoses were also detected in aphids present on other crops common in Michigan soybean production regions. *Pandora neoaphidis* was associated with cadavers of *A. pisum* in alfalfa and clover during the same period when soybean aphid infection was detected. Because aphid-pathogenic fungi can infect a range of aphid species on different plants (Latgé and Papierok 1988, Nielsen 2002, Shah et al. 2004), and dispersal of infective conidia across a variety of habitats is the common pathway through which insect pathogens reach their hosts (Tanada and Kaya 1993), *A. pisum* on alfalfa and clover may be an important source for fungal propagules that infect soybean aphid. Overall, mortality of soybean aphid and other aphid species due to fungal infection was confirmed in Michigan. The results also implicate infected winged aphid morphs as potential agents for fungal dispersal, and *A. pisum* in alfalfa and clover as a source of fungal propagules for soybean aphid.
ACKNOWLEDGMENTS

We are grateful to Drs. Ann Hajek (Cornell University) and Richard Humber (USDA-ARS, Ithaca, NY) for their advice during this study. Dr. Charlotte Nielsen (University of Copenhagen) kindly shared her expertise to study aphid pathogens and helped identify our pathogen samples. Drs. Hajek and Nielsen also provided helpful comments on our manuscript. We thank our Michigan State University (MSU) student workers (Matt Kaiser, Ananda Jenkins, Jared Natzke, Momoko Minakawa, and Paul Thomas) for their assistance in field sampling. We also thank Drew Corbin and Joe Simmons (National Science Foundation Long-Term Ecological Research Program at the MSU Kellogg Biological Station) and Chris Sebolt (MSU) for access and maintenance of soybean plots. Support for this study was provided by the Project GREEEN initiative of MSU, and USDA North Central Region IPM, Competitive Grants Program.

LITERATURE CITED


Female fighting and host competition among four sympatric species of *Melittobia* (Hymenoptera: Eulophidae)

Robert W. Matthews¹ and Leif D. Deyrup²

**Abstract**

*Melittobia* is a genus of parasitic wasps well known for high levels of inbreeding and violent male combat. Casual observations of groups of sisters of *M. femorata* placed with hosts revealed a surprising incidence of body mutilations (broken or missing tarsi, antennae, and wings). Replicated conspecific groups of 1, 2, or 3 females of *M. femorata, M. digitata,* and *M. australica* and interspecific groups of *M. femorata* and *M. australica* (2:1) were observed over their first 10 days in newly established cultures, and the incidence of mutilation was recorded. In some groups females were dye-fed, allowing us to subsequently chart their individual activity patterns on or near the host based on patterns of their colored fecal droppings. For *M. australica* and *M. digitata,* no conspecific females in any group size ever showed mutilation. However, in *M. femorata* nearly 3/4ths of the females in conspecific groups of two or three acquired body damage beginning about the time of first oviposition on the host. In 4 of 5 replicates of the interspecific groups, *M. femorata* females killed the female of *M. australica.* Patterns of dyed fecal droppings that developed over several days showed that individual females in groups of both *M. femorata* and *M. australica* increasingly restricted their activities to a small portion of the host. These “micro” territories were non-overlapping and appeared to be actively defended. In contrast, *M. digitata* females in groups never displayed obvious territoriality or interference. Possible reasons for these differences in female behavior are discussed.

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*Melittobia* are small external parasitoids that attack solitary bees, wasps, and their associates (Balfour-Browne 1922, Buckell 1928, Dahms 1983b, Krombein 1967). This cosmopolitan genus includes 12 species, some of which coexist geographically, often upon the same hosts (Matthews et al. 2009). Across eastern North America, a common shared host is the mud-dauber wasp, *Trypoxylon politum* Say (Hymenoptera: Sphecidae). Another common host sphecid, *Sceliphron caementarium* Drury, coexists with *T. politum,* but extends its range to include the western United States.

All *Melittobia* species appear to have a generally similar life history. Upon finding a host prepupa, the female parasitoid stings it and feeds upon exuded host fluids. This stimulates egg maturation and within 2 to 4 days, she begins to oviposit on the host; over the ensuing 10 days, she ultimately may lay hundreds of eggs in clusters on individual large hosts.

Most of these eggs develop into female offspring, either of an early brachypterous form or a later macropterous dispersal form (Schmieder, 1933, Cônsoli and Vinson 2002). Males, which generally comprise 5% or less of the offspring, emerge at a low but continuous rate throughout female emergence (Adams

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Before dispersing, the females mate with these males, which are very likely brothers; thus, inbreeding appears to be the usual situation.

Males of *Melittobia* are known for their lethal combat (Hamilton 1979, Hartley and Matthews 2003, Deyrup et al. 2006, Innocent et al. 2007). Females, however, are generally considered docile and even supportive of one another; for example, *M. digitata* females non-aggressively queue up to await male courtship and cooperate with one another to chew out of the host’s nest. (Donovan 1976, Deyrup et al. 2005). This female docility may not be the rule, however. Much of the available information on *Melittobia* is based on research conducted with this one species that is sold under the name “WOWBug” (Carolina Biological Supply Co., Burlington, NC). Because of its ready availability and ease of rearing on artificial hosts such as the common blowfly, *Sarcophaga (Neobellieria) bullata*, *M. digitata* is becoming a model organism for laboratory and classroom work. However, two less-studied species, *M. femorata* Dahms and *M. australica* Girault, are actually the most commonly collected *Melittobia* species on *T. politum* in the southeastern United States (J.M. González and R.W. Matthews, unpublished data). Also sympatric but generally more northerly and less widespread is *M. acasta* (Walker), which may have been accidentally introduced from Europe by way of Canada at least 40 years ago (González et al. 2004).

The extent of parasitoid competition in arthropod communities is unresolved, but thought to be widespread (Godfray 1994, Hawkins 2000), especially since multiple species often attack the same host. Competition may be manifested in various ways and at different times in the parasitoid-host interaction. Both interference and exploitative competition can occur and there are numerous examples, especially from the biological control literature (Hawkins 2000). Several parasitic wasps have been reported to defend a host resource, their eggs, or their offspring from conspecifics (e.g., Field and Calbert 1999, Hardy and Blackburn 1991, Wilson 1961). Interactions among female parasitoids often are mediated via chemical markings that appear to deter conspecific females from superparasitism (Hoffmeister and Roitberg 1997, Petersen and Hardy 1996). Among host searching female ectoparasitoids, competition between congeneric species has been little studied.

In our laboratory on various occasions we have noted both intra- and interspecific aggression, body damage, and death when combinations of *Melittobia* females have been placed on a common host. Field collections of host *T. politum* cocoons have revealed natural multiparasitism by two or rarely three *Melittobia* species on at least five occasions: three from Georgia, and one from both Alabama and New York (González and Matthews, unpublished data). Thus, to better understand competitive interactions among host-seeking females we undertook the studies reported here.

**MATERIALS AND METHODS**

All four *Melittobia* species were originally obtained from parasitized cocoons of the mud dauber wasp, *Trypoxylon politum* Say (Hymenoptera: Sphecidae). The *M. femorata* stock originated from Arnoldsville, Oglethorpe Co., GA; *M. digitata* from Athens, Clarke Co., GA; *M. australica* from Gainesville, Alachua Co., FL; and *M. acasta* from Townsend, Blount Co., TN. Prior to this study, laboratory cultures of each species had been continuously maintained from one to four years at the University of Georgia. Reculture protocol for each new generation was to haphazardly select five mated females of unknown age and place them on a naked *T. politum* prepupa in small vials maintained in a dark incubator at 25°C. New cultures were established every 21 days except for *M. femorata* whose reculture cycle varied from 90-120 days.

All experiments and controls used 1 to 2-day-old mated females that had eclosed from a single stock culture of each species. As hosts for these parasitoids,
we used naked *T. politum* prepupae extracted from local field-collected nests and individually placed in small plastic boxes (50 mm × 25 mm × 18 mm, Carolina Biological Supply Co., Cat. No. ER-14-4584). Experiments were conducted in the same individual plastic boxes and were maintained in a constant-temperature chamber at 25°C.

For some studies, we marked individual females by feeding them 20% fructose and water dyed with McCormick® food coloring. After females imbibe this fluid, it is easily visible in their crops through their semi-translucent cuticle (see Matthews et al. 2009); different colors served to identify individual females. In addition, because the color is retained in the female’s fecal matter, this technique allowed us to track each female’s activity through the pattern of her fecal droppings on the floor of the plastic box.

**Female competition in *M. femorata***. In 28 boxes, mated 2-day-old unfed *M. femorata* females of the long-winged morph were concurrently placed with individually boxed *T. politum* prepupae in the following design: A single female in 6 boxes, 2 females in 13 boxes, and 3 females in 9 boxes. Boxes were maintained at ambient room temperatures and checked daily over the following 10 days, noting the females’ behavior and recording any body damage. In order to track individual females and their movements, 15 additional cultures were established with 3 females of *M. femorata* marked by dye-feeding as outlined above.

**Interspecific competition in *M. femorata* and *M. australica***. To determine how *M. femorata* fared when confronted with another species on the host, we set up five boxes containing one dye-fed *M. australica* and two dye-fed *M. femorata*. These boxes were observed daily for 10 days and body damage and fecal dropping patterns were recorded. For comparison with intraspecific competition between individuals, we concurrently set up 20 boxes of three dye-fed *M. digitata* females and 20 boxes of three dye-fed *M. australica* females; *M. acasta* was unavailable for this comparison.

**Female competition in *M. digitata*, *M. australica*, and *M. acasta***. To further examine these interactions, a subsequent experiment used unfed females in a design that examined inter- and intra-specific interactions in three *Melittobia* species by comparison with solitary females. Three treatments placed two females of different species on a naked *T. politum* host (average weight = 0.253g ± 0.060 SD) in the 3 possible combinations: *M. digitata* vs. *M. acasta*, *M. digitata* vs. *M. australica*, and *M. australica* vs. *M. acasta*. Another three treatments placed conspecific pairs of each of the three *Melittobia* species. Controls consisted of cultures of each species established by a single female. Each treatment and control was replicated 10 times. *M. femorata* was not available for these comparisons.

Each treatment replicate and associated control was checked daily for the first 8 days, then twice weekly for the next 10 days, noting oviposition, feeding, and “jousting.” At day 18 all emerged adults were sexed and counted to assess the effects of inter- and intraspecific competition on fecundity and reproductive success relative to solitary foundress control cultures of each species at the same stage.

**RESULTS**

**Intraspecific female competition**. In the treatments containing three dyed *M. femorata* females, 1 to 4 days after being placed on a host the females’ activities became increasingly localized, each focused upon a particular portion of the host’s body. From the distribution patterns of dyed fecal droppings it was apparent that each female *M. femorata* was developing a more or less exclusive “micro” territory (Fig. 1), and that the boundaries between them were relatively distinct. Undyed females in the groups of two or three in the other set of cultures
appeared to behave similarly. Females of *M. australica* also displayed similar territoriality in all 20 cultures. However, fecal droppings of *M. digitata* females displayed no grouping pattern in any of the replicates. During the course of oviposition (roughly days 2-10), the frequency of aggression and incidence of body mutilation (manifested as missing tarsomeres and antennal flagellomeres and tattered and broken wings) increased among groups of *M. femorata* females. We regularly observed females biting at other females and even rolling around in locked combat (Fig. 2). In addition, many females were noted to walk about with their wings raised as though damaged. Normally, wings are held flat over their abdomens.

At least one female with damage occurred in every replicate (9/9 for groups of three females and 13/13 for groups of two females), and in several replicates all females in a group exhibited some type of body damage (Table 1). Overall, 16 of the 25 females in the foundress pairs replicates and 20 of the 25 females in the three foundress groups had body damage.

By contrast, none of the females in any of the 20 groups of three *M. digitata* or *M. australica* acquired body damage over the 10-day period. Periodic observation revealed no indication of agonistic interactions among females of *M. digitata*; however, while never overtly hostile, individual *M. australica* were sometimes seen to follow or approach other females on the host and appeared to disturb the other female with proximity or nudging.

**Progeny production.** In the final experiment, counts of adult progeny as of day 18 indicated that among both the single female control and the two conspecific female cultures, *M. digitata* was the most prolific, followed by *M. acasta* and *M. australica* (Table 2). Pair-wise interspecific comparisons of the average numbers of progeny produced showed that *M. acasta* outperformed both of the other two species when in direct competition, and that *M. digitata* did better than *M. australica*. However, *M. australica* was significantly less productive than either competitor (Tables 2 and 3). This contrasts to the intraspecific competition results where no significant differences in total progeny production were found between single female and two female cultures (Tables 2 and 3) though the variance in all experiments was great and the number of replicates relatively few.
Fig. 2. Two egg-laden female *M. femorata* locked in combat. Although these encounters do not tend to be lethal, females often mutilate one another.

Table 1. Incidence of damage among cofoundresses of *Melittobia femorata* in different sized foundress groups during the first 10 days of their being simultaneously placed with a *Trypoxylon politum* prepupa host.

<table>
<thead>
<tr>
<th>Initial No. of females</th>
<th>No. of replicates with female damage/Total No. of replicates</th>
<th>Total No. of females*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With body damage</td>
</tr>
<tr>
<td>1</td>
<td>0/6</td>
<td>0</td>
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<tr>
<td>2</td>
<td>13/13</td>
<td>16</td>
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<tr>
<td>3</td>
<td>9/9</td>
<td>20</td>
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</table>

*Discrepancy in total numbers due to loss of three females that escaped or were accidentally killed.*
Table 2. Numbers of *Melittobia* emerging by day 18 from each interspecific, intraspecific, and single female treatment on *Trypoxylon politum* prepupae. Values are means ± S. D.

<table>
<thead>
<tr>
<th>Treatment (N)</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. acasta</em> + <em>M. australica</em> (10)</td>
<td>230.8 ± 54.4</td>
<td>17.3 ± 6.3</td>
<td>248.1 ± 56.0</td>
<td>20.6 ± 41.8</td>
<td>2.1 ± 4.0</td>
<td>22.7 ± 45.7</td>
<td>20.6 ± 41.8</td>
<td>2.1 ± 4.0</td>
<td>22.7 ± 45.7</td>
</tr>
<tr>
<td><em>M. australica</em> + <em>M. digitata</em> (10)</td>
<td>48.4 ± 30.2</td>
<td>1.2 ± 1.0</td>
<td>49.6 ± 30.6</td>
<td>95.6 ± 74.2</td>
<td>5.5 ± 3.1</td>
<td>101.1 ± 75.0</td>
<td>213.6 ± 128.4</td>
<td>2.8 ± 2.9</td>
<td>216.4 ± 127.9</td>
</tr>
<tr>
<td><em>M. digitata</em> + <em>M. acasta</em> (10)</td>
<td>297.7 ± 149.6</td>
<td>11.2 ± 6.0</td>
<td>308.9 ± 150.2</td>
<td>213.6 ± 128.4</td>
<td>2.8 ± 2.9</td>
<td>216.4 ± 127.9</td>
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<tr>
<td><em>M. acasta</em> + <em>M. acasta</em> (9)</td>
<td>397.7 ± 181.5</td>
<td>15.7 ± 5.9</td>
<td>413.3 ± 181.2</td>
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<td></td>
<td>417.0 ± 160.5</td>
<td>13.6 ± 4.9</td>
<td>430.6 ± 160.3</td>
</tr>
<tr>
<td><em>M. australica</em> + <em>M. australica</em> (9)</td>
<td>269.9 ± 140.2</td>
<td>6.6 ± 1.3</td>
<td>276.4 ± 141.1</td>
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<tr>
<td><em>M. digitata</em> + <em>M. digitata</em> (8)</td>
<td>417.0 ± 160.5</td>
<td>13.6 ± 4.9</td>
<td>430.6 ± 160.3</td>
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<tr>
<td><em>M. acasta</em> (9)</td>
<td>285.6 ± 90.7</td>
<td>14.9 ± 5.6</td>
<td>300.4 ± 89.7</td>
<td>196.2 ± 193.0</td>
<td>5.7 ± 4.7</td>
<td>201.8 ± 192.9</td>
<td>339.6 ± 133.7</td>
<td>8.0 ± 2.5</td>
<td>347.6 ± 133.6</td>
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<td><em>M. australica</em> (6)</td>
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<tr>
<td><em>M. digitata</em> (9)</td>
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Female competition: *M. femorata* and *M. australica*. In the mixed species cultures, *M. australica* often appeared to pressure a female of *M. femorata* to abandon her territory, and in some instances caused her to move completely off of the host early in their association. However, after the *M. femorata* became physogastric (abdomens swollen with eggs), the tables turned, and in four of the five replicates the *M. australica* female exhibited damage and was eventually decapitated. In only one case did *M. femorata* and *M. australica* appear to share the same area on the host, with no evidence of any body damage.

Interestingly, in the cultures co-housing *M. femorata* and *M. australica* females, the onset of microterritoriality in *M. femorata* seemed to be delayed (3-5 days after being placed on host) relative to its onset for a single foundress; unfortunately, small sample sizes obviate firm conclusions.

Female competition: *M. acasta* and *M. australica*. In 8 of the 10 replicates, apparent signs of fierce and fatal competition were observed in females of both species within six days after introduction upon the host. Evidence of battles included damaged heads, broken and missing tarsi, tattered wings, and immobility. By 10 days the *M. australica* female was killed by *M. acasta* in 7 replicates, resulting in the very low numbers of progeny realized by *M. australica* (Table 2). In the three remaining replicates in which battles were not extreme enough to lead to immobility or death, both species nonetheless showed signs of struggle.

Female competition: *M. digitata* and *M. australica*. Based on daily observations, *M. australica* appeared to dominate over *M. digitata* during the first 12 days of the study, as *M. digitata* suffered more injuries and mortality (The *M. digitata* female was apparently killed in 2 replicates during first 10 days; in one other replicate both females were found dead after 4 days with no evident body damage to either). In the remaining 7 replicates both females survived with no injuries or evident aggression, though the daily checks revealed that the *M. australica* female was more often on the host. However, by the measure of number of adult females produced by day 18 of the study (Table 2), *M. digitata* dominated with significantly more progeny by every measure (Table 3).

Table 3. Statistical comparisons of progeny production of three *Melittobia* species in the inter- and intraspecific experimental treatment groups. $P$ values are for two sample assuming unequal variance t-test (2-tailed).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Comparison</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interspecific</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>australica &amp; digitata</em></td>
<td>Total <em>digitata</em> vs. ave. of 2 <em>digitata</em></td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Total <em>australica</em> vs. ave. of 2 <em>australica</em></td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Total of both vs <em>australica</em></td>
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</tr>
<tr>
<td></td>
<td>Total of both vs <em>digitata</em></td>
<td>0.001</td>
</tr>
<tr>
<td><em>australica &amp; acasta</em></td>
<td>Total <em>acasta</em> vs. ave. of 2 <em>acasta</em></td>
<td>0.262</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
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<tr>
<td><em>M. australica</em></td>
<td>Single female vs 2 females</td>
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</table>
**Female competition: *M. digitata* and *M. acasta***. When *M. digitata* and *M. acasta* shared a host, no aggression or body damage was observed between the females during the first 10 days. Both species realized high adult progeny production, averaging over 200 for *M. digitata* and nearly 300 for *M. acasta* (Table 2) and not significantly different from that realized in intraspecific competition (Table 3). Interestingly, in the heavy fighting that was observed between emerging males of these two species, *M. acasta* dominated, killing most *M. digitata*.

**DISCUSSION**

Territoriality has been widely documented in insects; however, much of the literature focuses on males in various forms of intrasexual selection (Baker 1983). Territoriality or intense intraspecific competition involving partitioning and defense of resources among conspecific female insects is relatively uncommon in most insect groups, but has been recorded for some tephritid flies (Diptera: Tephritidae) (Prichard 1969, Shelly 1999), water striders (Hemiptera: Gerridae) (Nummelin 1988), aphids (Hemiptera: Aphididae) (Inbar 1998), and webspinners (Embioptera) (Bradoo and Joseph 1970). Egg-brooding females of an African arachnophilic embiid viciously attacked experimentally-introduced conspecifics and at times succeeded in “plucking a leg or few antennal segments off the intruders” (Bradoo and Joseph 1970). Among the Hymenoptera, both ants (Formicidae) (Hölldobler and Wilson 1990) and parasitic wasps (Chalcidoidea) (Griffiths and Godfray 1988) often establish and defend foraging territories. Some parasitic wasps have been reported to defend a host resource, their eggs, or their offspring from conspecifics (Field and Calbert 1999, Hardy and Blackburn 1991, Wilson 1961).

Under field conditions, dispersing *Melittobia* females are temporally and spatially clumped, and usually crawl rather than fly to locate hosts (Freeman and Ittyeipe 1976). Potential hosts also may be clumped and persistent in favored locations. Thus, multiple parasitism is probably a rather common phenomenon. Molumby (1996), for example, found 1 to 5 (mean = 1.8) *M. femorata* females per host in midsummer *T. politum* nests in Mississippi. Some sort of response to such encounters would be warranted, and could be expected to differ for each species (and combination thereof).

Despite superficial similarities in host and lifestyle and overlapping geographic ranges, the behavior and life history of the four species in this study all differ from one another in significant ways; *M. femorata* in particular is not a typical member of its genus (Matthews et al. 2005, Matthews and González 2008). In addition to two distinctly separated non-overlapping adult generations on a single host, it shows striking differences in life history and morphology (Matthews and González 2008). Distinctly smaller than the other species, *M. australica* might be predicted to lose out in more interspecific battles, as in fact it did (Tables 2 and 3); interestingly, it also is the only species among those studied that does not belong to the acasta group of Dahms (1983a). The contrast between such an extreme degree of intraspecific female pugnacity in *M. femorata* and *M. acasta*, and its absence in *M. digitata* and *M. australica* was unexpected, particularly since *M. digitata*, *M. femorata*, and *M. acasta* are thought to be closely related and were placed in the same species group by Dahms (1984a) on the basis of morphology.

**Why should females of *M. digitata* and *M. australica* tolerate conspecifics?** Their communal oviposition is clearly facultative, since a single female has the ability to produce large numbers of eggs sufficient to fully consume the host upon hatching. Perhaps any disadvantages are outweighed by benefits accruing to larvae or the mixing of broods. Genetic studies could be enlightening.

One should not discount the possibility that the context in which we observe these interactions is not the same as the one in which the pugnacity
evolved. While mud-dauber wasps are commonly assumed to be the principal host of all these species, this could be simply a sampling bias brought on by the conspicuous nature of the highly visible, long-lasting nests. In addition, while today's high mud dauber nest densities provide a good likelihood that two or more female *Melittobia* emerging from the same clutch may jointly colonize a nearby host, this phenomenon may be relatively recent, an artifact of human activities such as bridge and barn building. Perhaps other solitary bees and wasps were the principal original hosts for the four *Melittobia* species, such that each species' fundamental behavioral ecology and selection pressures may have been very different from that carried over into the laboratory from mud dauber nests.

For *M. digitata* and *M. australica*, one laboratory study has compared progeny production of groups of one to five conspecific females given a single blowfly host (Silva-Torres and Matthews 2003). While absolute numbers from this smaller artificial host cannot be directly compared to our results, the relationships would be expected to be similar. In that study, as in ours, both alone and with up to five females of their own species, *M. digitata* produced more offspring than *M. australica* for every group size. Offspring of both species developed slightly faster when in competition than under sole foundress conditions.

Given that multiple foundresses of *M. femorata* readily attack one another on a new potential host, it is interesting to note that newly mated *M. femorata* females cooperate to chew a common exit hole (Deyrup and Matthews 2007a), just as *M. digitata* do (Deyrup et al. 2005). Comparing the behaviors of host feeding and cooperative escape-chewing in *M. digitata*, Deyrup and Matthews (2007b) found they were very similar, and suggested that the two behaviors have a similar biological basis. In *M. femorata* we have the seeming contradiction of a species in which cooperative chewing for escape and aggressive interactions coexist; it may be instructive that aggression only occurs when oviposition commences, days after host feeding has occurred.

While there appear to be no published papers on interspecific female competition in *Melittobia*, it most likely occurs in nature. As noted above, we have on occasion found females of 2 (and once, 3) species in a single mud dauber cocoon. This observation suggests that females' host-searching behavior must be somewhat flexible, and that both inter- and intraspecific host sharing does occur. Whether host sharing females can somehow assess a competitor's size and/or reproductive status and make conditional decisions about whether to stay or leave remains to be studied. Our laboratory experiments were admittedly artificial in that both females were simultaneously introduced to the host and had no opportunity to leave to search for another. In nature, two females would most likely arrive at different times, giving one a head start, and later arrivals would have a fight-or-flee option.

What selects for one species to behave aggressively, but not another? Genetic analysis of female relatedness, experimental manipulation of host searching cues and discovery context, and further life history research may ultimately lead to answers. Certainly one could hardly ask for a more amenable group than *Melittobia* with which to address that question; these four sympatric parasitoids are commonly found, easily reared, readily manipulated, and appear to display a continuum of aggressive interactions in both sexes, promising that such further study will be both agreeable and rewarding.

ACKNOWLEDGMENTS

Funding that supported part of this research was provided by a National Science Foundation grant to R. W. Matthews. We thank Kathryn Hauth who first noticed the mutilation occurring among groups of *M. femorata* females during an undergraduate independent study. We thank Joe R. Williamson, Aubrey Roche, and Rachel Bodiford for laboratory assistance.
LITERATURE CITED


ALFALFA SNOUT BEETLE, OTIORRHYNCHUS LIGUSTICI L. (COLEOPTERA: CURCULIONIDAE): METHODS FOR EGG COLLECTION AND LARVAL REARING

Elson J. Shields¹, Gabor Neumann and Antonio M. Testa

ABSTRACT

The alfalfa snout beetle, Otiorhynchus ligustici L., is the most serious pest of alfalfa in northern New York State. Recent research efforts focused on the biological control of this insect require the availability of all life stages. With a 2-year lifecycle and a mandatory diapause, the artificial rearing of a laboratory culture appears to be a non-viable option at present, but methods described here can be used to obtain sufficient numbers of eggs and larvae over an extended period of time for research purposes. The crowding of adult beetles in egg production units (cups) had a significant, negative effect on egg production per beetle but the total egg production per cup was still higher with higher number of beetles per cup resulting in a significant saving of labor per egg produced. Larval survival rates in alfalfa-planted cans were surprisingly low given the protected conditions of the greenhouse. The larval survival rates were not significantly different among the dates for the second instar and later instars, suggesting that larval mortality occurs in the first instar in alfalfa-planted cans.

The alfalfa snout beetle, Otiorhynchus ligustici L. (Coleoptera: Curculionidae), was introduced into the United States from Europe via wooden sailing ships carrying soil as ballast (Lindroth 1957, York et al. 1971). The beetle was first recorded in New York State in 1896 at the Port of Oswego and was first recorded as a pest of alfalfa when alfalfa was introduced into the area in the 1920s (York et al. 1971). In subsequent years, this flightless and parthenogenetic insect has spread to nine Northern New York counties, infested over 200,000 hectares of cropland and has become the most serious pest of alfalfa in northern New York State (Schroeder et al. 1994, Ferguson et al. 1995, Shields et al. 1999). The larvae feed on the lateral roots and later on the tap roots of the host plants. Alfalfa snout beetle has a 2-year lifecycle. The biology and life history of alfalfa snout beetle has been studied and described by several authors in Eurasia and North America (Vassiliev 1914 in York 1974, Lincoln and Palm 1941, Hanuss 1958, Nyilas 1962, York 1974, Jermy and Balázs 1990) and is very similar throughout Europe and Northern New York. Most larvae mature by late fall and move down in the soil to varying depths depending on soil type, temperature, and other factors. Mature larvae remain quiescent deep in the soil for ca. 8 months before pupation the following summer. After eclosion, adults remain in the pupal cells and only move to the soil surface the following spring after spending the second winter in the soil (Lincoln and Palm 1941). Adults start moving up to the soil surface when spring soil temperatures warm to 3°C and appear on the surface from late April to early May throughout the geographical distribution in the US. After reaching the surface, adults feed on the available host plants after which oviposition commences (Schroeder et al. 1995).

Research efforts focused on this insect require the availability of all life stages for an extended period of time; usually large numbers of individuals are needed. With a 2-year lifecycle and a mandatory diapause, the artificial rearing of a laboratory culture appears to be a non-viable option. However, the adults

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can be collected in the spring in large numbers and placed in cold storage at 4°C for 4 months without significant death, extending the adult availability from 3 weeks to 4 months. Adults will survive in cold storage for 4 months with low mortality and a few individuals will survive in cold storage for up to 9 months (EJS, personal observation). Adults can be removed from cold storage, fed and used to produce eggs. The purpose of this report is to illustrate a series of economically cost effective procedures to produce eggs, collect eggs and rear larvae to predetermined stages using alfalfa plants in containers. These techniques are currently being used in ongoing research projects focused on biological control and developing a snout beetle-resistant alfalfa.

MATERIALS AND METHODS

Egg production. Alfalfa snout beetle adults were field collected in late April 2006 near Great Bend, NY, immediately after emergence. The adult beetles were kept in cold storage (4°C) for 4 weeks on moist filter paper. The beetles were then placed into paper cups (82 mm diameter, 70 mm depth). The cups contained a layer of approximately 1 cm of autoclaved soil to encourage egg laying. The soil was sifted through an 18 mesh screen (1 mm opening) to remove the large particles. The soil was lightly moistened as needed. Fresh alfalfa foliage was provided to the beetles every other day as food.

The impact of adult crowding on egg production was investigated by placing adult females singly, two, four, eight, or 16 beetles/cup. Each treatment had 30 replicates. The cups were monitored daily and eggs were collected 6 and 12 days after the detected onset of oviposition. The total number of eggs was recorded for each cup. The average egg production per beetle in cups was calculated by dividing the total number of eggs by the number of beetles in the cup. A regression analysis was conducted with the number of beetles in a cup being the independent factor and the per beetle egg production being the response. Because of the heteroskedasticity of the data, the weighted least-square method was used.

Egg collection. The soil from the cups was rinsed in a 60-mesh screen (250 micron openings) to separate the eggs and large soil particles from the small soil particles. The remaining soil and eggs were placed into 300 ml of a 40% sugar solution in a 500 ml glass beaker. Eggs were suspended in the solution or floated to the surface, while the majority of the soil sank to the bottom of the beaker. The soil on the bottom of the beaker was stirred gently with a plastic stirring rod to free any eggs trapped in the soil. The egg-sugar solution was then decanted from the beaker through a 60 mesh sieve that retained the eggs. Eggs were then washed off the sieve with water into a 50 ml beaker. In water, the eggs sank to the bottom of the container while the remainder of the debris (frass, leftover plant material) floated to the top and could be simply decanted leaving a small volume of the water on the bottom of the beaker with the eggs. The eggs were then poured onto a filter paper in a filter funnel. The eggs were surface sterilized with a 5% bleach solution by pouring the bleach solution over the eggs. After one minute, the eggs were immediately rinsed with deionized water.

Egg hatch rate. Eggs from adult beetles were collected using the sugar flotation method described above. Three different methods of egg incubation were used: 1) control: 100 eggs were rinsed in deionized water and placed on a moist filter paper in a Petri dish (10 cm diameter); 2) treatment 1: 100 eggs were suspended in a 0.5% agar solution (10°C temperature) and left undisturbed for one hour; they were then pipetted off onto a filter paper to drain the excess agar and were placed onto a second filter paper moistened with deionized water in a Petri dish; 3) treatment 2: a Petri dish was filled with Cornell mix (consisting of 1:2 peat moss-vermiculite mixture). One hundred eggs were suspended in 0.5% agar solution (10°C temperature) and left undisturbed for one hour. Eggs were
then placed onto a filter paper to absorb the excess moisture. The eggs were then moved from the filter paper using a soft brush onto the surface of potting soil. These Petri dishes were left uncovered and were lightly moistened daily with a water sprayer bottle.

All eggs were incubated at 23°C in the dark. The number of eggs hatching was determined daily for a total of 14 days and the proportion of eggs hatching was recorded. Each treatment was replicated four times. Proportional data was transformed using arcsine square root transformations and then mean hatch rates in the different treatments were compared using ANOVA and Tukey’s HSD procedure. Statistical analyses were conducted using SAS™ system for Windows™, release 8.02 (SAS Institute Inc., Cary, N.C.).

Inoculation of alfalfa plants with eggs. Alfalfa plants were grown in plastic waste paper cans (21 cm width, 27 cm length, and 33 cm depth). The waste paper cans had four small holes (approximately 1 cm diameter) drilled in the bottom to allow for water drainage during watering and the bottom of the cans was lined with fine mesh fiberglass window screen to prevent the escape of larvae from the cans. The cans were then filled with Cornell Mix. Alfalfa seeds were planted in the cans and allowed to grow and establish for 6 weeks before being inoculated with snout beetle eggs.

Alfalfa snout beetle eggs were collected as described above and suspended in 0.5% agar solution and cooled to 10°C at a concentration of 5 eggs/ml. The eggs remained in the agar solution for approximately one hour before application into the soil around the plants. The use of the dilute agar solution thickened the liquid so the eggs remained suspended without gravitational settling. Each can was inoculated with 500 eggs by spreading 100 ml of egg suspension on the surface of the potting soil. A subset of the eggs used to inoculate the cans was returned to the laboratory and monitored for hatching. The inoculated cans were incubated in a growth chamber at a constant temperature of 24°C. A total of 22 cans were inoculated.

Alfalfa snout beetle larval survival in the cans. Larvae were recovered from the cans by breaking down the cans and sifting the soil at eight different times after inoculation: 22 days (three cans), 28 days (three cans), 35 days (two cans), 40 days (four cans), 49 days (three cans), 54 days (two cans), 60 days (two cans), and 68 days (three cans). The larval instars were determined by measuring the width of the head capsule of the larvae. The presence of 1st instar larvae was not tabulated due to the small size of the instar and the difficulty of accurately counting the larvae accurately. The proportion of surviving alfalfa snout beetle larvae was calculated by dividing the number of recovered larvae by 500 (the number of eggs inoculated). Proportional data was transformed using arcsine square root transformation and then mean survival rates at the different times were compared using ANOVA and Tukey’s HSD procedure.

RESULTS AND DISCUSSION

Egg production. The mean egg production per beetle was the highest, 82 ± 9.5 (mean ± SE), when only a single beetle was present in a cup and the lowest, 43.1 ± 1.9, when 16 beetles were in the same cup. The mean egg production per beetle was 66.1 ± 5.9 with two beetles in the same cup, 62.3 ± 3.0 with four beetles, and 51.5 ± 2.9 with eight beetles in a cup. There was a negative linear relationship ($F = 35.25$, df = 1, 148, $P < 0.000$, $r^2 = 0.19$) between the number of beetles in a cup and the per beetle egg production (Fig. 1). The mean total egg production per cup was the highest, 689.1 ± 31.1, with 16 beetles in a cup and the lowest, 82.0 ± 9.5, with a single beetle per cup. The mean total egg production per cup was 132.10 ± 11.70 with two beetles in the same cup, 249.1 ± 11.9 with four beetles, and 412.1 ± 23.5 with eight beetles in a cup. The high egg production variability which is the most obvious in the single insect per
cup data is typical of this parthenogenetic species, where egg production from individual insects commonly range from zero to several hundred per individual (Lincoln and Palm 1941, York 1974).

It appears that the crowding of the beetles results in decreased oviposition per beetle but it is not known whether this effect would be observed over the entire lifetime of the beetle. Since we were mostly interested in the efficiency of harvesting the eggs, only the first 12 day period was investigated. It is not clear why adult beetles would decrease their egg production when crowded, but one possible explanation could be that in natural conditions, oviposition under crowded conditions increases the intraspecific competition for larval resources.

Our main objective was to produce a large number of eggs efficiently. Although crowding the beetles (16 per cup) resulted in reduced per beetle egg production compared to the single beetle per cup, the total egg production per cup was still much higher, resulting in a significant saving of labor per egg produced. If the availability of beetles is not the limiting factor, then crowding the beetles is the most labor efficient method. However, if beetle availability is an issue, egg production is the greatest when a single beetle is caged individually.

**Egg hatch rate.** The percentages of hatching eggs were 47.0 ± 3.2% in the control, 51.0 ± 4.6% in treatment 1, and 46.5 ± 2.5 in treatment 2. Other researchers have reported a similar hatch rate ranging between 50-60%
The proportions of hatching eggs among the three different incubation methods were not significantly different ($F = 0.48$, df = 2, 9, $P = 0.631$). Therefore, we conclude that the sucrose-flotation method had no adverse effects on snout beetle egg hatching rate. Suspending insect eggs in agar solution with a specific density is a widespread method for insect egg applications, because the eggs neither sink nor float on the surface of the suspension so the rate of application is even (Fery et al. 1979, McEwen 1996, Abel et al. 2000).

**Larval survival.** The survival of alfalfa snout beetle on alfalfa plants grown in waste paper cans ranged between 3.13 ± 0.07% and 4.20 ± 0.20%. Survival rates did not change significantly with increasing time since inoculation ($F = 0.75$, df = 7, 14, $P = 0.638$) (Table 1.). The hatching rate we observed did not differ from early research conducted on snout beetle (Lincoln and Palm 1941). The larval survival rates were surprisingly low given the protected conditions of the greenhouse but were higher than reported by Schroeder et al. (1994). The cans had sufficient alfalfa plants to support a higher number of larvae with a large root mass in every container, so food availability was not considered a limiting factor. It would appear that a large amount of larval mortality occurs from the 1st instar larvae failing to find a root as a food source shortly after hatching.

**ACKNOWLEDGMENTS**

We thank the Northern New York Agricultural Development Program for their long-term financial support for research on alfalfa snout beetle. Without their support, little research on this invasive insect would be attempted or completed. We thank Luanne Belgodere for her help in the laboratory.

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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. Swanson1,2 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* Le Conte (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 m$^2$ 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) (Blesser 1993, Dirig 1994). Interruption of naturally occurring disturbance regimes (i.e., fire, drought, grazing) has contributed to the success 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 (Hastin 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), *Pirophorum* (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 quadrant. The same researcher (JS) made this estimation before the beetles arrived, during the beetle
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 × 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 × 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 (± SD) number of beetles per lupine stem was 2.0 (± 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 (± SD) percent cover of lupine per quadrat before the beetles arrived was 31.8 (± 1.3) % (Table 3) and declined to 7.8 (± 0.5) % by 10 June. Before the beetles appeared, the mean (± SD) number of stems with flowers per clump was 16.2 (± 8.5) which declined to 2.5 (± 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* larval 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.

<table>
<thead>
<tr>
<th></th>
<th><strong>Females (n=14)</strong></th>
<th><strong>Males (n=14)</strong></th>
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<tbody>
<tr>
<td></td>
<td>East</td>
<td>West</td>
</tr>
<tr>
<td>Before</td>
<td>92.5</td>
<td>7.4</td>
</tr>
<tr>
<td>During</td>
<td>87.6</td>
<td>12.3</td>
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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).

<table>
<thead>
<tr>
<th>Sex</th>
<th>% Time Perching</th>
<th>% Time Flying</th>
<th>¹P. Simplex Flower</th>
<th>¹L. perennis Flower</th>
<th>Other Flower</th>
<th>Other Leaf</th>
<th>OTHER %</th>
<th>Under leaf</th>
<th>Flower ¹</th>
<th>Leaf ⁶</th>
<th>Soil</th>
<th>Grass</th>
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<td>Female</td>
<td>Before</td>
<td>90.7</td>
<td>21.9</td>
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<td>0.0</td>
<td>3.6</td>
<td>5.4</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>During</td>
<td>92.9</td>
<td>11.0</td>
<td>1.3</td>
<td>48.0</td>
<td>0.0</td>
<td>16.6</td>
<td>5.3</td>
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<td></td>
<td>During</td>
<td>31.8</td>
<td>18.2B</td>
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<td>8.7</td>
<td>7.6</td>
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</table>

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.

<table>
<thead>
<tr>
<th>Q</th>
<th>¹Mean % of <em>L. sayi</em> population</th>
<th>²% Cover <em>P. simplex</em></th>
<th>³% Cover <em>L. perennis</em> 6 June</th>
<th>⁴% Cover <em>L. perennis</em> ¹¹ June (n=26)</th>
<th>⁵% Larval damage sites before <em>L. sayi</em> (n=63)</th>
<th>⁶% Larval damage sites during <em>L. sayi</em></th>
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<tbody>
<tr>
<td></td>
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<td>S</td>
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Table 3. Continued.

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<th>1Mean % of ( L. ) sayi population</th>
<th>2% Cover ( P. ) simplex</th>
<th>3% Cover ( L. ) perennis 6 June</th>
<th>( L. ) sayi damage sites June (n=26)</th>
<th>4% Cover ( L. ) perennis 6 June before ( L. ) sayi (n=63)</th>
<th>5% Larval damage sites during ( L. ) sayi</th>
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</table>

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);

3,4\( L. \) 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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**LITERATURE CITED**


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ABSTRACT

The gut contents of a brown trout (Salmo trutta) included horsehair worms (Gordius robustus, Nematomorpha: Gordiida) emerging from a camel cricket (Ceuthophilus sp., Orthoptera: Gryllacrididae). This provides more evidence of secondary ingestion than most previous reports of predation on horsehair worms, but it also illustrates the difficulty of distinguishing in practice between direct and secondary predation.

The term “secondary ingestion” has been used when food items in a predator’s gut were not eaten directly by that predator but were consumed instead by one of its prey (e.g., Neill and Allen 1956). The same term may be appropriately applied to parasites in the gut of a predator that are released from the body of one of its prey, at least when those parasites do not survive the digestive process.

The life cycles of horsehair worms (Phylum Nematomorpha) include a juvenile stage parasitic within terrestrial insects and a free-living aquatic adult stage (Poinar 2001, Hanelt and Janovy, Jr. 2003). In at least some species, terrestrial hosts infected by maturing horsehair worms are more likely than uninfected individuals to enter water (Thomas et al. 2002). The slow-moving adult worms do not feed and sometimes are found in clumps of from several to many individuals (Cochran et al. 2004).

Predation on horsehair worms was reviewed by Cochran et al. (1999). Subsequently, Poinar (2001) reported that an adult Gordius was brought to nestlings by a bird in Chile. Kinziger et al. (2002) provided additional accounts of predation in Minnesota and Missouri but overlooked an earlier mention of an unidentified horsehair worm in the gut of a hellbender (Cryptobranchus alleganiensis) from the latter state (Peterson et al. 1989). Schmidt-Rhaesa et al. (2003) and Martin and Cochran (2005) reported additional cases of horsehair worms recovered among the gut contents of trout.

Most previously reported cases of predation on horsehair worms involved fish, and most were interpreted to have resulted from fish having preyed directly upon free-living adult worms. However, Cochran et al. (1999) observed that many fish tested in laboratory trials rejected adult horsehair worms, and they recognized the possibility that at least some horsehair worms found among the gut contents of fish may have been ingested secondarily (i.e., before having emerged from their invertebrate hosts). Ponton et al. (2006) staged secondary predation in the laboratory by generalist predators (fishes and ranid frogs) consuming crickets infected by horsehair worms. Depending on the predator, 18-35% of the worms were able to escape by wriggling out through the predator’s mouth, nose, or gills, but most of them were presumably digested.

There is some evidence for secondary predation on horsehair worms in the field. Bolek’s (2000) report of a dog regurgitating a Gordius robustus suggests the possibility that it had eaten an insect containing the worm. Bolek and Coggins

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(2002) found *G. difficilis* within carabid beetles among the gut contents of two green frogs (*Rana clamitans*). The purpose of this note is to describe another apparent case of secondary predation on a horsehair worm.

On 31 August 2006, at approximately 20:00, I collected a brown trout (*Salmo trutta*) by angling in Gilmore Creek below the spillway at the south end of the Saint Mary’s University Campus in Winona, Winona County, Minnesota. The trout measured 32 cm in total length. In addition to fragments of a dragonfly nymph, the trout contained in its stomach a partially digested camel cricket (*Orthoptera: Gryllacrididae: Ceuthophilus* sp.), from which a living male horsehair worm (*Gordius robustus* Leidy) had partially emerged. Two female *G. robustus* extended from the stomach into the intestine, which also contained snails. At some point after the gut contents of the trout were initially examined and preserved in ethanol, a second male *G. robustus* partially emerged from the camel cricket. The camel cricket and *G. robustus* have been placed in the Milwaukee Public Museum invertebrate collection.

The gut contents described above are consistent with a scenario by which the trout consumed the camel cricket just prior to the emergence of the male *G. robustus*. It is also possible that the female worms emerged from the same cricket. Thomas et al. (2002) determined that 5 of 41 crickets infected by *Paragordius tricuspidatus* contained more than one worm. Hanelt and Janovy, Jr. (2004) observed in a laboratory study the successful maturation of multiple *P. varius* within individual crickets. They did not indicate whether these worms were smaller than single worms that matured within separate hosts, but the female worms in the present study were notably short (120 and 123 mm).

Although direct and secondary predation would seem to be mutually exclusive events, an intermediate scenario is possible. A predator might consume both horsehair worm and its insect host during the time that the worm is emerging, as observed in the laboratory by Ponton et al. (2006). Indeed, the emergence itself might draw the attention of the predator, and natural selection might therefore favor rapid emergence and separation from the host. Hanelt and Janovy, Jr. (2004) reported that *P. varius* in laboratory studies began emerging from their hosts within two seconds of the hosts being placed in water and that most worms had exited completely within 90 seconds. However, they also stated that worms formed mating aggregations even while emerging from hosts. Thomas et al. (2002) stated that emergence from a host could be immediate or could take several minutes after the host entered the water, and Ponton et al. (2006) stated that emergence may take as long as 10 minutes.

Neill and Allen (1956) discussed the difficulty of interpreting items that are resistant to digestion and that persist for relatively long times in digestive tracts. For example, vertebrate prey may be digested much more quickly than the invertebrates they themselves have consumed, and it might be wrongly concluded that the invertebrates were preyed upon directly. In the case of insect hosts containing horsehair worms, differences in digestibility are possibly not as extreme, and it might be less likely to find secondarily ingested horsehair worms in the absence of at least some remains of their invertebrate hosts. However, given recent advances in the culture of horsehair worms in captivity (Hanelt and Janovy, Jr. 2004), it would be desirable to test this possibility via laboratory experiments.

*Gordius robustus* has not been previously reported to parasitize camel crickets but has been reported from other orthopterans (Schmidt-Rhaesa et al. 2003). The camel cricket *Ceuthophilus stygius* is parasitized by *Chordodes morgani* in Kentucky (Studier et al. 1991).

Although *Gordius robustus* has been reported recently at several locations in Minnesota (Martin and Cochran 2005), this is the first collection from Gilmore Creek. An earlier report of *G. robustus* from Gilmore Creek (Cochran
et al. 1999) was revised to *G. difficilis* by Cochran et al. (2004), and Martin and Cochran (2005) found the latter species to be more common in cold spring-fed streams in southeast Minnesota. Indeed, on 30 June 2006, while angling in the same pool where the trout containing the *G. robustus* was collected during the present study, I collected two separate *G. difficilis* that became entangled in the treble hook of my lure while it was being retrieved. Moreover, Martin and Cochran (2005) listed several prior collections of *G. difficilis* among the gut contents of brown trout collected in Gilmore Creek. Two of the trout were captured in the same pool as the trout that contained a *G. robustus* during the present study (19 July 2003 and 18 June 2004). The two *Gordius* species can be distinguished by differences in diameter, color, and, in males, the presence or absence of a parabolic fringe of hairlike processes anterior to the cloaca (Bolek and Coggins 2002, Martin and Cochran 2005).

Additional specimens of *G. robustus* were collected farther downstream on the Saint Mary’s University campus on 22 September 2006 and 30 October 2007. The worm collected on the latter date, an adult female, was of special interest because it was found moving in a terrestrial environment, a steep shaded bank approximately 1 meter above the waterline. No host was evident in the immediate vicinity.

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**LITERATURE CITED**


ABUNDANCE OF RICE ROOT APHID AMONG SELECTED PLANT SPECIES AND ON PLANTS GROWN WITH DIFFERENT SOIL-SURFACE MEDIA

Louis S. Hesler1 and S. Dean Kindler2

ABSTRACT

The rice root aphid, *Rhopalosiphum rufiabdominalis* (Sasaki), is distributed worldwide and colonizes a wide range of plants. However, relatively little is known about the suitability of different host plants, optimal rearing techniques, and the aphid’s impact on plant fitness. To improve understanding of these factors, laboratory experiments were conducted to compare the abundance of rice root aphid on plants grown using three different soil-surface media and among selected monocotyledonous and dicotyledonous plants. Rice root aphid was more abundant on plants grown with a sandy soil surface than a surface with fine wood chips or only bare non-sandy soil. Rice root aphid was more abundant on ‘Elbon’ rye than on ‘Bart 38,’ ‘Dart,’ ‘Fletcher’ and ‘Ramona 50’ wheat. More winged rice root aphids were produced on Elbon rye than on Dart wheat, but the number of winged aphids on Elbon rye did not differ from that on other wheat lines. Rice root aphid was more abundant on Elbon rye and ‘TAM 110’ wheat than on ‘Marmin,’ ‘Marshall’ and ‘Sharp’ wheat. Additional observations with monocotyledonous plants showed that abundance of rice root aphid on ‘Kivu 85’ triticale was comparable to that on Elbon rye. Rice root aphid did not reproduce on potato or soybean, although winged adults persisted up to 24 days on caged potato plants. The implications of differential abundance of rice root aphid on plants are discussed in regard to colony rearing, future experiments and possible pest management considerations.


Rice root aphid is an indirect pest of many crop plants because of its ability to vector disease agents such as barley yellow dwarf virus (BYDV) (Paliwal 1980, Jedlinski et al. 1981) and sugarcane yellow leaf virus (Schenck and Lehrer 2000). Rice root aphid is often one of the most abundant aphids infesting wheat and other small-grain crops in North America (Palmer 1939, Kieckhefer and Gustin 1967, Paliwal 1980, Chapin et al. 2001). Despite its lengthy history

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and abundance on small-grain crops, tests of direct impact by rice root aphid on plant fitness are lacking.

A critical first step in evaluating the effect of a potential pest aphid on plant fitness is to determine the range of host plants that are capable of supporting large populations of the aphid. Once identified, these plants may then be used as rearing hosts and as test plants for evaluating any impacts of aphid infestations (Blackman 1990). The identification of suitable rearing plants is important, as pretest conditions in which aphids are held may directly affect experimental outcomes (Smith et al. 1994). For instance, the use of a particular plant species or line for rearing and subsequent impact testing may predispose test aphids to feed on it and lead to exaggerated estimates of impact.

It is also important to determine particular conditions that support suitable numbers of aphids on rearing plants or experimental plants or that facilitate the execution of experiments (Blackman 1990, Tsai and Liu 1998). For instance in laboratory experiments with the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), a thin layer of light-colored quartzite sand serves as a useful contrasting surface for tracking this generally dark colored aphid during infestation and evaluative counting, when it may become dislodged from test plants and fall onto the soil surface of experimental arenas (Hesler and Tharp 2005). As bird cherry-oat aphid and rice root aphid closely resemble one another morphologically (Richards 1960, Pike et al. 1990), light-colored sand may also be a useful soil surface in experiments with rice root aphid, even though a dark soil mix is suitable for rearing and for experiments (Paliwal 1980, Kindler et al. 2004). Anecdotal observations have also indicated that population growth of rice root aphid may be facilitated by the placement of a thin layer of fine wood chips on the soil surface around the base of rearing plants (Kindler et al. 2004), but a layer of wood chips may also hinder tracking the aphids during infestation and evaluative counting. Thus, the population growth of rice root aphid needs comparison among different soil-surface treatments, such as sand and wood chips, to determine their utility in future experiments. The objective of this research is to determine the suitability of selected plants and soil media for rice root aphid by measuring the abundance of rice root aphid over time when placed on selected monocotyledonous and dicotyledonous plants.

**MATERIALS AND METHODS**

*Aphids.* Rice root aphids used in the experiments were obtained from a virus-free, multiclonal stock colony maintained on ‘Elbon’ rye plants (Kindler et al. 2004) growing in a 15.2-cm diameter pot covered with a cylindrical cellulose nitrate cage (Hesler and Tharp 2005). The colony was maintained in a growth chamber (Controlled Environments Inc., Pembina, ND, USA) under constant conditions (13 h light at 19°C, 11 h dark at 18°C) at the USDA North Central Agricultural Research Laboratory (NCARL), Brookings, South Dakota. A non-viruliferous colony of rice root aphid was established by collecting numerous individuals from a winter wheat field near Brookings in autumn 1999, placing them on sachets of Parafilm® (American National Can Co., Greenwich, CT, U.S.A.) containing 20% sucrose solution, removing neonate offspring, and transferring them to noninfested rye plants (Hesler and Tharp 2005). This procedure was repeated about once per year with colony aphids, and occasionally leaf tissue was tested serologically (Agdia, Elkhart, IN, U.S.A.) to ensure that colony plants were free of BYDV. The integrity of the colony was also checked weekly by examining a few hundred individuals to ensure no contamination by morphologically similar species such as bird cherry-oat aphid. Winged aphids became present in cages 3 weeks after initial infestation and aggregated on the inner surface of each cylindrical cage. The colony was perpetuated by regularly infesting one-week-old rye plants with winged rice
root aphids obtained from caged plants infested 3 to 4 weeks earlier (Kindler et al. 2004). Voucher specimens of the aphids are deposited at NCARL.

**Abundance of rice root aphid on Elbon rye using different soil-surface media.** Experimental plants were prepared by germinating seeds between layers of moist paper towels held in plastic containers in the dark at 20°C (Hesler and Tharp 2005). After 24 to 48 h, 25 to 40 individual seedlings exhibiting uniform root and coleoptile growth were planted into a 2:1:1 mixture of Vienna soil (fine-loamy, mixed Calcic Hapludolls), perlite, and coarsely ground coconut shells (Coir, J. R. Johnson Supply Inc., Roseville, MN, U.S.A.). Pots were thinned to 20 seedlings 5 or 6 days later.

Three types of soil-surface media treatments were tested, and they were applied to pots when rye plants were 7-d old. Treatments consisted of adding volumes of 5 oz. of soil mixture, 8 oz. of fine cedar-wood chips, or 5 oz. of light-colored quartzite sand to each test pot and spreading each soil treatment over the soil surface and around the base of test plants. After treatments were applied, the modified soil surface in each pot was sprayed lightly with water. Then, each pot of 20 seedlings was infested with 28 winged rice root aphids selected randomly from the cylindrical cages that had covered colony plants infested 3 to 4 weeks earlier. Infested plants were immediately caged and placed into a growth chamber (13 h light at 19°C, 11 h dark at 18°C) as a randomized complete block design with four replications.

Abundance of rice root aphids was measured 24 d later when plants were about 40-cm tall with 3 to 4 leaves and had dense aphid populations around their basal half. Aphids were counted immediately from plants clipped at the soil surface of each quadrant in an experimental pot (4 plants total). Winged aphids on the cut plants were more likely to escape and were counted first. The numbers of all aphids on each set of 4 plants was summed for each treatment replicate. Additional plants from the pots were gently dug to confirm that rice root aphids had not infested roots, consistent with our previous, unpublished observations. As a second measure of abundance, the number of winged aphids on the inner surface of each treatment cage was also counted. The four-plant counts and counts of winged aphids from cages were subjected to separate analyses of variance (PROC ANOVA; SAS Institute 2002), and treatment means were separated by Tukey’s HSD method. A significance level of $\alpha = 0.05$ was used for statistical tests.

**Abundance of rice root aphid on selected monocotyledonous plants.** The abundance of rice root aphid on monocot plants was evaluated in two separate, quantitative experiments and a third experiment in which abundance was observed but not quantified. The first experiment compared rice root aphid abundance on Elbon rye to that on wheat lines ‘Fletcher,’ ‘Dart,’ ‘Baart 38,’ and ‘Ramona 50.’ These lines express symptoms of BYDV infection (Oswald and Houston 1953; NGRP 2007a, b, c) and were evaluated as possible hosts of rice root aphid for studies on virus transmission and yield effects. A second experiment compared abundance of rice root aphid on Elbon rye to that on two spring-wheat lines, ‘Marshall’ and ‘Sharp,’ and two winter-wheat lines, ‘Marmin’ and ‘TAM 110.’ Marshall, Sharp, Marmin, and TAM 110 are lines that are regionally adapted to the northern Great Plains. Each of these two experiments was conducted identically to the soil-media experiment. In addition to these quantitative tests, abundance of rice root aphids was also evaluated qualitatively on ‘Kivu 85’ triticale, which in preliminary tests appeared to support large populations of rice root aphids. Sets of triticale plants were infested and maintained in the same manner as the quantitative experiments with wheat and rye. A set of Elbon colony plants was infested at the same time and placed into a separate chamber (13 h light at 19°C, 11 h dark at 18°C). After 4 weeks, aphid abundance on triticale and rye was observed *in situ.*
Abundance of rice root aphid on selected dicotyledonous plants. Potato and soybean were evaluated as hosts of rice root aphid in separate experiments. In the first experiment, three potato plants per pot were used. To achieve this, three cuttings of potato tuber (each from a different tuber) were planted about 2 inches deep in a 15.2-cm diameter pot filled with a modified soil mix. The mix was modified to enhance potato growth by adding an equal volume of sand to the original soil mix used with monocots. One of three common, locally available lines of potato (‘Irish Cobbler,’ ‘Norkotah Russet,’ or ‘Red Pontiac’) was planted per pot. A separate control treatment of Elbon rye (20 plants per pot) was also included. Potato plants were three-weeks old and rye plants were one-week old when they were infested on the same date with 28 winged rice root aphids. Infested treatment plants were placed into a growth chamber (13 h light at 19°C, 11 h dark at 18°C) according to a randomized complete block design with four replications. After 24 days, aphids were counted on the potato plants in each pot and from 4 randomly selected rye plants per pot. In a second experiment, seeds of soybean line ‘91B91’ were planted in 15.2-cm diameter pots with soil mix and thinned to two to three plants two weeks later. Each of three pots of soybean was infested with 28 winged rice root aphids, immediately caged, and placed into a growth chamber (16 h light at 22°C, 8 h dark at 19°C). A set of three Elbon colony plants was infested at the same time and placed into a separate chamber (13 h light at 19°C, 11 h dark at 18°C). After 2 weeks, aphid abundance on soybean and rye were observed in situ.

RESULTS

Abundance of rice root aphid on Elbon rye using different soil-surface media. Abundance of rice root aphid on Elbon rye varied with soil-surface media ($F = 1.28$, df = 2, 14, $P = 0.0012$). Plants grown with a sandy soil surface ($\bar{x} \pm SE = 900.1 \pm 92.8$ per 4 plants) had more aphids than a surface with fine wood chips ($\bar{x} \pm SE = 494.8 \pm 54.2$ per 4 plants) or only soil ($\bar{x} \pm SE = 453.8 \pm 53.9$ per 4 plants). In addition, more winged aphids ($F = 37.59$, df = 2, 14, $P < 0.001$) were collected from cages in the sand treatment ($\bar{x} \pm SE = 240.6 \pm 23.9$) than with treatments of wood chips ($\bar{x} \pm SE = 60.4 \pm 8.9$) or only soil ($\bar{x} \pm SE = 52.1 \pm 11.7$).

Abundance of rice root aphid on selected monocotyledonous plants. In the first experiment (Table 1), rice root aphid was more abundant on Elbon rye than on Bart 38, Dart, Fletcher and Ramona 50 wheat ($F = 8.10$, df = 4, 12, $P = 0.002$). More winged rice root aphids were collected from cages of Elbon rye than from those of Dart wheat, but the number of winged aphids on Elbon rye did not differ from that of other wheat lines ($F = 3.70$, df = 4, 12, $P = 0.035$). In the second experiment (Table 1), rice root aphid was more abundant on Elbon rye and TAM 110 wheat than on Marmin, Marshall and Sharp wheat ($F = 28.13$, df = 4, 12, $P < 0.001$), and more winged aphids were collected from cages of Elbon rye and TAM 110 wheat than from cages of other wheat plants ($F = 18.67$, df = 4, 12, $P < 0.001$). Additional observations showed that rice root aphid became highly abundant on Kivu 85 triticale, with abundance appearing comparable to that on Elbon rye infested for the same length of time. Stems and lower leaves of rye and triticale were virtually covered with dense colonies of rice root aphids, and winged aphids were abundant on the inner surfaces of cages.

Abundance of rice root aphid on selected dicotyledonous plants. Winged rice root aphids were found on potato and soybean plants within a few hours after being introduced into test cages, and they remained active in test arenas for at least several days. In potato tests, alates were generally observed on plants rather than on the inner surface of cages. After two weeks, however, dead alates began to appear increasingly on the soil surface of the potato test arenas, but several alates survived on potato throughout the 4-week test period. However, no offspring were found on the shoots, roots, or tubers of potato plants.
after 24 days. After 24 days in the potato test, Elbon rye had 1816.8 ± 466.5 \((x \pm SE)\) aphids per 4 plants and 290.8 ± 170.8 \((x \pm SE)\) winged aphids per cage. Most of the winged aphids on soybean died in about one week and no offspring were found on soybean plants, but after 4 weeks Elbon rye was heavily infested with hundreds of rice root aphids per stem and many alates were found on the inner surface of cages.

**DISCUSSION**

The abundance of rice root aphid was greater on plants growing above a sandy soil surface than with surfaces of wood chips or soil mix. Our test was not designed to determine why rice root aphids were more abundant in the sand treatment, and this may be addressed in future studies. Nonetheless, the use of light colored sand could facilitate tracking aphids during infestation and counting, and the use of a sandy surface is recommended especially for laboratory and greenhouse experiments involving infestations of shoots with rice root aphids. The sandy surface could also enhance colony production of rice root aphids on Elbon rye.

Rice root aphid had differential population growth among various plant species and no population growth on potato, which had been reported previously as a host (Essig 1944). The population of rice root aphids in this study was collected from a field of ‘Roughrider’ wheat, and results showed that it was well adapted to rye, wheat and triticale, but not potato or soybean. Previous results have shown that this population of rice root aphid also becomes only moderately abundant on barley and oats and is poorly adapted to rice and other grasses (Kindler et al. 2004), but other North American populations of rice root aphid have been well adapted to barley and oats (Paliwal 1980). Collectively, these results suggest that the population of rice root aphid in this study may represent a biotype based on its differential survival and development on particular host plants (Eastop 1973, Diehl and Bush 1984, Drés and Mallet 2002).

The rice root aphid did not reproduce on potato and soybean, but alates had prolonged survival on potato and limited survival on soybean. The survival of
alates on potato and soybean raises the question of whether rice root aphid may be a vector of plant-disease viruses in these crops, but its ability to transmit viruses to potato and soybean is unknown. Several congeneric aphid species do not colonize potato or soybean, but they are a vector of stylet-borne plant-disease viruses to these crops. For instance, both corn leaf aphid, *Rhopalosiphum maidis* (Fitch), and bird cherry-oat aphid transmit potato leafroll virus to potato (Halbert et al. 2003) and soybean mosaic virus to soybean (Halbert et al. 1981), and *Rhopalosiphum insertum* (Walker) is capable of transmitting potato virus Y to potato (van Hoof 1980).

Rice root aphid may be prevalent in regions of North America where wheat is grown near soybean and potato (Kieckhefer and Gustin 1967, Al-Raeesi et al. 1992, Chapin et al. 2001, Kindler et al. 2004), but it has not been considered significant in the epidemiology of aphid-borne viruses in potato and soybean. There is no record of rice root aphid colonizing soybean, and it composes <<1% of alates captured in traps within soybean fields (Halbert et al. 1981). Alate rice root aphids have not been trapped in Minnesota and North Dakota potato fields (DiFonzo et al. 1997). However, as rice root aphid (as *Cerosipha californica*) has been recorded on potato in California (Essig 1944), and given the prolonged survival of alates on potato in the present study, tests of its ability to transmit viruses to potato may be warranted.

Rice root aphids in this study differed in abundance among wheat lines, with greatest numbers on TAM 110 wheat and with decreased abundance of winged aphids on Dart wheat. The differential abundance of rice root aphid among wheat lines has some implications for rearing and experimentation. First, TAM 110 was the only wheat line with an abundance of rice root aphid comparable to that on Elbon rye. Abundance of rice root aphid on Kivu 85 triticale, a wheat × rye cross, was also comparable to that of Elbon rye. Thus, TAM 110 and Kivu 85 may be equally suitable to Elbon rye as rearing hosts. However, the differential abundance of rice root aphid among wheat and triticale lines suggests that experiments to test for an impact of rice root aphid on the growth and grain yield need to be designed to maintain an equal number of aphids across test plants over time (Lamb and MacKay 1995). From a pest management standpoint, the differential abundance of rice root aphids among wheat lines suggests inherent variation in wheat that could be exploited to develop and eventually deploy lines that limit aphid infestations (Webster 1991).

One objective of this study was to identify wheat lines that support large numbers of rice root aphid and also readily express symptoms of BYDV infection for possible use in studies on virus transmission and yield effects. The wheat lines Baart 38, Dart, Fletcher and Ramona 50 are moderately to extremely susceptible to BYDV (Oswald and Houston 1953; NGRP 2007a, b, c), and rice root aphid was fairly abundant on these lines, although relatively low numbers of aphids were produced on Dart. Thus, Baart 38, Fletcher and Ramona 50 may be useful as colony plants for maintaining rice root aphids and BYDV, and in future experiments to determine the effects of rice root aphid and BYDV on wheat growth and yield.

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LITERATURE CITED


Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious invasive pest of ash trees (*Fraxinus* spp.) in North America. Much of EAB’s range expansion has been attributed to human-assisted movement of infested items such as ash logs and firewood. It is unclear the amount of time that logs cut from live EAB-infested ash trees should be restricted from movement until they are no longer capable of producing viable EAB adults. In March and April 2004, we cut log sections from EAB-infested green ash (*F. pennsylvanica* Marsh) trees in Ann Arbor, Washtenaw County, Michigan. Log sections (mean length = 24.8 cm; diam. = 11.6 cm) were stood upright on one cut end and stored beneath a hardwood forest canopy. Adult EAB were allowed to freely emerge from log sections during summer 2004. When logs were dissected in November 2004 to January 2005, approximately one half of the total EAB life stages that were present in the logs were dead, while the other half either emerged as adults in summer 2004 or were live prepupae. Also, adults emerged from a subset of these log sections when reared in the laboratory in January to February 2005. These data suggest that EAB adults can emerge from logs for two successive emergence periods after infested ash trees have been cut.

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious invasive pest of ash trees (*Fraxinus* spp.) and, as of May 2008, it was established in the US states of Illinois, Indiana, Maryland, Michigan, Ohio, Pennsylvania, and West Virginia; and the Canadian province of Ontario (Haack 2006; http://www.emeraldashborer.info). EAB is native to Asia and was first discovered in North America in the Detroit metropolitan area of Michigan in 2002 (Yu 1992, Haack et al. 2002, Poland and McCullough 2006). Most of the range expansion of EAB has been attributed to inadvertent human-assisted movement of infested ash nursery stock, logs, and firewood. A federal quarantine was imposed to limit human-assisted dispersal of EAB by regulating movement of these articles (Federal Register 2003).

EAB larvae develop through four instars as they feed in the phloem of ash trees. When fourth instar larvae have completed feeding, they excavate pupation chambers in the outer sapwood or bark of ash trees (Cappaert et al. 2005, Wei et al. 2007). In southern Michigan, most EAB larvae overwinter as prepupae in their pupation chambers after they have developed from eggs laid in early summer of that same year. However, some EAB larvae do not complete larval development the same year they eclosed from eggs and overwinter as larvae in the phloem of ash trees (Cappaert et al. 2005). Some of these larvae complete feeding the following spring and emerge as adults later that same summer. However, a percentage of these larvae do not complete feeding and become prepupae until late summer or fall and overwinter a second time before emerging as adults. Thus, logs cut in mid-summer may have both newly initiated and fully developed larvae.

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EAB prefer to oviposit on live ash trees, but they will occasionally oviposit on freshly cut ash logs (Anulewicz et al. 2008). However, larvae that develop from eggs laid on cut logs rarely complete their development (Anulewicz et al. 2008; T.M. Poland and D.G. McCullough, pers. comm.). Therefore, it is unclear the length of time logs and firewood cut from EAB infested trees should be restricted from movement to assure the absence of live EAB life stages. We present information here from a recent study (Petrice and Haack 2006b) where data were generated suggesting that EAB adults can emerge from log sections two successive emergence-periods after infested trees are cut.

In April 2004, before adult EAB emerged, we cut 136 logs from green ash, *F. pennsylvanica* Marsh, trees that contained live EAB life stages in Ann Arbor, Washtenaw County, MI. Logs were cut approximately 50-cm long and averaged 12 cm in diameter. In May and June 2004, prior to EAB adult emergence, each log was cut into two equal sections, with one section treated with an insecticide and one section untreated to serve as a control (see Petrice and Haack 2006b for more details). Log sections averaged (mean ± SE) 24.8 ± 0.3 cm long and 11.6 ± 0.2 cm in diam. All exit holes present on log sections from previous years (2003 and earlier) were marked with white caulk. Data presented here represent only the control log sections. Log sections were stood upright on one cut end and placed under a hardwood-forest canopy during June to October 2004 and EAB adults were allowed to freely emerge during this period. During November 2004 to January 2005, 126 log sections were brought back to the laboratory and dissected. We recorded the number of EAB adults that had emerged from the sample log sections during summer 2004 based on the presence of new exit holes at the time of dissection. We also recorded the number of dead EAB adults, pupae, prepupae (larvae that have completed feeding and excavated pupation chambers in the bark or wood), and larvae present in log sections; the number of live EAB prepupae in each log section. Live prepupae, which were apparently undamaged during dissection, were reared in petri dishes at 24°C. Their final stage of development at death (e.g., prepupae, pupae) or whether they successfully developed to adults was recorded. On 4 January 2005, the 10 remaining log sections that had been cut in April 2004 were brought into the laboratory and placed in cardboard tubes to monitor for adult emergence.

Log dissections revealed that 30.9 ± 2.2 % of the total EAB in log sections had emerged as adults during summer 2004, 22.4 ± 1.9% were live prepupae, 17.0 ± 1.6% died as larvae, 9.6 ± 0.9 % died as prepupae, 0.1 ± 0.1 % died as pupae , and 20.1 ± 1.8% died as callow adults. Furthermore, 18% of the 151 live prepupae that were dissected from log sections in November 2004-January 2005 and reared in petri dishes developed to adults, while 17% died as pupae and 65% died as prepupae. A total of 8 adults emerged in February 2005 from 5 of the 10 log sections that were cut in April 2004 and reared in the laboratory during January-February 2005 (Table 1).

The logs for this study were only moderately infested with EAB and, therefore, competition for food among larvae was low. This likely enhanced EAB survival as compared to logs that would have been heavily infested. We suspect adults that emerged in summer 2004 were fourth instars or prepupae at the time logs were cut in April 2004. While adults that emerged in the laboratory in 2005 were likely younger instars in April 2004. These earlier-instar larvae would have had to continue developing during summer 2004, with adult emergence occurring in late summer 2004 or early summer 2005. Development may have been protracted because of log moisture loss and cooler temperatures that resulted from the log sections being stored in the shade. Alternatively, given that some EAB larvae may require a two-year life cycle (Cappaert et al. 2005; Wei et al. 2007), it is equally likely that live prepupae we found in log sections in November 2004-January 2005 and adults that emerged in February 2005 may have needed two seasons to complete development regardless of when logs were cut from infested trees. We assume all EAB individuals that emerged in
2004 and 2005 had resulted from eggs laid in 2003 or possibly one year earlier. Although highly unlikely, it could be argued that the EAB adults that emerged in 2005 resulted from eggs laid on the log sections during summer 2004 because the log sections were exposed to natural attack. As mentioned above, EAB oviposition has been recorded on cut logs but is evidently rare (Anulewicz et al. 2008; T.M. Poland and D.G. McCullough, pers. comm.).

Surprisingly, even though the log sections were cut to very short lengths (25 cm) within 1-2 months after they were initially cut from trees in March and April, they still contained live EAB prepupae in November 2004-January 2005 that were able to develop into adults in the laboratory. Petrice and Haack (2006a) found that when firewood logs cut from EAB-infested trees remained uncovered outdoors in either the sun or shade, EAB survival was reduced the following summer compared to logs stored under tarps. This difference was likely a result of logs desiccating more when they were exposed to ambient conditions. Therefore it would be assumed that desiccation would have greatly reduced survival of EAB in the short, small-diameter log sections used in the current study. If log sections in the present study would have remained outdoors an additional 4-5 months until the 2005 summer emergence, it is likely that further desiccation would have lowered EAB survival even more. Nevertheless, our data show that EAB adults can be reared from short, small-diameter logs during two successive emergence periods after logs are cut from infested trees. Based on these results, ash logs and firewood potentially infested with EAB should be held at least two summers after trees are cut to allow EAB adults time to emerge prior to any log movement if the objective is to prevent human-assisted movement of EAB. Nevertheless, current EAB quarantine regulations prohibit movement of all hardwood firewood and ash logs to areas outside of EAB quarantine zones unless they have been treated with an approved method (Federal Register 2003).

ACKNOWLEDGMENTS

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LITERATURE CITED


**ERYTHRODIPLAX UMBRATA (ODONATA: LIBELLULIDAE): NEW FOR MICHIGAN**

Julie A. Craves\(^1\,2\) and Darrin S. O’Brien\(^2\)

**ABSTRACT**

Two band-winged dragonlets, *Erythrodiplax umbrata* (Linnaeus), collected in Wayne County, Michigan on 6 October 2007 represent the first records for this genus and species in the state, as well as the northernmost record for the species. They were found during a period in which many individuals were seen or photographed in Ohio, which prior to 2006, had only two records.

*Erythrodiplax* (Brauer) is a primarily Neotropical genus of 56 species (Gar- rison et al. 2006), six of which occur regularly in North America north of Mexico (Dunkle 2000). *Erythrodiplax umbrata* (Linnaeus), the band-winged dragonlet, is found in South America south to Argentina, Central America, Mexico, the Greater Antilles, and the southern United States. Prior to 2006, there were only four records outside the southern U.S., all represented by specimens. One was taken at Cedar Bog, Champaign County, Ohio on 11 June 1934 (Borror 1935). Two teneral individuals were collected in Indiana by B. E. Montgomery on 1 September 1934, a male in Gibson County, and a female in Pike County (Borror 1935). Kansas has two records, a teneral male collected by G. F. Hevel on 11 July 1964 in Labette County and a female taken on 8 June 1999 in Sedgwick County by R. J. Beckemeyer (Beckemeyer 2004).

On 11 August 2006, a male *E. umbrata* was photographed at Armleder Park, Cincinnati, Hamilton County, Ohio, which remained present until at least 23 August (Abbott 2007, Hull 2007). In 2007, Ohio had a spate of records for this species. One was photographed on 29 August at Headlands Dunes State Nature Preserve, Lake County and two more were seen at this location on 10 September (Rosche 2007). Two adult males were found and photographed on 4 September at Frohring Meadows, Geauga County and another was photographed there on 18 September (Rosche 2007). An adult male was found on 14 September at the Leroy Wetlands, Lake County. Multiple individuals, including juveniles, were observed at this site through 22 October; the peak number was at least 20 juveniles on 26 September (Rosche 2007, J. Pogacnik, pers. comm.). A male was photographed at Cuyahoga Valley National Park, Cuyahoga County on 8 October and two teneral individuals on 17 October (Gardella 2007a, L. Gardella, pers. comm., *contra* Rosche 2007). None of these individuals were collected.

Bearing the recent Ohio findings in mind, on 6 October we took advantage of unseasonably warm (30°C) and sunny weather to do a final survey of adult odonates at the Detroit River International Wildlife Refuge, Humbug Marsh Unit, located along the lower Detroit River, in Wayne County, Michigan. Part of this unit is an 18 ha brownfield owned by Wayne County. The only surface water on the brownfield site were rainwater puddles unintentionally created by construction equipment earlier in the summer. These puddles were restricted to a 3 ha section approximately 300 m from the Detroit River.

Immediately upon entering the site, JAC spotted a male *E. umbrata* at an 8 × 4 m puddle (42°06′53″N, 83°11′38″W). As we attempted to photograph

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it, a second male flew in and the two chased each other. For about 15 min, they alternated between the original puddle and another of similar size approximately 20 m away. Although they were wary and difficult to approach, JAC was eventually able to capture both. Voucher specimens have been deposited into the Univ. Michigan Museum of Zoology, Insect Division, and have been cataloged by the Michigan Odonata Survey.

This location is roughly 40 km farther north than the northernmost record for Ohio (at Headlands Dunes State Nature Preserve) and over 220 km farther north than the previous northernmost specimen, the one taken by Borror in 1934 outside of Columbus (Borror 1935).

**DISCUSSION**

*Erythrodiplax umbrata* inhabits marshy ponds, pools, and lakes, often temporary water (Dunkle 2000, Abbott 2005, Garrison et al. 2006). The Michigan dragonlets were in < 3-month-old depressions created by earth-moving equipment. All the 2007 Ohio records were found in similar pools and puddles of recent vintage. The Frohring Meadows park was under construction and the dragonlets there, as well as the one at Headland Dunes, were found in "simple scrapes" (L. Rosche, pers. comm.). Leroy Wetlands is a newly created wetland complex and the site containing the dragonlets had held water for < 2 months (Pogacnik 2007). The Cuyahoga Valley National Park site is a mitigated wetland and the dragonlet was in what was described by the observer as a “mud puddle” (Gardella 2007b).

These northern records of *E. umbrata* constitute a substantial northern range expansion for this species. Hickling et al. (2005) documented a northward shift in the range margins in 34 of 37 species of non-migratory British Odonata between 1960-1970 and 1985-1995. Catling (1996) reported that the range of *Enallagma civile* (Hagen), (Odonata: Libellulidae), had moved north by at least 200 km in southern Ontario between 1959 and 1996. Authors of both these papers noted that these range shifts could be associated with global climate change. More short-term climatic events might also help explain the recent northward movements of *E. umbrata*. For much of 2006, Texas and Oklahoma, core areas of the range of *E. umbrata* in the U.S., experienced severe to extreme drought (NWSCPC 2008) with 2007 the driest year in the 112-year record in the southeastern U.S. (NCDC 2008a). The drought was coupled with above-average temperatures in 2006-2007 over the south and southeast (NCDC 2008b). These conditions may have pushed *E. umbrata* north in search of breeding sites as the shallow ponds and puddles dried up over much of their range or above-average temperatures created unsuitable thermal conditions in surviving aquatic environments.

The presence of teneral *E. umbrata* in northeast Ohio suggests they were able to breed in the temporary ponds near which they were found. The two Michigan males on 6 October were fully pruinose adults. No *E. umbrata* were present at the Michigan site in over a dozen previous weekly visits or one subsequent visit and no nymphs were found during larval sampling in the puddles on 13 October. These puddles will be checked again in 2008, although they are likely to be destroyed early in the spring season.

**ACKNOWLEDGMENTS**

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recent Ohio records: Lou Gardella, Bob Glotzhober, John Pogacnik, and Larry Rosche. Additional photographers that documented the Ohio records include William Hull, Sally Isacco, Linda Gilbert, and Judy Semroc.

LITERATURE CITED


Gomphus spicatus (Odonata: Gomphidae) Rediscovered in Illinois and Libellula semifasciata (Odonata: Libellulidae) Recorded Near Wisconsin

Robert B. DuBois¹ and Craig R. Stettner²

Gomphus spicatus Hagen (Odonata: Gomphidae), commonly called dusky clubtail, is a common and widely distributed dragonfly in a variety of ponds, lakes, and slow streams throughout its range in the north-eastern and north-central United States and adjacent areas of southern Canada (Donnelly 2004). It is known in Illinois only from a few adult records from Cedar, Fox and Sand lakes in Lake County during June 1892 (Needham and Hart 1901). Needham and Hart (1901) also mentioned that many nymphs thought to be this species were taken from Sand and Clear lakes, but no dates were given and it is not known where these nymphs were deposited. Since then, no specimens of G. spicatus are known to have been taken in Illinois (T. Cashatt, Illinois State Museum, pers. comm.). We checked the Odonata housed at the Field Museum in Chicago, including both the adult collection (J. Boone, pers. comm.) and larval collection (D. Summers, pers. comm.) without locating any specimens of G. spicatus from Illinois. Further, personnel with the Illinois Natural History Survey were not aware of any recent Illinois specimens (E. DeWalt, pers. comm.).

We collected one adult male of G. spicatus and observed several other males during the mid-afternoon of 10 June 2007 near the Dead River in Illinois Beach State Park (South Unit), Lake County, Illinois. The specimen is deposited in the Odonata Collection of the Wisconsin Department of Natural Resources (WDNR), which is housed at the WDNR Superior Service Center. The males of G. spicatus were perched on a gravel access road where it intersected a hiking trail immediately adjacent to the river, less than 1 km from its mouth at Lake Michigan. The Dead River at the site is a slowly flowing stream that is blocked by a sand bar much of the year, forming an elongated pond. On 22 June 2007, one of us (CRS) returned to the South Unit and observed several G. spicatus at each of two interdunal wetlands (pannes) on the calcareous moist sands of the lake plain, about 800 m north of the mouth of the Dead River (about 600 m from the original G. spicatus site). Our findings suggest that these sites, which are located less than 30 km from the three lakes where G. spicatus was found over 100 years ago, likely provided breeding habitat for G. spicatus in 2007. Further surveys of Odonata at the Dead River and the nearby pannes are recommended to determine if populations of G. spicatus are persisting in those areas, and surveys of the odonate faunas of Clear, Fox, and Sand lakes would be helpful as well.

A single adult female Libellula semifasciata Burmeister (Odonata: Libellulidae), commonly called painted skimmer, was also collected on the hiking trail along the Dead River on 10 June 2007 and is deposited in the WDNR Odonata Collection. A number of adult L. semifasciata had been observed by CRS along the same hiking trail on several previous occasions and several individuals were again present along that trail on 22 June 2007. The finding of L. semifasciata evidently breeding at the Dead River site, which is within 8 km of the Wisconsin state line, is noteworthy because that species has not been found in Wisconsin since Muttkowski (1908) reported it from Milwaukee County in 1903 (Smith et al. 2003; Wisconsin Odonata Survey 2008). Populations of L. semifasciata may persist, and should be looked for, in the southern tier of counties of Wisconsin, especially in Kenosha County near Lake Michigan.

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LITERATURE CITED


A NEW STATE RECORD FOR **OLIXON BANKSII** (HYMENOPTERA: RHOPALOSOMATIDAE) IN MISSOURI.

Diane L. Wood\textsuperscript{1, 2} and M. Anthony Maupin\textsuperscript{1}

The cosmopolitan family Rhopalosomatidae is comprised of four genera and 37 species (Townes 1977, Goulet and Huber 1993, Fernandez and Sarmiento-M 2002, Lohrmann and Ohl 2007). It is represented in America north of Mexico by three genera, each with one species: *Olixon banksii* Brues, *Rhopalosoma nearticum* Brues, *Liosphex varius* Townes (Town 1977, Goulet and Huber 1993, MacGowan 1998). Rhopalosomatids have been reported only as ectoparasitoids of immature crickets (Goulet and Huber 1993).

*Olixon banksii* occurs primarily in the eastern United States (Ramsdell and Taylor 2006). It is rarely collected (Maes et al. 1993, McGown 1998, Krauth 2000), most often in pitfall traps (Ramsdell and Taylor 2006).

During an insect inventory of southeast Missouri, four males and three females of *O. banksii* were collected in pitfall traps placed in stands of *Arundinaria gigantea* (Walt.) Muhl. (Gleason and Cronquist 1991) in Bollinger, Cape Girardeau, and New Madrid counties during July 2006 and July 2007. All were brachypterous and had only mesothoracic wings. This is the first known recorded collection of *O. banksii* from Missouri.

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LITERATURE CITED


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PRESENCE OF THE “THREATENED” TRIMEROTROPIS HURONIANA (ORTHOPTERA: ACRIDIDAE) IN RELATION TO THE OCCURRENCE OF NATIVE DUNE PLANT SPECIES AND THE EXOTIC CENTAUREA BIEBERSTEINII

Jordan M. Marshall1 and Andrew J. Storer2

ABSTRACT

Trimerotropis huroniana Wlk. is a “Threatened” species in Michigan and Wisconsin with a distribution limited to open dune systems in the northern Great Lakes region of North America. Pitfall traps were utilized in the Grand Sable Dunes of Pictured Rocks National Lakeshore, MI, along with an herbaceous plant survey, to identify the relationship of T. huroniana with native dune plant species, Ammophila breviligulata Fern. (American beachgrass, Poaceae), Artemisia campestris L. (field sagewort, Asteraceae), and the exotic invasive plant Centaurea biebersteinii DC. [=Centaurea maculosa, spotted knapweed, Lamarck] (Asteraceae). The absence of C. biebersteinii resulted in an increased likelihood of capturing T. huroniana. This was most likely due to the increased likelihood of encountering A. campestris in areas without C. biebersteinii. The occurrence of A. breviligulata was independent of C. biebersteinii presence. A significant positive linear relationship occurred between the percent cover of A. campestris and the traps that captured T. huroniana. There was no significant relationship between A. breviligulata percent cover and the traps that captured T. huroniana. The occurrence and distribution of T. huroniana is closely related to the presence and abundance of A. campestris. Habitat conservation and improvement for T. huroniana should include increases in A. campestris populations through the removal of C. biebersteinii.

INTRODUCTION

With its U.S. distribution limited to sensitive open dune systems of the northern Great Lakes in Michigan and Wisconsin, Trimerotropis huroniana Wlk. (Orthoptera: Acrididae) is considered critically imperiled and listed as “Threatened” by Michigan and “Endangered” by Wisconsin (Hubbell 1929, Otte 1984, Ballard, Jr. 1989, Sjogren 2001, Scholtens et al. 2005). This locust has historically occurred in similar dune systems in Ontario, Canada (Hubbell 1929, Otte 1970). Now, however, T. huroniana may be extirpated from Ontario (Ontario Ministry of Natural Resources 2005).

Ammophila breviligulata Fern., Artemisia campestris L., and Calamovilfa longifolia (Hook.) Scribn. (prairie sandreed, Poaceae) are three native dune plant species identified as the most likely food plants for T. huroniana (Rabe 1999, Scholtens et al. 2005). Scholtens et al. (2005) suggested that the presence of T. huroniana was not related to the presence of native plant species. The landscape scale of their survey efforts in an attempt to delineate population distribution within the known range of this locust species may not have been adequate to determine finer scale correlations. Also, Scholtens et al. (2005) performed a qualitative assessment of the plant communities within dunes

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where *T. huroniana* occurs. Such a survey technique may not have provided the detail necessary to identify relationships between a rarely occurring organism and its food resources. A localized comparison of *T. huroniana* occurrences with the important dune food plants may provide a clearer understanding of mechanisms influencing the distribution of *T. huroniana*.


The objective of this study was to test the hypotheses that *T. huroniana* occurrence was independent of the presence of *C. biebersteinii*, *A. campestris*, and *A. breviligulata*.

**METHODS AND MATERIALS**

Areas with and without *C. biebersteinii* were utilized within the Grand Sable Dunes of Pictured Rocks National Lakeshore in the Upper Peninsula of Michigan (46°39'38"N, 86°1'54"W). *C. biebersteinii*, along with other major vegetation cover types, was mapped within the Grand Sable Dunes during the summer of 2000 (B. Leutscher, personal communication). The majority of the Grand Sable Dunes are covered by herbaceous dune plant communities, with natural dune stabilization occurring as *Pinus banksiana* Lamb. (Jack pine, Pinaceae) and Northern Hardwood forests invade.

The three largest delineated areas of *C. biebersteinii* (10.7, 6.3, 4.8 ha), which had been established for at least five years (B. Leutscher, personal communication), were selected for this study. A transect (500-600 m) was established along the long axis of each area of *C. biebersteinii*. In areas of native dune plant communities without *C. biebersteinii* adjacent to each *C. biebersteinii* area, transects of comparable length were established. Along each transect in the survey area, two arrays of five pitfall traps (8.5 cm diameter, 12.5 cm height) were installed on a linear 5-meter spacing following the transect approximately 200-250 m apart (10 traps per transect). Approximately 75 ml of 50 percent propylene glycol (Preston LowTox® Antifreeze) was used in each trap as a killing agent and preservative. Pitfall traps were open for one week and then closed for approximately three weeks to reduce the likelihood of population depressions due to trapping. At the time of closing, traps were emptied and upon re-opening, new propylene glycol was added to each trap. A total of five trapping cycles were carried out from 2 May 2003 to 28 August 2003, however for analysis, only the final two trapping cycles from 23-30 July and 21-28 August (3 transects × 2 trap groups × 5 traps × 2 trapping cycles = 60 traps/treatment with and without *C. biebersteinii*) were used. These cycles were the only with *T. huroniana* captures due to the late season activity of adults (Rabe 1999).

A plant survey was conducted within five 1-m² quadrats along each transect within 5 m of each trap (3 transects × 2 trap groups × 5 quadrats = 30 quadrats/treatment with and without *C. biebersteinii*) identifying percent cover of *C. biebersteinii*, *A. campestris*, and *A. breviligulata*. Mean percent cover for each taxon was calculated for individual transects. A chi-squared analysis was used to test the hypothesis that traps capturing *T. huroniana* were independent of *C. biebersteinii* presence, as well as to test the hypothesis that the presence of *A. campestris* and *A. breviligulata* were independent of *C. biebersteinii* presence.
Simple linear regression was used to test for the relationship between the percent cover of *A. campestris*, as well as *A. breviligulata*, and the traps that captured *T. huroniana*.

**RESULTS AND DISCUSSION**

Traps that captured *T. huroniana* were not independent of the presence of *C. biebersteinii* (Table 1). Traps installed in areas without *C. biebersteinii* were more likely to capture *T. huroniana* than traps in areas with *C. biebersteinii*. This relationship may be due to the increased likelihood of encountering *A. campestris* in quadrats without *C. biebersteinii* (Table 2). Along with *A. campestris*, two dune grasses occurred in the Grand Sable Dunes, however, *C. longifolia* was rare and *A. breviligulata* was the dominant grass species. Usually these two grass species singularly dominate, as in the Grand Sable Dunes, or co-dominate suitable *T. huroniana* habitat and are also known plants fed on by this locust (Scholtens et al. 2005), however, the presence of *A. breviligulata* was independent of the presence of *C. biebersteinii* ($\chi^2 = 0.33, \text{df} = 1, P = 0.567$). The number of traps that captured *T. huroniana* was not related to the percent cover of *A. breviligulata* ($F = 0.25, \text{df} = 1,4, P = 0.644, R^2 = 0.059$).

As *A. campestris* percent cover increased, the number of traps along each transect that captured *T. huroniana* also increased (Fig. 1). This relationship corroborates the suggestions made by Rabe (1999) and Scholtens et al. (2005) that *A. campestris* is one of the important plant species in the distribution of *T. huroniana*. As a native dune plant species and an important component of *T. huroniana* habitat, changes in *A. campestris* distribution and occurrence would be expected to alter *T. huroniana* distribution and occurrence.

*Trimerotropis huroniana* habitat conservation may be enhanced by increasing the dune coverage of *A. campestris* by reducing the coverage of *C. biebersteinii*. The occurrence of *A. breviligulata* was independent of *C. biebersteinii* presence and suggests that this dune grass may not be the most influential

| Table 1. Traps capturing *Trimerotropis huroniana* in areas with and without *C. biebersteinii* in the Grand Sable Dunes, Pictured Rocks National Lakeshore, MI. |
|-----------------------------------|-------------------|-------------------|
| *Trimerotropis huroniana*         | Captured          | Not Captured      |
| *C. biebersteinii*                | Present           | 3                 | 57                |
| *C. biebersteinii*                | Absent            | 10                | 50                |

$\chi^2 = 4.23, \text{df} = 1, P = 0.039$

<table>
<thead>
<tr>
<th>Table 2. Number of quadrats sampled encountering <em>Artemisia campestris</em> and <em>C. biebersteinii</em> in the Grand Sable Dunes, Pictured Rocks National Lakeshore, MI.</th>
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<tr>
<td><em>Artemisia campestris</em></td>
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<td>Present</td>
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<td><em>C. biebersteinii</em> Present</td>
</tr>
<tr>
<td><em>C. biebersteinii</em> Absent</td>
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$\chi^2 = 13.01, \text{df} = 1, P < 0.001$
factor in determining the occurrence of *T. huroniana* in the Grand Sable Dunes, however, increasing the coverage and distribution of this dune grass would also be beneficial to *T. huroniana*.

Efforts within the Grand Sable Dunes, Pictured Rocks National Lakeshore, to control *C. biebersteinii* by hand pulling have been carried out by the National Park Service but the availability of funding has limited the size and recurrence of such operations (B. Leutscher, personal communication). A more viable option may be classical biological control. While early biological control agents selected for *C. biebersteinii* control have been plagued with limited efficiency, parasitoid activity, and predation, more recent control agents have demonstrated effective reductions in *C. biebersteinii* density and biomass (Myers 2000, Long et al. 2003, Marshall et al. 2005, Corn et al. 2006, Story et al. 2006). Based on the results of this study, reducing the populations of *C. biebersteinii* in the dune habitat of *T. huroniana* would increase populations of *A. campestris* to the benefit of this “Threatened” locust.

**ACKNOWLEDGMENTS**

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LITERATURE CITED


NOTES ON DIANTHIDIJUM SIMILE (CRESSON) (HYMENOPTERA: MEGACHILIDAE) IN MICHIGAN

Mark F. O’Brien

ABSTRACT

*Dianthidium simile* (Cresson) is a small ground-nesting megachilid bee restricted to sandy areas in Michigan, often bordering lakeshores. Females dig their nests in sand, at the base of dried clumps of grass. Nests are small clusters of cells, formed from conifer resin and sand grains, with each 1-1.5 cm in length by 8.5 mm in diameter. Collection records and field observations indicate a flight period from late June to early September. This is the first report on the behavior of this species.

Here I report on the behavior and nesting habits of *Dianthidium simile* (Cresson), a colorful, fairly small but robust member of the Megachilidae (Hymenoptera) that nests fossorially, most often in sandy lacustrine regions. It is a member of the tribe Anthidiini, a diverse group of megachilids that use a variety of materials to line their nests, ranging from plant resins to trichomes (Michener 2000). Members of the genus *Dianthidium* are known to use resins and small stones and debris to construct the brood cells in the ground, in trap nests, and on above-ground substrates. *D. simile* ranges from the Great Lakes region to Maine, and south to Georgia (Krombein, et al. 1979). Fischer (1951) reported rearing two specimens from a partly rotted log, but nothing else has been published about its biology. Although Romankova (2004) included it in the list of the anthidiines of Ontario, no new biological information was presented for it.

The Megachilidae are known for their diverse array of nesting sites, nesting materials, and behavior, even within a genus. Members of the genus *Dianthidium* display a range of nesting preferences: multi-celled nests on small shrubs; vertical faces of gravel pits; and edges of dunes amongst grass rhizomes. All of the published behavioral observations have one commonality: cells made of resin with a matrix of sand grains and plant debris. Several western North American species of *Dianthidium* have been studied, and the plasticity of nesting behavior within this genus of small megachilids is evident from those studies. *Dianthidium ulkei* (Cresson) was briefly studied by Hicks (1933) near Boulder, Colorado. He reported that the bees constructed short tunnels in natural cavities in soil. Nests of one or two cells were constructed with pebbles and plant debris in a resin matrix. Frolich and Parker (1985) studied the nesting and mating behavior of *D. ulkei* in a greenhouse, and were able to induce females to nest in wood cavities. The nests were lined with a resin, pebble, and soil matrix. Krombein (1967) described the nests of a number of southwestern species from trap nests, as well as a nest of the Floridian species *D. floridiense* Schwarz. An Arizona nest of *Dianthidium pudicum pudicum* (Cresson) was studied briefly by Clement (1974), and in that instance the nest was found in the fork of small branches of a small tree (*Larrea tridentate* (DC.) Coville, creosote bush). The aerial, external nest was triangular in shape (held in the “V” between 2 branches), and contained 10 cells. The nest was comprised of resin,

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small pebbles, and plant debris. Fischer's (1951) report on *Dianthidium concinnum* (Cresson) also revealed that the 10-celled nest was constructed externally on a branch of a small elm in Kansas. The nests were comprised of the same materials as above. Fischer (1951) made some additional observations on *D. sayi*, Cockerell (now *D. curvatum sayi* Cockerell [Krombein et al. 1979]) which nested in the soil in a vertical bank in Kansas. The cells were also constructed of soil and plant resin. Michener and Michener (1999) studied an aggregation of the same species for several years in a vertical sandy bank adjacent to the Oldman River in Alberta, Canada.

**OBSERVATIONS**

In Michigan, *D. simile* has been collected predominantly from lacustrine sandy areas bordering the Great Lakes, but there are a few records towards the center of the state (Fig. 1). Adults have been collected from 30 June to 12 September, with most records from late July to early August. Specimen labels indicate that they visit the following flowers: *Coreopsis lanceolata* L., *Rudbeckia hirta* L., and *Polanisia graveolens* Raf.

I collected adults, excavated several nests, and made the following observations at two sites in Michigan: in the Lower Peninsula at Ludington State Park (LSP), Mason Co., in a stretch of vegetated back dunes well away from Lake Michigan from 1745 -1845 hr on 7 August 1990, and in the Upper Peninsula (22°C) at 1400 hr, on 29 July 1994 in Mackinac Co. in back dunes along Lake Michigan at Big Knob State Forest Campground (BSF) area. Both sites were similar in that areas of bare sand were intermingled with grasses and other herbaceous plants, and were at the edges of old dunes that bordered ecotones leading to conifer-dominated woods. These sites were all more or less horizontal, with a slight slope leading away from the nesting area.

**LSP:** I collected 8 females at the site, and at least 10 *D. simile* were seen nesting at the base of dead clumps of grass, in an area 30 × 15 cm. Cells were intermingled with dead rhizomes, often just below the surface. Bees were flying in with provisions every 2-3 minutes. I dug up a few clumps of cells, but not the entire aggregation. Cells were 1.0 – 1.5 cm long × 0.8 – 0.9 cm in diameter. Thirteen cells were in one cluster, all facing up, nearly perpendicular to the substrate (Fig. 2). Another nest (Fig. 3) had 7 cells arranged around a grass stem, also pointing upwards. These nests were placed in sealed containers but were never subjected to cool temperatures. During January-February 1991, 4 females and 3 males emerged.

**BSF:** Nests were located in a back dunes-beach area closest to the edge of woods in stable grassy/shrubby area. I estimated that there were 20-24 nest entrances in one square meter. I also observed several females coming and going from the nest site. All nest entrances were located at the base of old clumps of dried grass, all facing south, and partially obscured by dead grass leaves. In one 30 × 30 cm area, I counted 7 nest entrances. All cells were made of coniferous resin embedded with sand grains. I collected 6 females there.

Most clumps of cells were at the very basal rhizome portion of the grasses, just below the surface, varying from 1-5 cm deep. Some nests that appeared to be older were 3 cm deep. All were very close to the main stems of grass. None of the cells were grouped like those from LSP. The nests that I found had no more than 3 or 4 cells grouped together. All cells were made of coniferous resin embedded with sand grains and small bits of plant debris.

Six clumps of cells were placed in small snap-top containers, and sometime over the winter, 4 males and 3 females emerged from the cells. No parasitoids emerged from the collected cells. The pungent aroma of honey and pine resin was quite noticeable after the nests were placed into small containers.
Figure 1. Map of Michigan, with dots representing collection localities of *Dianthidium simile* from specimens in the Univ. of Michigan Museum of Zoology and Michigan State Univ. Cook Arthropod Research Collection. The two study sites are designated as follows: BSF = Big Knob State Forest Campground, and LSP = Ludington State Park.
Figure 2. Excavated nest of *Dianthidium simile*, Mason Co., MI. The scale is in mm.

Figure 3. Excavated nest of *Dianthidium simile*, Mason Co., MI. The scale is in mm.
DISCUSSION

*Dianthidium simile* is an interesting inhabitant of the dune ecosystem, and it would be desirable to know how much variation exists in nest site selection across its range. Romankova (2004) listed many Ontario sites that also appear to be located near water and a similar flight period (July-August). In Michigan, most of the localities are around the margins of the state, with a few in the interior (Fig. 1). Based on observations, I would predict all of the sites to be in sandy substrates.

Nests from BSF differed from those found at LSP. For example, the LSP nests had most of the cells oriented in the same direction and the clumps of cells were more connected forming a mass of cells in a single plane, whereas the BSF cells were more random in orientation and fewer than 5 cells in a cluster was typical (Fig. 4). Of course, there was a mixture of old (previous years) and new cells in the BSF matrix of cells amidst the grass rhizomes, whereas the LSP cells all appeared recently constructed.

Although there were some differences in the maximum number of cells and orientation between nests in Mason and Mackinac Counties, that may only be a reflection on the ability of a bee to dig amongst grass rhizomes and construct adjacent cells. Otherwise, the two sites are consistent in terms of substrate, cell size, materials used in lining the cells, and location of the nests. Based upon the two sites, it appears that a given nesting area supports many individuals nesting in close proximity, as reported for the closely related *D. curvatum sayi* (Michener and Michener 1999).

I am skeptical of Fischer’s (1951) report of rearing two *D. simile* from a partially-rotted log. Without further attribution as to the site and specimen verification, it’s a bit anomalous compared to what I have seen for *D. simile* in Michigan, however, if the rotted log came from a sandy area, the record would certainly be more credible.

Figure 4. Excavated cells from nests of *Dianthidium simile*, Mackinac Co., MI. The scale is in mm.
ACKNOWLEDGMENTS

I thank Virginia Scott (now at the University of Colorado Museum) for her assistance with records from the Michigan State University. Mike Arduser of the Missouri Dept. of Conservation kindly supplied me with some updated references and greatly improved the manuscript.

All specimens and nest materials collected by me are deposited in the collection of the Insect Division at the University of Michigan Museum of Zoology.

LITERATURE CITED


DO GENERALIST TIGER SWALLOWTAIL BUTTERFLY FEMALES SELECT DARK GREEN LEAVES OVER YELLOWISH – OR REDDISH-GREEN LEAVES FOR OVIPOSITION?

Rodrigo J. Mercader¹, Rory Kruithoff¹, and J. Mark Scriber¹, ²

ABSTRACT

In late August and September, using leaves from the same branches, the polyphagous North American swallowtail butterfly species *Papilio glaucus* L. (Lepidoptera: Papilionidae) is shown to select mature dark green leaves of their host plants white ash, *Fraxinus americana* L. (Oleaceae) and tulip tree, *Liriodendron tulipifera* L. (Magnoliaceae) rather than the pale green or yellowish-green mature leaves in laboratory oviposition arenas. In early August, similar results were observed for black cherry, *Prunus serotina* Ehrh. (Rosaceae). Dark green leaves were preferred over light green and yellowish green leaves. These green leaves of black cherry were the most nutritious leaves for larval growth indicating a clear correlation between adult preference and larval performance on this plant. However, tulip tree leaves in the summer did not elicit different oviposition responses between green and light green leaves. A field evaluation of oviposition preferences for young expanding reddish leaves of red bay, *Persea borbonia* (L.) Spreng (Lauraceae) versus slightly older expanded green leaves of the same branch also suggested avoidance of “young” red leaves in Florida by *Papilio troilus* L. and *Papilio palamedes* Drury during the spring season (March-April).

Many intrinsic factors (e.g., female age, time since last oviposition, egg load, time since last mating; Singer 1983, Miller and Strickler 1984, Bossart and Scriber 1999) and extrinsic factors (plant volatiles, leaf color, texture, leaf shape, contact chemosensory cues, and species of host plant) influence the choice of host plant by ovipositing females (Courtney and Kibota 1990; Thompson and Pellmyr 1991; Carter et al. 1999; Frankfater and Scriber 1999, 2003; Renwick 2002; Mercader and Scriber 2007). Additional ovipositional-determining factors not directly related to the plant may include avoidance of natural enemies (Redman and Scriber 2000, Murphy 2004), microclimate temperature and/or humidity preferences (Grossmueller and Lederhouse 1985), and seasonal thermal constraints on voltinism which can select for the most nutritious hosts (Nylin 1988, Scriber and Lederhouse 1992). Lack of availability or low abundance of some host species can also result in local host plant preferences (Rausher 1978, Fitt 1986, Scriber 1986, Scriber et al. 2006).

The selection of host plants by polyphagous species is governed both by factors affecting the rank order of preference and also by the “specificity” (Courtney and Kibota 1990, Mercader and Scriber 2005, 2007). It has been seen that *Papilio glaucus* L. generally selects (specializes on) the host plant species that support fast larval growth in thermally-constrained areas (thus allowing the possibility of an extra generation), but use a wider array of potential hosts in thermally-relaxed areas (i.e., where enough Degree-days accumulate seasonally to complete development of the extra generation on all host plant species, even those of rather low suitability/nutritional quality; Scriber and Lederhouse 1992). This voltinism-suability hypothesis suggests that preference performance

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relationships may exert strong selection pressures, where allelochemical toxin barriers are not involved, interacting with leaf nutritional quality (bottom up regulation) and natural enemies (top-down regulation).

We know that different species of tree leaves differ in their ability to support growth of lepidopteran species (Scriber and Slansky, Jr. 1981, Slansky, Jr. and Scriber 1985, Mattson and Scriber 1987). The seasonal variation in leaf suitability (Scriber 1984) has less frequently been evaluated for its impact on host selection (Finke and Scriber 1988). The neural limitations hypothesis of host selection (Bernays 2001) predicts that polyphagous species incur costs in the form of prolonged decision making time and increased error rates in host selection relative to more specialized insects. Therefore, the ability of polyphagous insects to detect differences in host plant quality is expected to be lower, as has been previously noted in other butterfly species (Janz and Nylin 1997). Given the likely constraints on information processing in the highly polyphagous \textit{P. glaucus}, we used 2-choice oviposition arenas to determine if \textit{P. glaucus} was capable of selecting between leaves that were light green or reddish-green versus leaves (fully expanded, but) with dark green color, as would be encountered late in the growing season. It is known that for black cherry trees different colors reflect different leaf water and nitrogen content which declines seasonally in green leaves (from 5.1% Nitrogen and 80% water) to 1.5 - 2.0% N and 65% water in yellowish green leaves, and less nitrogen (<1%) and water (<55%) in reddish green, yellow-brown leaves (Scriber 1977). The correlations of color (possibly with nutritional value) and oviposition preference of \textit{Papilio} females for green leaves over yellowish-green mature leaves in the fall and potential toxins/deterrents for younger expanding reddish spring leaves are addressed here.

**MATERIALS AND METHODS**

Most \textit{P. glaucus} oviposition assays were conducted using wild females of \textit{P. glaucus} collected in Clinton Co. and Allegan Co. in southern Michigan. Due to small sample sizes black cherry assays were supplemented using females obtained from Clarke Co. Georgia and sent to our lab by Express Mail.

Oviposition preferences using leaves from the same branches were conducted in the Fall (September, when most leaves were changing color) using white ash (\textit{Fraxinus americana} L.; \(n = 5\) females) and tulip tree (\textit{Liriodendron tulipifera} L.; \(n = 9\) females). In mid-August, again using leaves from the same branches light green and dark green black cherry leaves (\textit{Prunus serotina} Ehrh.; \(n = 10\) females) and green versus light green tulip tree leaves (\(n = 8\) females) were assessed. Leaf petioles were inserted into water-filled vials with rubber caps, and the leaves were draped along the inside wall of clear, round, large plastic dishes on rotating platforms in front of a bank of incandescent lights (Fig. 1; Scriber 1993). Adults were fed using a 15% honey water solution and eggs were counted and removed daily. Only females laying 9 or more eggs were included in the analyses. The proportion of eggs laid on each leaf was compared within each paired comparison using Bonferroni corrected Paired Wilcoxon Tests using the R Statistical Package V 2.4 (R Development Core Team 2006) with a 0.05 alpha level to test if there were differences in preference between leaves of different ages.

A similar ovipositional study was conducted in late summer, using light green and dark green leaves of black cherry and tulip tree. The eggs from two laboratory reared families that originated from individuals collected in Clarke Co. Georgia were held at 27°C and newly hatched neonate larvae were transferred using camel hair brushes to host plant leaves. Larvae from the two families were reared at 24°C (18:6 h L:D photoperiod) on green (\(n = 14\) from one family and \(n = 26\) from the other family) and light green or yellowish-green (\(n = 14\) and \(n = 18\), respectively) cherry leaves, similar to those used in oviposition assays and weighed ten days after emergence. We tested the effect of leaf age
(green or yellowish-green black cherry leaves) on larval growth with a Nested ANOVA (leaf age nested within larval family) using the R statistical package V 2.4 (R Development Core Team, 2006).

In addition to these lab experiments, we evaluated the differences in field oviposition preferences between “new” expanding reddish leaves compared to more fully-expanded slightly older green leaves of red bay for Papilio palamedes Drury and Papilio troilus L. during the spring (late March - early April) in Levy Co. and Highlands Co. Florida.

RESULTS

In studies done in September, when the leaves of most trees were beginning to change color, females of P. glaucus presented with a choice of light green (or yellowish-green leaves) and dark green leaves from the same branch of white ash trees, showed a clear preference for the green leaves (Figure 1). Nine females that laid 9 or more eggs all placed the majority (84.6%) of these on the green versus yellowish-green leaves (Fig 2A). The same result was obtained for 5 females offered tulip tree leaves (Figure 2B); few were placed on the light green or yellowish-green leaves and most (95.5%) were placed on the green (from the same branches).

In the second study (conducted in August), using black cherry and tulip tree. Females placed in assays with green and yellowish-green black cherry leaves (n = 10) selected green leaves about three times as often as yellowish-green leaves (72.8 ± 5.5 vs. 27.2 ± 5.5, mean ± SE, Figure 2C). However, these summer assays with tulip tree leaves showed mixed results with no strong preference for dark green versus light green tulip leaves (Figure 2D and 3B).

The growth rates of resulting neonate larvae on these green and light green leaves verified that suitability for larval growth was better on the dark green leaves than on the yellowish-green leaves of black cherry (Fig. 3A). The means ± SE for family #20196 and 20192 for larval weight were significantly higher on the green (97.5 ± 6.8 mg) versus the yellowish-green leaves (64.6 ± 5.1 mg) at 10 days ($F = 199.59$, df = 1, 1, $P = 0.04$).

In Highlands County, Florida the newly expanding red bay (Persea borbonia (L.) Spreng.) leaves were red or reddish-green, and a careful search of more than 1500 such red bay leaves from 21 different trees in March 2007, yielded no eggs or larvae of Papilio. However, very often on the adjacent one or two more expanded green leaves larvae were found feeding and resting in leaf rolls. Sixteen neonate and early instar larvae and one egg were found on green leaves (of more than 2500 searched) of these same 21 trees. Apparently the ovipositing P. troilus and P. palamedes avoid these red leaves of red bay (Fig. 4). Over a three year period in Levy Co. Florida, eggs of P. glaucus were found on young (still expanding) green ash (Fraxinus pennsylvanica Marshall) leaves (> 15 eggs or neonates on more than 2000 leaves), but not on any young reddish-green leaves of the same branch (Fig 5).

DISCUSSION

We have shown here that female oviposition preferences of P. glaucus are for greener leaves rather than light green, yellowish-green, leaves of black cherry and white ash (Figs 1 & 2). P. glaucus females also prefer green over yellowish-green tulip tree leaves in the fall, but in the summer they do not always prefer green over light green leaves from the same branch (Fig 2). This might indicate that cues between these August tulip tree leaves are insufficiently different to be distinguished or of similar chemistry. It seems that this generalist butterfly discerns host quality through contact chemoreception since volatiles would be confused/confounded in these multi-choice lab arenas.
Fig. 1. The experimental oviposition arena showing eggs on the dark green ash leaves but not on the yellowish-green ash leaves. The inset shows the stacks of arenas on the turntable in front of the light bank.
Fig. 2. A). Median proportion of eggs laid by *P. glaucus* butterflies using green (G) and yellowish-green (YG) leaves of white ash (WA) in the fall. B). Median proportion of eggs laid by *P. glaucus* butterflies using green (G) and yellowish-green (YG) leaves of tulip tree (TT) in the fall. C). Median proportion of eggs laid by *P. glaucus* butterflies using green (G) and light green (LG) leaves of tulip tree. D). Median proportion of eggs by *P. glaucus* butterflies using green (G) and light green (LG) leaves of black cherry (BC). In each panel, bars around the medians represent the inter-quartile ranges. Pairwise differences between proportions of eggs laid on each leaf were analyzed separately for each leaf species using Paired Wilcoxon tests. Leaves within each panel with the same letter did not have a significant difference at a Bonferroni corrected α < 0.05.
Fig. 3. A). Black cherry showing fully expanded (new) and fully expanded (over-mature) leaves. B). Tulip tree leaves showing the indeterminant growth, with continuous production of new leaves.
Fig. 4. Red bay leaves which had *Papilio* eggs deposited and larvae feeding on the green, but not on the red leaves (March 24-27, 2007; Highlands Co. Florida). Both *P. palamedes* and *P. troilus* form leaf rolls during all instars on red bay (see inset).
Fig. 5. White ash leaves with red expanding tip leaves ( Levy Co., Florida; March 2007). Upper right insert shows the new expanded green leaves where eggs are placed and where larvae feed.
White ash and black cherry tree leaves have been documented to show general seasonal decline in the amounts of leaf water and nitrogen seasonally, although tulip tree continuously generates new leaves all season (Scriber and Slansky, Jr. 1981, Scriber 1984). Accelerated differences in the fall can occur with abscission layer formation. In fact, the same branch (of black cherry, for example) can have all stages of leaf quality from dark green (3.2% - 2.8% N and 76% - 65% water), to light green (2.8 - 2.2% N), yellowish-green (2.1 - 1.8% N) reddish-green (1.5% N) and yellow-brown (1.2 – 0.8% N, and <55% water (Scriber 1977, and unpublished). While we did not evaluate the nutritional differences between differently colored tulip tree and ash leaves here, we did determine that the dark green black cherry leaves support a 3-fold faster growth rate than the yellow-green leaves.

Since neonate larvae of these tree-feeding lepidoptera need to start feeding near the location on a particular tree chosen by the mother, it has been assumed that strong selection for highly nutritional oviposition substrates might occur (Zalucki et al. 2002). Even in polyphagous Papilio species, the selection of the “wrong” young leaves (i.e., on a toxic, or nearly toxic unsuitable host) could be a fatal “mistake” (Straatman 1962, Wiklund 1975, Berenbaum 1981, Larsson and Ekbom 1995, Renwick 2002, Scriber 2002a, Graves and Shapiro 2003). Consequently, the general lack of correlation of adult oviposition preference and larval growth performance in many, if not most, herbivorous insects has been somewhat of an enigma (Thompson 1988, 1998; Mayhew 2001; Bossart 2003). The reasons for a general lack of genetic linkage of preference and performance remains largely unknown (Bossart and Scriber 1999, Berenbaum and Feeny 2008). However, it is known that abiotic factors can interact with nutritional quality to result in strong preference-performance correlations in Papilio in accord with the “voltinism-suitability hypothesis” (Nylin 1988; Scriber and Lederhouse 1992; Scriber 1996, 2002b, 2005). In this scenario, thermal constraints on seasonal degree-day accumulations may (on a poor host) result in fewer generations than might be possible on a host species that supports rapid growth. The selection of the “best” leaves available on a non-toxic host species (Feeny 1995, Scriber et al. 2007) is generally assumed to be always favored, in order to minimize time exposed to natural enemies (predators, parasites, and disease; Slansky, Jr. 1983, Bernays 1998, Scriber 2004) or freeze susceptibility (Fordyce and Shapiro 2003, Tesar and Scriber 2003). However, fast growth is not always possible (e.g., tree leaves are generally poorer than herbaceous plants and roots are notoriously poor in nutritional quality, resulting in slow growth of root feeders, etc. (Slansky, Jr. and Scriber 1985).

From both the ecological and evolutionary perspectives, it remains unclear if rapid growth rates are always selected for (Slansky, Jr. 1993, Benrey and Denno 1997). In addition, adaptations for “compensatory feeding” may be invoked when diets are unbalanced in carbohydrates, protein, energy, or minerals (Scriber 1984, Slansky, Jr. and Scriber 1985, Mattson and Scriber 1987, Simpson and Simpson 1990, Fageria and Scriber 2001, Slansky, Jr. and Wheeler 1992, Trier and Mattson 2003 “diet-induced thermogenesis”). This feeding compensation may be via diet “self-selection” (Waldbauer and Friedman 1991) or “organismal stoichiometry” (Raubenheimer and Simpson 2004). However, larvae can not always compensate for eggs placed on older, over-mature leaves since higher risks as well as slower growth may be involved for the neonate larva, harder to consume and digest leaves, with higher vulnerability to enemies (Ayres and Scriber 1994, Zalucki et al. 2002). These slower growth rates were observed here with P. glaucus on older light green or yellowish-green versus newer dark green leaves of black cherry.

Field assessments of green versus younger expanding reddish leaves of red bay in Florida suggest that the Lauraceae-specialized P. palamedes and P. troilus butterflies avoid “reddish” leaves (Fig. 4). Our extensive searches of 21 trees resulted in no eggs or neonates feeding on red leaves (more than 1500),
but we found 16 neonate larvae and one egg on green leaves (>2500 searched). Similarly, in Florida, *P. glaucus* on green ash prefers green leaves and appears to avoid reddish-green leaves in the spring (JMS personal observation, and Fig. 5).

It is likely that these *Papilio* spp. may be using cues other than gustation to detect host plant quality. It is known, that unlike most invertebrates (Lee et al. 1987), *Papilio* species including *P. glaucus* (Briscoe 2000) have long wavelength red receptors that enable them to see and select green leaves (Kelber 1999). While most invertebrates lack red receptors (Menzel and Backhaus 1991), and peak reflectance from leaf anthocyanins lies in this region of about 630 nm (Lee et al. 1987), notable exceptions with a long wavelength receptor tuned to 610 nm include the Papilionidae (Arikawa and Üchiyama 1996). One species, *Papilio aegeus* Donovan, uses this red receptor to avoid red leaves (Kelber 1999) and, perhaps *P. glaucus* can do the same (Briscoe 2000).

It has been suggested that some young leaves gain a protection from insect herbivores by a “delayed-greening” strategy for newly flushed leaves (which are reddish; Dominy et al. 2002). This “delayed greening” appeared to be true for ash leaves in Levy Co. Florida in 2007 (Fig. 5). This “delayed greening” has been suggested by Coley and Aide (1989) to be due to a high anthocyanin content, which, aside from being red, has fungicidal properties. However, this “delayed greening” may also protect leaves by keeping them devoid of nutritive value until they reach full size (Figs. 4 and 5, Dominy et al. 2002).

Regardless of the ecological/evolutionary advantages of selecting younger nutritious host leaves by *P. glaucus*, the physiological/chemical mechanisms permitting them to distinguish the best leaves remains unknown. Leaf color may play some unknown role in these *Papilio* as in the related *Battus philenor* L. (Papilionidae), which can learn leaf color and shape (Rausher 1978, Weiss and Papaj 2003, Miller and Strickler 1984). It is known that other *Papilio* species have red receptors to see green (Kelber 1999), as is the case for *P. glaucus* (Briscoe 2000), however, we do not really know if these specific color stimuli were used by the *P. palamedes* and *P. troilus* (or even *P. glaucus*) in these studies. Our ovipositional assays primarily detect differences in contact chemoreception and therefore the responses observed in our assays suggest that females of the polyphagous *P. glaucus* can detect and utilize leaf surface cues correlated with color. In these and related *Papilio*, the final and most important signal seems to come from chemosensory cues to the female tarsi as they taste the leaves before ovipositing (Feeny 1995; Nishida 1995; Frankfater and Scriber 1999, 2003).

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Fungal pathogens infecting soybean aphid and aphids on other crops grown in soybean production areas of Michigan

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ABSTRACT

Seasonal prevalence of fungal pathogens infecting soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), was assessed from 2004 to 2006 in two Michigan soybean production areas. In 2005 and 2006 field-collected soybean aphids were incubated, and fungal infection was detected at both sites early in August 2005 during soybean pod development and high soybean aphid densities. Significantly higher proportions of winged aphid morphs were infected (20 and 90% infection at the two sites) than wingless aphid morphs (1 and 3% infection). All cases of mycosis examined involved one pathogen species, *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Entomophthorales: Entomophthoraceae). In 2004 and 2005, we surveyed for pathogens of the soybean aphid in soybean as well as pathogens in other aphid species feeding on other crop plants (alfalfa, clover, corn, and wheat) by inspecting for sporulating aphid cadavers every 2 to 3 wk during the soybean growing season. Aphid cadavers were most abundant in alfalfa, especially in August; were less common in clover, corn, and soybean; and were not found in wheat. *Pandora neoaphidis* was associated with cadavers of *Acythosiphon pisum* (Harris) (Hemiptera: Aphididae) in alfalfa and clover during the same period when soybean aphid infection was detected. Overall, mortality of soybean aphid and other aphid species due to fungal infection was confirmed in Michigan. The results also implicate infected winged soybean aphid morphs as potential agents for fungal dispersal, and *A. pisum* in alfalfa and clover as a source of fungal propagules for soybean aphid.

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a pest of soybean from Asia, recently established in the soybean production regions of North America (Ragsdale et al. 2004). Heavy infestations are associated with soybean yield loss (Ragsdale et al. 2007). Insecticide use, which was previously uncommon in soybean, became widespread for control of this aphid (Myers et al. 2005). In Michigan, outbreaks of soybean aphid were reported in 2000, 2001, 2003, and 2005 (DiFonzo and Hines 2002, T. N., pers. obs.).

Aphid pathogens, especially certain entomophthoralean fungi, may play a role in soybean aphid suppression (Wu et al. 2004, Nielsen and Hajek 2005). In China, *Neozygites (=Entomophthora* fresenii) (Nowakowski) Batko (Entomophthorales: Entomophthoraceae) is one of the most common pathogens infecting soybean aphid, and its prevalence was positively correlated with humidity and aphid density (Wu et al. 2004). In North America, fungal disease was found in up to 84% of aphids sampled in New York State in 2003 and 2004 (Nielsen and Hajek 2005), and 3 to 70% of aphids sampled in Minnesota between 2002 and 2006 (K. Koch, personal communication). *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Entomophthorales: Entomophthoraceae) was the most abundant pathogen detected in both studies.

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Obligate aphid pathogenic fungi from the order Entomophthorales are widely distributed across temperate regions and infect a range of aphid species inhabiting various vegetation (Latgé and Papierok 1988, Nielsen 2002, Shah et al. 2004). Natural epizootics of aphid diseases often occur under favorable biotic and abiotic conditions (Latgé and Papierok 1988, Shah et al. 2004, Zhang et al. 2006). Many crop plants besides soybean that are cultivated in Michigan’s soybean production areas harbor aphids, including corn and wheat that are often rotated with soybean, and occasionally alfalfa (Blackman and Eastop 2000, USDA-NASS 2007).

In this study in Michigan, we quantified seasonal prevalence of fungal pathogens infecting soybean aphid, which allowed for regional comparisons with earlier studies in New York (Nielsen and Hajek 2005) and Minnesota (K. Koch, personal communication). In addition, we quantified abundance of sporulating aphid cadavers on other crop plants common in the soybean production region of lower Michigan, allowing for consideration of potential sources of inoculum near soybean fields.

**MATERIALS AND METHODS**

**STUDY SITES.** Sampling of aphids and their pathogens were conducted at two Michigan locations: Kellogg Biological Station, Long-Term Ecological Research site, Hickory Corners (42°24’N, 85°23’W) and Entomology Farm, East Lansing (42°41’N, 84°29’W). Sites were managed by Michigan State University and were separated by 80 km. Soybean was sampled for soybean aphid at the Hickory Corners site in 2004 and at both sites in 2005 and 2006. Alfalfa, corn, wheat, and clover were also sampled for other aphids at the Hickory Corners site in 2004 and 2005. All crops sampled at both sites were cultivated under (rain-fed) condition without irrigation. We sampled four replicated plots of each crop through the soybean growing season in Michigan (June through September). The size of different crop plots at Hickory Corners were either 9 by 27 m (soybean [2004, 2005], wheat [2004, 2005], corn [2004, 2005], and clover [2004]) or 1 ha (soybean [2006] and alfalfa [2004, 2005]). All plots were set in the same experimental area devoted to crop rotational experiments, replications were randomized, and no insecticide or fungicide was used. The plot sizes of soybean at the Entomology Farm were 17 × 33 m.

**SOYBEAN APHID DENSITY.** Soybean aphid populations were sampled four to eight times during the soybean growing season from 2004 to 2006 at each site. Soybean growth stages were noted using the scale of Fehr and Caviness (1977). The sampling periods were spread across a) mid-June, during early vegetative growth (V2-V3) when aphids may first migrate to soybean; b) mid-July, during flowering (R1-R2) when aphids may be multiplying on soybean; c) early August, during soybean pod fill (R3-R5) when aphids may be reaching peak densities; and d) late August, during plant senescence (R6) when aphids may be declining in density. Field aphid density was estimated by visually inspecting a random sample of 25 plants per plot (a total of 100 plants per site). On each plant, up to 50 aphids were counted, after which the number of aphids was estimated using a series of count ranges: 51-100, 101-500, 501-1,000, and 1,001-5,000. The high-end range was based on our field observations and past studies (DiFonzo and Hines 2002) that indicated aphid densities varied widely once populations surpassed several hundreds per plant but did not exceed 5,000 per plant during this period.

**LATENT Fungal INFECTIONS IN FIELD-COLLECTED SOYBEAN APHID.** In 2005 and 2006, prevalence of aphid pathogenic fungi infecting soybean aphid at each site on each sampling date was estimated by incubating field-collected live aphids in the laboratory. Aphid-infested leaves were collected from at least 20 soybean plants, taken randomly across the four plots. The aphids were kept cool while being transported to the laboratory. Up to 100 aphids were selected for incubation
from the entire leaf collection representing each site. The selection consisted of all individuals of the winged morph (alate adults), which were always found in small numbers or absent, and all or a portion of the wingless morphs (apterous adults and nymphs that were third instar or older). The selected aphids were dislodged from the leaves and incubated on fresh soybean leaflets encaged in 1-ounce plastic portion cups lined with 2% water agar (Nielsen and Hajek 2005). Aphids, in groups of five individuals per cage, were incubated at 21°C with a photoperiod of 16:8 (L:D) h. Because different rates of fungal infection between aphid morphs have been noted (Nielsen and Hajek 2005), winged morphs were monitored for infection separately from wingless morphs. Aphid mortality was checked daily for 4 d, and aphid cadavers were removed from the cages and positioned in a 5-mm gap between two microscope slides (Nielsen and Hajek 2005). The slides were kept under high humidity at 15°C overnight to promote sporulation of fungal pathogens from the aphid cadavers. Discharged conidia that adhered to the slides were mounted in a drop of 90% lactic acid, and the morphology of conidia was examined under the compound light microscope for identification of aphid pathogens (Balazy 1993, Humber 1997, Keller 1991).

Mean aphid density per plant was calculated for each sampling date and site using the midpoint of the count ranges and soybean plots as replicates (n = 4). Prevalence of fungal infection was estimated for each sampling date and study site as a percentage of aphids from which sporulation by aphid pathogens was observed. The calculation was carried out separately for winged and wingless aphid morphs using aphid collections across the entire study site. Aphids collected from different plots were pooled together to estimate infection rates because of patchy aphid distribution in the soybean field and scarce collection of winged aphids. The chi-square test for independence (Gomez and Gomez 1984) was used to compare percent infection between the two aphid morphs.

Active fungal infection of aphids in soybean and other crops. At the Hickory Corners site in 2004 and 2005, soybean, alfalfa, corn, wheat, and clover were sampled for aphids and sporulating aphid cadavers. Soybean, alfalfa, and corn were sampled from mid-June through early September. Wheat was sampled from mid-June through mid-July until harvest, and clover, which was an under-story crop in wheat plots, was sampled from late July through early September 2004. Clover was not available in 2005 due to frost kill in the spring. For each crop, a random sample of 25 plants was inspected per plot (a total of 100 plants for each crop type per sampling date). The entire plant above the ground was inspected, and counts of aphids and aphid cadavers were recorded for each aphid species. In 2005 (but not in 2004), aphid cadavers found were brought back to the laboratory for identification of any pathogen involved. Fungal sporulation from aphid cadavers was promoted under high humidity, and aphid pathogens were identified using the same methods used for fungal pathogens of soybean aphid (Nielsen and Hajek 2005). Two sampling methods were used to quantify infection in soybean aphid (rearing pathogens from field-collected aphids and cadaver inspection) to maximize ability to detect infection. Infection in all other aphid species was sampled using cadaver inspection to check for similarity of infection patterns in other crop-infesting aphids common to soybean production areas.

Mean densities of aphids and aphid cadavers per plant were calculated for each crop type, sampling date, and aphid species using plots as replicates (n = 4). Numbers of aphid cadavers were compared among crops by year of sampling, using analysis of variance (PROC GLM, SAS Institute 2004). The independent variables included in the model were crop type, sampling date, plot, and the interaction between crop type and sampling date. Crop type was considered as a fixed variable, and sampling date and plot were considered random variables. The cadaver counts (number of aphid cadavers per plant [X]) were transformed into a logarithmic scale (log_{10} [100X + 1/6]) to satisfy the assumption of normality for analysis of variance.
RESULTS AND DISCUSSION

Latent fungal infections in field-collected soybean aphid. Soybean aphid occurred at relatively high densities in 2005 (Fig. 1) and relatively low densities in 2006 (data not presented graphically, peak aphid densities per plant were 3.6 in Hickory Corners and 0.5 in East Lansing) at both soybean sites. Fungal pathogens infecting soybean aphid were detected on 1 August 2005 at both study sites during soybean pod development (Fig. 1). All cases of infection detected involved one pathogen, *P. neoaphidis*, which was also the dominant pathogen of soybean aphid detected in New York and Minnesota (Nielsen and Hajek 2005; K. Koch, personal communication). Additional aphid pathogens were detected in the New York study, which was likely due to higher sampling intensity, but *P. neoaphidis* was by far the dominant species. Our findings were also similar to those of Nielsen and Hajek (2005) in that the fungal infection was associated with high aphid densities late in the growing season (Fig. 1). In 2006 when aphid densities were very low at both study sites throughout the season, no infection was detected.

When fungal infection was detected on 1 August 2005, significantly greater proportions of alate adults were infected than the wingless morphs at both study sites (N = 100 aphids per site): 20% alate adults and 1% wingless morphs were infected at the Hickory Corners site ($\chi^2 = 67.40$, df = 1, $P < 0.01$; Fig. 1a), and 90% alate adults and 3% wingless morphs were infected at the East Lansing site ($\chi^2 = 203.13$, df = 1, $P < 0.01$; Fig. 1b). We acknowledge that sample sizes were limited for quantifying infection rates among the alate adults compared with wingless aphids. The uneven sample sizes between the two aphid morphs reflected the fact that alate adults were always rare relative to wingless morphs during this study (Fig. 1). Regardless of the uneven sample sizes, our results (significantly higher proportions of alate adults were infected compared with wingless aphids) were consistent at both of our study sites and with the study in New York (Nielsen and Hajek 2005). These results suggest that movement of winged aphids infected by *P. neoaphidis* may play a role in the onset of disease (Zhang et al. 2006).

Active fungal infection of aphids in soybean and other crops. At the Hickory Corners site, sporulating aphid cadavers were found in soybean, alfalfa, clover, corn, but not wheat during the soybean growing season (Fig. 2c,d) (only dates when cadavers were detected are shown; other sampling dates were 14 June and 15 July in 2004 and 14 June, 12 July, 25 July, and 31 August in 2005). Of six aphid species found infesting these five crops during the two year study (Fig. 2a,b), fungal sporulation was most often observed on cadavers of *Acyrthosiphon pisum* (Harris) (69 cadavers, 57 on alfalfa and 12 on clover), followed by cadavers of *Theroaophis trifolii* (Monell) (19 cadavers, 18 on alfalfa and one on clover), *Rhopalosiphum maidis* (Fitch) on corn (7 cadavers), *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) on corn (1 cadaver), and soybean aphid (1 cadaver). (Fig. 2 c,d). Two aphids, *Rhopalosiphum padi* (L.) on corn and wheat, and *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) on wheat, were detected but fungus-killed individuals were never found. The interaction between crop type and sampling date was significant in 2004 ($F = 8.47$; df = 13, 66; $P < 0.0001$) and 2005 ($F = 6.09$; df = 10, 54; $P < 0.0001$). In 2004, the numbers of cadavers found on alfalfa were greater than on clover and corn on 16 August, but there were no differences for other dates (Fig. 2c). In 2005, cadavers were only found on alfalfa on 27 June and 15 August (Fig. 2d). There were differences in plot sizes among crops (i.e., large plots for alfalfa and smaller plots for other crops), which could possibly contribute to differences in cadaver densities among crops. But greater numbers of aphid cadavers often
Figure 1. Mean soybean aphid densities (±SE) (lines) and percent infection found in winged (white bars) and wingless (black bars) aphid morphs monitored at two Michigan soybean sites in (a) Hickory Corners and (b) East Lansing during 2005. Percent infection was estimated on four dates. In 2006 (not shown), aphid densities were very low at both study sites, and no infection was detected. Percent infection was measured by incubating field-collected aphids (numbers of winged and wingless aphids, respectively, assessed are listed in brackets on top of graph). An asterisk over bars within a date indicates a significant difference in percent infection between winged and wingless aphids ($\chi^2$ test, $\alpha = 0.05$). Codes below the dates are soybean growth stages (Fehr and Caviness 1977). V, vegetative stages; R, reproductive stages.
Figure 2. Abundance of aphids (a, b) and sporulating aphid cadavers (c, d) among soybean, alfalfa, clover, corn, and wheat monitored in Hickory Corners, Michigan, in 2004 and 2005. Sampling dates on which cadavers were found on at least one crop are shown. Patterns of bar graphs indicate species composition of aphids (a, b) and aphid cadavers (c, d) in soybean (soy), alfalfa (alf), clover (clv), corn (crn), and wheat (wht). Different letters within a date in (c) and (d) indicate significant differences in cadaver abundance by Tukey’s test ($\alpha = 0.05$).
found in alfalfa appeared to be attributed to higher disease incidences associated with aphid species found in alfalfa (A. pisum and T. trifolii) and not to the larger plot sizes of alfalfa.

In 2005 when disease was identified from aphid cadavers, P. neoaphidis was associated with A. pisum on alfalfa, and Zoophthora sp. was associated with T. trifolii on alfalfa. Shah et al. (2004) found that A. pisum was highly susceptible to P. neoaphidis, and soybean aphid is also a suitable host of P. neoaphidis based on observations in this and other studies (Nielsen and Hajek 2005; K. Koch, personal communication). Zoophthora occidentalis (Thaxter) Batko (Entomophthorales: Entomophthoraceae) was infrequently detected from soybean aphid in New York (Nielsen and Hajek 2005) but was not observed in Minnesota (K. Koch, personal communication) or during the present study.

A few cadavers of R. maidis and M. euphorbiae were found on corn in 2004 (associated pathogens not identified) (Fig. 2c). In Idaho, P. neoaphidis and Conidiobolus spp. have been reported from these aphids (Feng et al. 1990). We did not detect diseased aphids on wheat. Although two aphid species (R. padi and S. avenae) were found infesting wheat, aphid densities were low (≤ 1.1 aphids per plant) from mid-June to mid-July when plants were mature and drying. Relatively low susceptibility to fungal infection has been previously reported for R. padi to P. neoaphidis (Shah et al. 2004).

There was a consistent under-reporting of fungal incidence by counting mycotized cadavers encountered in the field versus by the collection of living aphids that were incubated in the laboratory to allow development of any pathogens they carried. For instance, at the Hickory Corners site, infection was detected only by incubating field-collected aphids (Fig. 1a) and not by inspecting aphid cadavers (Fig. 2d). Similarly, Nielsen and Hajek (2005) observed consistently higher infection rates by incubating live aphids than collecting cadavers. In other systems involving different aphid species and crops, relative sensitivities of these sampling techniques varied widely (Nielsen and Hajek 2005). It seems prudent to utilize both methods when first assessing fungal pathogen infections of insect pests.

In conclusion, we observed fungal infection in soybean aphid populations (Fig. 1) with P. neoaphidis to be the most dominant aphid pathogen, but soybean aphid cadavers were rarely seen (Fig. 2c). Infections (as measured by percent infection of field-collected and laboratory-incubated aphids) were associated with high aphid densities late in the soybean growing season, and were primarily detected in the winged aphid morph. The same pathogen was the dominant species in other studies reported from the midwestern and eastern US (Nielsen and Hajek 2005; K. Koch, personal communication). The seasonal patterns observed in this and other studies implicate that fungi have the potential to disrupt annual life cycles of soybean aphid by infecting late season aphid populations, especially migratory populations. On the other hand, fungi may have limited potential for within-season control of soybean aphid. In our study, mycoses were also detected in aphids present on other crops common in Michigan soybean production regions. Pandora neoaphidis was associated with cadavers of A. pisum in alfalfa and clover during the same period when soybean aphid infection was detected. Because aphid-pathogenic fungi can infect a range of aphid species on different plants (Latgé and Papierok 1988, Nielsen 2002, Shah et al. 2004), and dispersal of infective conidia across a variety of habitats is the common pathway through which insect pathogens reach their hosts (Tanada and Kaya 1993), A. pisum on alfalfa and clover may be an important source for fungal propagules that infect soybean aphid. Overall, mortality of soybean aphid and other aphid species due to fungal infection was confirmed in Michigan. The results also implicate infected winged aphid morphs as potential agents for fungal dispersal, and A. pisum in alfalfa and clover as a source of fungal propagules for soybean aphid.
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LITERATURE CITED


Female Fighting and Host Competition among Four Sympatric Species of Melittobia (Hymenoptera: Eulophidae)

Robert W. Matthews¹ and Leif D. Deyrup²

Abstract

Melittobia is a genus of parasitic wasps well known for high levels of inbreeding and violent male combat. Casual observations of groups of sisters of M. femorata placed with hosts revealed a surprising incidence of body mutilations (broken or missing tarsi, antennae, and wings). Replicated conspecific groups of 1, 2, or 3 females of M. femorata, M. digitata, and M. australica and interspecific groups of M. femorata and M. australica (2:1) were observed over their first 10 days in newly established cultures, and the incidence of mutilation was recorded. In some groups females were dye-fed, allowing us to subsequently chart their individual activity patterns on or near the host based on patterns of their colored fecal droppings. For M. australica and M. digitata, no conspecific females in any group size ever showed mutilation. However, in M. femorata nearly 3/4ths of the females in conspecific groups of two or three acquired body damage beginning about the time of first oviposition on the host. In 4 of 5 replicates of the interspecific groups, M. femorata females killed the female of M. australica. Patterns of dyed fecal droppings that developed over several days showed that individual females in groups of both M. femorata and M. australica increasingly restricted their activities to a small portion of the host. These “micro” territories were non-overlapping and appeared to be actively defended. In contrast, M. digitata females in groups never displayed obvious territoriality or interference. Possible reasons for these differences in female behavior are discussed.

Melittobia are small external parasitoids that attack solitary bees, wasps, and their associates (Balfour-Browne 1922, Buckell 1928, Dahms 1983b, Krombein 1967). This cosmopolitan genus includes 12 species, some of which coexist geographically, often upon the same hosts (Matthews et al. 2009). Across eastern North America, a common shared host is the mud-dauber wasp, Trypoxylon politum Say (Hymenoptera: Sphecidae). Another common host sphecid, Sceliphron caementarium Drury, coexists with T. politum, but extends its range to include the western United States.

All Melittobia species appear to have a generally similar life history. Upon finding a host prepupa, the female parasitoid stings it and feeds upon exuded host fluids. This stimulates egg maturation and within 2 to 4 days, she begins to oviposit on the host; over the ensuing 10 days, she ultimately may lay hundreds of eggs in clusters on individual large hosts.

Most of these eggs develop into female offspring, either of an early brachypterous form or a later macropterous dispersal form (Schmieder, 1933, Cônsoli and Vinson 2002). Males, which generally comprise 5% or less of the offspring, emerge at a low but continuous rate throughout female emergence (Adams

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Before dispersing, the females mate with these males, which are very likely brothers; thus, inbreeding appears to be the usual situation.

Males of *Melittobia* are known for their lethal combat (Hamilton 1979, Hartley and Matthews 2003, Deyrup et al. 2006, Innocent et al. 2007). Females, however, are generally considered docile and even supportive of one another; for example, *M. digitata* females non-aggressively queue up to await male courtship and cooperate with one another to chew out of the host’s nest. (Donovan 1976, Deyrup et al. 2005). This female docility may not be the rule, however. Much of the available information on *Melittobia* is based on research conducted with this one species that is sold under the name “WOWBug” (Carolina Biological Supply Co., Burlington, NC). Because of its ready availability and ease of rearing on artificial hosts such as the common blowfly, *Sarcophaga (Neobellieria) bullata, M. digitata* is becoming a model organism for laboratory and classroom work. However, two less-studied species, *M. femorata* Dahms and *M. australica* Girault, are actually the most commonly collected *Melittobia* species on *T. politum* in the southeastern United States (J.M. González and R.W. Matthews, unpublished data). Also sympatric but generally more northerly and less widespread is *M. acasta* (Walker), which may have been accidentally introduced from Europe by way of Canada at least 40 years ago (González et al. 2004).

The extent of parasitoid competition in arthropod communities is unresolved, but thought to be widespread (Godfray 1994, Hawkins 2000), especially since multiple species often attack the same host. Competition may be manifested in various ways and at different times in the parasitoid-host interaction. Both interference and exploitative competition can occur and there are numerous examples, especially from the biological control literature (Hawkins 2000). Several parasitic wasps have been reported to defend a host resource, their eggs, or their offspring from conspecifics (e.g., Field and Calbert 1999, Hardy and Blackburn 1991, Wilson 1961). Interactions among female parasitoids often are mediated via chemical markings that appear to deter conspecific females from superparasitism (Hoffmeister and Roitberg 1997, Petersen and Hardy 1996). Among host searching female ectoparasitoids, competition between congeneric species has been little studied.

In our laboratory on various occasions we have noted both intra- and interspecific aggression, body damage, and death when combinations of *Melittobia* females have been placed on a common host. Field collections of host *T. politum* cocoons have revealed natural multiparasitism by two or rarely three *Melittobia* species on at least five occasions: three from Georgia, and one from both Alabama and New York (González and Matthews, unpublished data). Thus, to better understand competitive interactions among host-seeking females we undertook the studies reported here.

**MATERIALS AND METHODS**

All four *Melittobia* species were originally obtained from parasitized cocoons of the mud dauber wasp, *Trypoxylon politum* Say (Hymenoptera: Sphecidae). The *M. femorata* stock originated from Arnoldsville, Oglethorpe Co., GA; *M. digitata* from Athens, Clarke Co., GA; *M. australica* from Gainesville, Alachua Co., FL; and *M. acasta* from Townsend, Blount Co., TN. Prior to this study, laboratory cultures of each species had been continuously maintained from one to four years at the University of Georgia. Reculture protocol for each new generation was to haphazardly select five mated females of unknown age and place them on a naked *T. politum* prepupa in small vials maintained in a dark incubator at 25°C. New cultures were established every 21 days except for *M. femorata* whose reculture cycle varied from 90-120 days.

All experiments and controls used 1 to 2-day-old mated females that had eclosed from a single stock culture of each species. As hosts for these parasitoids,
we used naked *T. politum* prepupae extracted from local field-collected nests and individually placed in small plastic boxes (50 mm × 25 mm × 18 mm, Carolina Biological Supply Co., Cat. No. ER-14-4584). Experiments were conducted in the same individual plastic boxes and were maintained in a constant-temperature chamber at 25°C.

For some studies, we marked individual females by feeding them 20% fructose and water dyed with McCormick® food coloring. After females imbibe this fluid, it is easily visible in their crops through their semi-translucent cuticle (see Matthews et al. 2009); different colors served to identify individual females. In addition, because the color is retained in the female’s fecal matter, this technique allowed us to track each female’s activity through the pattern of her fecal droppings on the floor of the plastic box.

**Female competition in *M. femorata***. In 28 boxes, mated 2-day-old unfed *M. femorata* females of the long-winged morph were concurrently placed with individually boxed *T. politum* prepupae in the following design: A single female in 6 boxes, 2 females in 13 boxes, and 3 females in 9 boxes. Boxes were maintained at ambient room temperatures and checked daily over the following 10 days, noting the females’ behavior and recording any body damage. In order to track individual females and their movements, 15 additional cultures were established with 3 females of *M. femorata* marked by dye-feeding as outlined above.

**Interspecific competition in *M. femorata* and *M. australica***. To determine how *M. femorata* fared when confronted with another species on the host, we set up five boxes containing one dye-fed *M. australica* and two dye-fed *M. femorata*. These boxes were observed daily for 10 days and body damage and fecal dropping patterns were recorded. For comparison with intraspecific competition between individuals, we concurrently set up 20 boxes of three dye-fed *M. digitata* females and 20 boxes of three dye-fed *M. australica* females; *M. acasta* was unavailable for this comparison.

**Female competition in *M. digitata*, *M. australica*, and *M. acasta***. To further examine these interactions, a subsequent experiment used unfed females in a design that examined inter- and intra-specific interactions in three *Melittobia* species by comparison with solitary females. Three treatments placed two females of different species on a naked *T. politum* host (average weight = 0.253g ± 0.060 SD) in the 3 possible combinations: *M. digitata* vs. *M. acasta*, *M. digitata* vs. *M. australica*, and *M. australica* vs. *M. acasta*. Another three treatments placed conspecific pairs of each of the three *Melittobia* species. Controls consisted of cultures of each species established by a single female. Each treatment and control was replicated 10 times. *M. femorata* was not available for these comparisons.

Each treatment replicate and associated control was checked daily for the first 8 days, then twice weekly for the next 10 days, noting oviposition, feeding, and “jousting.” At day 18 all emerged adults were sexed and counted to assess the effects of inter- and intraspecific competition on fecundity and reproductive success relative to solitary foundress control cultures of each species at the same stage.

**RESULTS**

**Intraspecific female competition**. In the treatments containing three dyed *M. femorata* females, 1 to 4 days after being placed on a host the females’ activities became increasingly localized, each focused upon a particular portion of the host’s body. From the distribution patterns of dyed fecal droppings it was apparent that each female *M. femorata* was developing a more or less exclusive “micro” territory (Fig. 1), and that the boundaries between them were relatively distinct. Undyed females in the groups of two or three in the other set of cultures
appeared to behave similarly. Females of *M. australica* also displayed similar territoriality in all 20 cultures. However, fecal droppings of *M. digitata* females displayed no grouping pattern in any of the replicates.

During the course of oviposition (roughly days 2-10), the frequency of aggression and incidence of body mutilation (manifested as missing tarsomers and antennal flagellomeres and tattered and broken wings) increased among groups of *M. femorata* females. We regularly observed females biting at other females and even rolling around in locked combat (Fig. 2). In addition, many females were noted to walk about with their wings raised as though damaged. Normally, wings are held flat over their abdomens.

At least one female with damage occurred in every replicate (9/9 for groups of three females and 13/13 for groups of two females), and in several replicates all females in a group exhibited some type of body damage (Table 1). Overall, 16 of the 25 females in the foundress pairs replicates and 20 of the 25 females in the three foundress groups had body damage.

By contrast, none of the females in any of the 20 groups of three *M. digitata* or *M. australica* acquired body damage over the 10-day period. Periodic observation revealed no indication of agonistic interactions among females of *M. digitata*; however, while never overtly hostile, individual *M. australica* were sometimes seen to follow or approach other females on the host and appeared to disturb the other female with proximity or nudging.

**Progeny production.** In the final experiment, counts of adult progeny as of day 18 indicated that among both the single female control and the two conspecific female cultures, *M. digitata* was the most prolific, followed by *M. acasta* and *M. australica* (Table 2). Pair-wise interspecific comparisons of the average numbers of progeny produced showed that *M. acasta* outperformed both of the other two species when in direct competition, and that *M. digitata* did better than *M. australica*. However, *M. australica* was significantly less productive than either competitor (Tables 2 and 3). This contrasts to the intraspecific competition results where no significant differences in total progeny production were found between single female and two female cultures (Tables 2 and 3) though the variance in all experiments was great and the number of replicates relatively few.
Fig. 2. Two egg-laden female *M. femorata* locked in combat. Although these encounters do not tend to be lethal, females often mutilate one another.

Table 1. Incidence of damage among cofoundresses of *Melittobia femorata* in different sized foundress groups during the first 10 days of their being simultaneously placed with a *Trypoxylon politum* prepupa host.

<table>
<thead>
<tr>
<th>Initial No. of females</th>
<th>No. of replicates with female damage/Total No. of replicates</th>
<th>Total No. of females*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With body damage</td>
</tr>
<tr>
<td>1</td>
<td>0/6</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>13/13</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>9/9</td>
<td>20</td>
</tr>
</tbody>
</table>

*Discrepancy in total numbers due to loss of three females that escaped or were accidentally killed.
Table 2. Numbers of *Melittobia* emerging by day 18 from each interspecific, intraspecific, and single female treatment on *Trypoxylon politum* prepupae. Values are means ± S. D.

<table>
<thead>
<tr>
<th>Treatment (N)</th>
<th><em>M. acasta</em></th>
<th><em>M. australica</em></th>
<th><em>M. digitata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Total</td>
</tr>
<tr>
<td><em>M. acasta</em> + <em>M. australica</em> (10)</td>
<td>230.8 ± 54.4</td>
<td>17.3 ± 6.3</td>
<td>248.1 ± 56.0</td>
</tr>
<tr>
<td><em>M. australica</em> + <em>M. digitata</em> (10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. acasta</em> + <em>M. acasta</em> (9)</td>
<td>397.7 ± 181.5</td>
<td>15.7 ± 5.9</td>
<td>413.3 ± 181.2</td>
</tr>
<tr>
<td><em>M. australica</em> + <em>M. australica</em> (9)</td>
<td>269.9 ± 140.2</td>
<td>6.6 ± 1.3</td>
<td>276.4 ± 141.1</td>
</tr>
<tr>
<td><em>M. digitata</em> + <em>M. digitata</em> (8)</td>
<td>339.6 ± 133.7</td>
<td>8.0 ± 2.5</td>
<td>347.6 ± 133.6</td>
</tr>
</tbody>
</table>
Female competition: *M. femorata* and *M. australica*. In the mixed species cultures, *M. australica* often appeared to pressure a female of *M. femorata* to abandon her territory, and in some instances caused her to move completely off of the host early in their association. However, after the *M. femorata* became physogastric (abdomens swollen with eggs), the tables turned, and in four of the five replicates the *M. australica* female exhibited damage and was eventually decapitated. In only one case did *M. femorata* and *M. australica* appear to share the same area on the host, with no evidence of any body damage.

Interestingly, in the cultures co-housing *M. femorata* and *M. australica* females, the onset of microterritoriality in *M. femorata* seemed to be delayed (3-5 days after being placed on host) relative to its onset for a single foundress; unfortunately, small sample sizes obviate firm conclusions.

Female competition: *M. acasta* and *M. australica*. In 8 of the 10 replicates, apparent signs of fierce and fatal competition were observed in females of both species within six days after introduction upon the host. Evidence of battles included damaged heads, broken and missing tarsi, tattered wings, and immobility. By 10 days the *M. australica* female was killed by *M. acasta* in 7 replicates, resulting in the very low numbers of progeny realized by *M. australica* (Table 2). In the three remaining replicates in which battles were not extreme enough to lead to immobility or death, both species nonetheless showed signs of struggle.

**Female competition: *M. digitata* and *M. australica*.** Based on daily observations, *M. australica* appeared to dominate over *M. digitata* during the first 12 days of the study, as *M. digitata* suffered more injuries and mortality (The *M. digitata* female was apparently killed in 2 replicates during first 10 days; in one other replicate both females were found dead after 4 days with no evident body damage to either). In the remaining 7 replicates both females survived with no injuries or evident aggression, though the daily checks revealed that the *M. australica* female was more often on the host. However, by the measure of number of adult females produced by day 18 of the study (Table 2), *M. digitata* dominated with significantly more progeny by every measure (Table 3).

Table 3. Statistical comparisons of progeny production of three *Melittobia* species in the inter- and intraspecific experimental treatment groups. *P* values are for two sample assuming unequal variance t-test (2-tailed).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Comparison</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interspecific</td>
<td><strong>australica &amp; digitata</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total digitata vs. ave. of 2 digitata</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Total australica vs. ave. of 2 australica</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Total of both vs 2 australica</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>Total of both vs 2 digitata</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td><strong>australica &amp; acasta</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total acasta vs ave. of 2 acasta</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>Total australica vs ave. 2 australica</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Total both vs 2 australica</td>
<td>0.913</td>
</tr>
<tr>
<td></td>
<td>Total both vs 2 acasta</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td><strong>digitata &amp; acasta</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total acasta vs ave. 2 acasta</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>Total digitata vs ave. 2 digitata</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>Total both vs 2 digitata</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>Total both vs 2 acasta</td>
<td>0.169</td>
</tr>
<tr>
<td>Intraspecific</td>
<td><em>M. digitata</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single female vs 2 females</td>
<td>0.268</td>
</tr>
<tr>
<td></td>
<td><em>M. australica</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single female vs 2 females</td>
<td>0.437</td>
</tr>
<tr>
<td></td>
<td><em>M. australica</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single female vs 2 females</td>
<td>0.120</td>
</tr>
</tbody>
</table>
Female competition: *M. digitata* and *M. acasta*. When *M. digitata* and *M. acasta* shared a host, no aggression or body damage was observed between the females during the first 10 days. Both species realized high adult progeny production, averaging over 200 for *M. digitata* and nearly 300 for *M. acasta* (Table 2) and not significantly different from that realized in intraspecific competition (Table 3). Interestingly, in the heavy fighting that was observed between emerging males of these two species, *M. acasta* dominated, killing most *M. digitata*.

**DISCUSSION**

Territoriality has been widely documented in insects; however, much of the literature focuses on males in various forms of intrasexual selection (Baker 1983). Territoriality or intense intraspecific competition involving partitioning and defense of resources among conspecific female insects is relatively uncommon in most insect groups, but has been recorded for some tephritid flies (Diptera: Tephritidae) (Prichard 1969, Shelly 1999), water striders (Hemiptera: Gerridae) (Nummelin 1988), aphids (Hemiptera: Aphididae) (Inbar 1998), and web spinners (Embioptera) (Brando and Joseph 1970). Egg-brooding females of an African arachno philic embiid viciously attacked experimentally-introduced conspecifics and at times succeeded in “plucking a leg or few antennal segments off the intruders” (Brando and Joseph 1970). Among the Hymenoptera, both ants (Formicidae) (Hölldobler and Wilson 1990) and parasitic wasps (Chalcidoidea) (Griffiths and Godfray 1988) often establish and defend foraging territories. Some parasitic wasps have been reported to defend a host resource, their eggs, or their offspring from conspecifics (Field and Calbert 1999, Hardy and Blackburn 1991, Wilson 1961).

Under field conditions, dispersing *Melittobia* females are temporally and spatially clumped, and usually crawl rather than fly to locate hosts (Freeman and Ittyeipe 1976). Potential hosts also may be clumped and persistent in favored locations. Thus, multiple parasitism is probably a rather common phenomenon. Molumby (1996), for example, found 1 to 5 (mean = 1.8) *M. femorata* females per host in midsummer *T. politum* nests in Mississippi. Some sort of response to such encounters would be warranted, and could be expected to differ for each species (and combination thereof).

Despite superficial similarities in host and lifestyle and overlapping geographic ranges, the behavior and life history of the four species in this study all differ from one another in significant ways; *M. femorata* in particular is not a typical member of its genus (Matthews et al. 2005, Matthews and González 2008). In addition to two distinctly separated non-overlapping adult generations on a single host, it shows striking differences in life history and morphology (Matthews and González 2008). Distinctly smaller than the other species, *M. australica* might be predicted to lose out in more interspecific battles, as in fact it did (Tables 2 and 3); interestingly, it also is the only species among those studied that does not belong to the *acasta* group of Dahms (1983a). The contrast between such an extreme degree of intraspecific female pugnacity in *M. femorata* and *M. acasta*, and its absence in *M. digitata* and *M. australica* was unexpected, particularly since *M. digitata*, *M. femorata*, and *M. acasta* are thought to be closely related and were placed in the same species group by Dahms (1984a) on the basis of morphology.

**Why should females of *M. digitata* and *M. australica* tolerate conspecifics?** Their communal oviposition is clearly facultative, since a single female has the ability to produce large numbers of eggs sufficient to fully consume the host upon hatching. Perhaps any disadvantages are outweighed by benefits accruing to larvae or the mixing of broods. Genetic studies could be enlightening.

One should not discount the possibility that the context in which we observe these interactions is not the same as the one in which the pugnacity
evolved. While mud-dauber wasps are commonly assumed to be the principal host of all these species, this could be simply a sampling bias brought on by the conspicuous nature of the highly visible, long-lasting nests. In addition, while today’s high mud dauber nest densities provide a good likelihood that two or more female Melittobia emerging from the same clutch may jointly colonize a nearby host, this phenomenon may be relatively recent, an artifact of human activities such as bridge and barn building. Perhaps other solitary bees and wasps were the principal original hosts for the four Melittobia species, such that each species’ fundamental behavioral ecology and selection pressures may have been very different from that carried over into the laboratory from mud dauber nests.

For M. digitata and M. australica, one laboratory study has compared progeny production of groups of one to five conspecific females given a single blowfly host (Silva-Torres and Matthews 2003). While absolute numbers from this smaller artificial host cannot be directly compared to our results, the relationships would be expected to be similar. In that study, as in ours, both alone and with up to five females of their own species, M. digitata produced more offspring than M. australica for every group size. Offspring of both species developed slightly faster when in competition than under sole foundress conditions.

Given that multiple foundresses of M. femorata readily attack one another on a new potential host, it is interesting to note that newly mated M. femorata females cooperate to chew a common exit hole (Deyrup and Matthews 2007a), just as M. digitata do (Deyrup et al. 2005). Comparing the behaviors of host feeding and cooperative escape-chewing in M. digitata, Deyrup and Matthews (2007b) found they were very similar, and suggested that the two behaviors have a similar biological basis. In M. femorata we have the seeming contradiction of a species in which cooperative chewing for escape and aggressive interactions coexist; it may be instructive that aggression only occurs when oviposition commences, days after host feeding has occurred.

While there appear to be no published papers on interspecific female competition in Melittobia, it most likely occurs in nature. As noted above, we have on occasion found females of 2 (and once, 3) species in a single mud dauber cocoon. This observation suggests that females’ host-searching behavior must be somewhat flexible, and that both inter- and intraspecific host sharing does occur. Whether host sharing females can somehow assess a competitor’s size and/or reproductive status and make conditional decisions about whether to stay or leave remains to be studied. Our laboratory experiments were admittedly artificial in that both females were simultaneously introduced to the host and had no opportunity to leave to search for another. In nature, two females would most likely arrive at different times, giving one a head start, and later arrivals would have a fight-or-flee option.

What selects for one species to behave aggressively, but not another? Genetic analysis of female relatedness, experimental manipulation of host searching cues and discovery context, and further life history research may ultimately lead to answers. Certainly one could hardly ask for a more amenable group than Melittobia with which to address that question; these four sympatric parasitoids are commonly found, easily reared, readily manipulated, and appear to display a continuum of aggressive interactions in both sexes, promising that such further study will be both agreeable and rewarding.

ACKNOWLEDGMENTS

Funding that supported part of this research was provided by a National Science Foundation grant to R. W. Matthews. We thank Kathryn Hauth who first noticed the mutilation occurring among groups of M. femorata females during an undergraduate independent study. We thank Joe R. Williamson, Aubrey Roche, and Rachel Bodiford for laboratory assistance.
LITERATURE CITED


The alfalfa snout beetle, *Otiorhynchus ligustici* L., is the most serious pest of alfalfa in northern New York State. Recent research efforts focused on the biological control of this insect require the availability of all life stages. With a 2-year lifecycle and a mandatory diapause, the artificial rearing of a laboratory culture appears to be a non-viable option at present, but methods described here can be used to obtain sufficient numbers of eggs and larvae over an extended period of time for research purposes. The crowding of adult beetles in egg production units (cups) had a significant, negative effect on egg production per beetle but the total egg production per cup was still higher with higher number of beetles per cup resulting in a significant saving of labor per egg produced. Larval survival rates in alfalfa-planted cans were surprisingly low given the protected conditions of the greenhouse. The larval survival rates were not significantly different among the dates for the second instar and later instars, suggesting that larval mortality occurs in the first instar in alfalfa-planted cans.

The alfalfa snout beetle, *Otiorhynchus ligustici* L. (Coleoptera: Curculionidae), was introduced into the United States from Europe via wooden sailing ships carrying soil as ballast (Lindroth 1957, York et al. 1971). The beetle was first recorded in New York State in 1896 at the Port of Oswego and was first recorded as a pest of alfalfa when alfalfa was introduced into the area in the 1920s (York et al. 1971). In subsequent years, this flightless and parthenogenetic insect has spread to nine Northern New York counties, infested over 200,000 hectares of cropland and has become the most serious pest of alfalfa in northern New York State (Schroeder et al. 1994, Ferguson et al. 1995, Shields et al. 1999). The larvae feed on the lateral roots and later on the tap roots of the host plants. Alfalfa snout beetle has a 2-year lifecycle. The biology and life history of alfalfa snout beetle has been studied and described by several authors in Eurasia and North America (Vassiliev 1914 in York 1974, Lincoln and Palm 1941, Hanuss 1958, Nyilas 1962, York 1974, Jermy and Balázs 1990) and is very similar throughout Europe and Northern New York. Most larvae mature by late fall and move down in the soil to varying depths depending on soil type, temperature, and other factors. Mature larvae remain quiescent deep in the soil for ca. 8 months before pupation the following summer. After eclosion, adults remain in the pupal cells and only move to the soil surface the following spring after spending the second winter in the soil (Lincoln and Palm 1941). Adults start moving up to the soil surface when spring soil temperatures warm to 3°C and appear on the surface from late April to early May throughout the geographical distribution in the US. After reaching the surface, adults feed on the available host plants after which oviposition commences (Schroeder et al. 1995).

Research efforts focused on this insect require the availability of all life stages for an extended period of time; usually large numbers of individuals are needed. With a 2-year lifecycle and a mandatory diapause, the artificial rearing of a laboratory culture appears to be a non-viable option. However, the adults...
can be collected in the spring in large numbers and placed in cold storage at 4°C for 4 months without significant death, extending the adult availability from 3 weeks to 4 months. Adults will survive in cold storage for 4 months with low mortality and a few individuals will survive in cold storage for up to 9 months (EJS, personal observation). Adults can be removed from cold storage, fed and used to produce eggs. The purpose of this report is to illustrate a series of economically cost effective procedures to produce eggs, collect eggs and rear larvae to predetermined stages using alfalfa plants in containers. These techniques are currently being used in ongoing research projects focused on biological control and developing a snout beetle-resistant alfalfa.

MATERIALS AND METHODS

Egg production. Alfalfa snout beetle adults were field collected in late April 2006 near Great Bend, NY, immediately after emergence. The adult beetles were kept in cold storage (4°C) for 4 weeks on moist filter paper. The beetles were then placed into paper cups (82 mm diameter, 70 mm depth). The cups contained a layer of approximately 1 cm of autoclaved soil to encourage egg laying. The soil was sifted through an 18 mesh screen (1 mm opening) to remove the large particles. The soil was lightly moistened as needed. Fresh alfalfa foliage was provided to the beetles every other day as food.

The impact of adult crowding on egg production was investigated by placing adult females singly, two, four, eight, or 16 beetles/cup. Each treatment had 30 replicates. The cups were monitored daily and eggs were collected 6 and 12 days after the detected onset of oviposition. The total number of eggs was recorded for each cup. The average egg production per beetle in cups was calculated by dividing the total number of eggs by the number of beetles in the cup. A regression analysis was conducted with the number of beetles in a cup being the independent factor and the per beetle egg production being the response. Because of the heteroskedasticity of the data, the weighted least-square method was used.

Egg collection. The soil from the cups was rinsed in a 60-mesh screen (250 micron openings) to separate the eggs and large soil particles from the small soil particles. The remaining soil and eggs were placed into 300 ml of a 40% sugar solution in a 500 ml glass beaker. Eggs were suspended in the solution or floated to the surface, while the majority of the soil sank to the bottom of the beaker. The soil on the bottom of the beaker was stirred gently with a plastic stirring rod to free any eggs trapped in the soil. The egg-sugar solution was then decanted from the beaker through a 60 mesh sieve that retained the eggs. Eggs were then washed off the sieve with water into a 50 ml beaker. In water, the eggs sank to the bottom of the container while the remainder of the debris (frass, leftover plant material) floated to the top and could be simply decanted leaving a small volume of the water on the bottom of the beaker with the eggs. The eggs were then poured onto a filter paper in a filter funnel. The eggs were surface sterilized with a 5% bleach solution by pouring the bleach solution over the eggs. After one minute, the eggs were immediately rinsed with deionized water.

Egg hatch rate. Eggs from adult beetles were collected using the sugar flotation method described above. Three different methods of egg incubation were used: 1) control: 100 eggs were rinsed in deionized water and placed on a moist filter paper in a Petri dish (10 cm diameter); 2) treatment 1: 100 eggs were suspended in a 0.5% agar solution (10°C temperature) and left undisturbed for one hour; they were then pipetted off onto a filter paper to drain the excess agar and were placed onto a second filter paper moistened with deionized water in a Petri dish; 3) treatment 2: a Petri dish was filled with Cornell mix (consisting of 1:2 peat moss-vermiculite mixture). One hundred eggs were suspended in 0.5% agar solution (10°C temperature) and left undisturbed for one hour. Eggs were
then placed onto a filter paper to absorb the excess moisture. The eggs were then moved from the filter paper using a soft brush onto the surface of potting soil. These Petri dishes were left uncovered and were lightly moistened daily with a water sprayer bottle.

All eggs were incubated at 23°C in the dark. The number of eggs hatching was determined daily for a total of 14 days and the proportion of eggs hatching was recorded. Each treatment was replicated four times. Proportional data was transformed using arcsine square root transformations and then mean hatch rates in the different treatments were compared using ANOVA and Tukey’s HSD procedure. Statistical analyses were conducted using SAS™ system for Windows™, release 8.02 (SAS Institute Inc., Cary, N.C.).

Inoculation of alfalfa plants with eggs. Alfalfa plants were grown in plastic waste paper cans (21 cm width, 27 cm length, and 33 cm depth). The waste paper cans had four small holes (approximately 1 cm diameter) drilled in the bottom to allow for water drainage during watering and the bottom of the cans was lined with fine mesh fiberglass window screen to prevent the escape of larvae from the cans. The cans were then filled with Cornell Mix. Alfalfa seeds were planted in the cans and allowed to grow and establish for 6 weeks before being inoculated with snout beetle eggs.

Alfalfa snout beetle eggs were collected as described above and suspended in 0.5% agar solution and cooled to 10°C at a concentration of 5 eggs/ml. The eggs remained in the agar solution for approximately one hour before application into the soil around the plants. The use of the dilute agar solution thickened the liquid so the eggs remained suspended without gravitational settling. Each can was inoculated with 500 eggs by spreading 100 ml of egg suspension on the surface of the potting soil. A subset of the eggs used to inoculate the cans was returned to the laboratory and monitored for hatching. The inoculated cans were incubated in a growth chamber at a constant temperature of 24°C. A total of 22 cans were inoculated.

Alfalfa snout beetle larval survival in the cans. Larvae were recovered from the cans by breaking down the cans and sifting the soil at eight different times after inoculation: 22 days (three cans), 28 days (three cans), 35 days (two cans), 40 days (four cans), 49 days (three cans), 54 days (two cans), 60 days (two cans), and 68 days (three cans). The larval instars were determined by measuring the width of the head capsule of the larvae. The presence of 1st instar larvae was not tabulated due to the small size of the instar and the difficulty of accurately counting the larvae accurately. The proportion of surviving alfalfa snout beetle larvae was calculated by dividing the number of recovered larvae by 500 (the number of eggs inoculated). Proportional data was transformed using arcsine square root transformation and then mean survival rates at the different times were compared using ANOVA and Tukey’s HSD procedure.

RESULTS AND DISCUSSION

Egg production. The mean egg production per beetle was the highest, 82 ± 9.5 (mean ± SE), when only a single beetle was present in a cup and the lowest, 43.1 ± 1.9, when 16 beetles were in the same cup. The mean egg production per beetle was 66.1 ± 5.9 with two beetles in the same cup, 62.3 ± 3.0 with four beetles, and 51.5 ± 2.9 with eight beetles in a cup. There was a negative linear relationship ($F = 35.25$, df = 1, 148, $P < 0.000$, $r^2 = 0.19$) between the number of beetles in a cup and the per beetle egg production (Fig. 1). The mean total egg production per cup was the highest, 689.1 ± 31.1, with 16 beetles in a cup and the lowest, 82.0 ± 9.5, with a single beetle per cup. The mean total egg production per cup was 132.10 ± 11.70 with two beetles in the same cup, 249.1 ± 11.9 with four beetles, and 412.1 ± 23.5 with eight beetles in a cup. The high egg production variability which is the most obvious in the single insect per
cup data is typical of this parthenogenetic species, where egg production from individual insects commonly range from zero to several hundred per individual (Lincoln and Palm 1941, York 1974).

It appears that the crowding of the beetles results in decreased oviposition per beetle but it is not known whether this effect would be observed over the entire lifetime of the beetle. Since we were mostly interested in the efficiency of harvesting the eggs, only the first 12 day period was investigated. It is not clear why adult beetles would decrease their egg production when crowded, but one possible explanation could be that in natural conditions, oviposition under crowded conditions increases the intraspecific competition for larval resources.

Our main objective was to produce a large number of eggs efficiently. Although crowding the beetles (16 per cup) resulted in reduced per beetle egg production compared to the single beetle per cup, the total egg production per cup was still much higher, resulting in a significant saving of labor per egg produced. If the availability of beetles is not the limiting factor, then crowding the beetles is the most labor efficient method. However, if beetle availability is an issue, egg production is the greatest when a single beetle is caged individually.

**Egg hatch rate.** The percentages of hatching eggs were $47.0 \pm 3.2\%$ in the control, $51.0 \pm 4.6\%$ in treatment 1, and $46.5 \pm 2.5\%$ in treatment 2. Other researchers have reported a similar hatch rate ranging between 50-60%
The proportions of hatching eggs among the three different incubation methods were not significantly different ($F = 0.48$, df = 2, 9, $P = 0.631$). Therefore, we conclude that the sucrose-flotation method had no adverse effects on snout beetle egg hatching rate. Suspending insect eggs in agar solution with a specific density is a widespread method for insect egg applications, because the eggs neither sink nor float on the surface of the suspension so the rate of application is even (Fery et al. 1979, McEwen 1996, Abel et al. 2000).

**Larval survival.** The survival of alfalfa snout beetle on alfalfa plants grown in waste paper cans ranged between $3.13 \pm 0.07\%$ and $4.20 \pm 0.20\%$. Survival rates did not change significantly with increasing time since inoculation ($F = 0.75$, df = 7, 14, $P = 0.638$) (Table 1.). The hatching rate we observed did not differ from early research conducted on snout beetle (Lincoln and Palm 1941). The larval survival rates were surprisingly low given the protected conditions of the greenhouse but were higher than reported by Schroeder et al. (1994). The cans had sufficient alfalfa plants to support a higher number of larvae with a large root mass in every container, so food availability was not considered a limiting factor. It would appear that a large amount of larval mortality occurs from the 1st instar larvae failing to find a root as a food source shortly after hatching.

**ACKNOWLEDGMENTS**

We thank the Northern New York Agricultural Development Program for their long-term financial support for research on alfalfa snout beetle. Without their support, little research on this invasive insect would be attempted or completed. We thank Luanne Belgodere for her help in the laboratory.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>2nd instar</th>
<th>3rd instar</th>
<th>4th instar</th>
<th>5th instar</th>
<th>6th instar</th>
<th>% survival</th>
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<td>3.1 ± 0.1</td>
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<td>3.8 ± 0.6</td>
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<td>11.0 ± 1.0</td>
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<td>2.00 ± 2.00</td>
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</table>

We thank the Northern New York Agricultural Development Program for their long-term financial support for research on alfalfa snout beetle. Without their support, little research on this invasive insect would be attempted or completed. We thank Luanne Belgodere for her help in the laboratory.
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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. Swanson1, 2 and Paula K. Kleintjes Neff1

ABSTRACT

Lycaeid 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) (Blesser 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), *Pirophorum* (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 quadrant. The same researcher (JS) made this estimation before the beetles arrived, during the beetle
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 × 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 × 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 (± SD) number of beetles per lupine stem was 2.0 (± 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 (± SD) percent cover of lupine per quadrat before the beetles arrived was 31.8 (± 1.3) % (Table 3) and declined to 7.8 (± 0.5) % by 10 June. Before the beetles appeared, the mean (± SD) number of stems with flowers per clump was 16.2 (± 8.5) which declined to 2.5 (± 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.

<table>
<thead>
<tr>
<th></th>
<th>Females (n=14)</th>
<th>Males (n=14)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>East</td>
<td>West</td>
</tr>
<tr>
<td>Before</td>
<td>92.5</td>
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<td>During</td>
<td>87.6</td>
<td>12.3</td>
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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).

<table>
<thead>
<tr>
<th></th>
<th>% Time Perching</th>
<th>% Time Flying</th>
<th><em>P. Simplex</em></th>
<th><em>L. perennis</em></th>
<th>Other</th>
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<tr>
<td>Female</td>
<td>Before</td>
<td>90.7</td>
<td>9.3</td>
<td>21.9</td>
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<td>1.0</td>
<td>65.4</td>
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</tr>
<tr>
<td></td>
<td>During</td>
<td>92.9</td>
<td>7.1</td>
<td>11.0</td>
<td>2.0</td>
<td>1.3</td>
<td>48.0</td>
<td>7.6</td>
<td>16.6</td>
<td>5.3</td>
<td>7.7</td>
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<td>Male</td>
<td>Before</td>
<td>53.3</td>
<td>46.7</td>
<td>44.3B</td>
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<td>1.2</td>
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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.

<table>
<thead>
<tr>
<th>Q</th>
<th>¹Mean % of <em>L. sayi</em> population</th>
<th>²% Cover <em>P. simplex</em></th>
<th>³% Cover <em>L. perennis</em> 6 June</th>
<th>⁴% Cover <em>L. perennis</em> 11 June (n=26)</th>
<th>⁵% Larval damage sites before <em>L. sayi</em> (n=63)</th>
<th>⁶% Larval damage sites during <em>L. sayi</em></th>
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<tbody>
<tr>
<td></td>
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<td>S</td>
<td>N</td>
<td>S</td>
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Table 3. Continued.

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<th>1Mean % of L. sayi population</th>
<th>2% Cover P. simplex</th>
<th>3% Cover L. perennis 6 June</th>
<th>perennis 11 June (n=26)</th>
<th>4% Cover L. damage sites before L. sayi (n=63)</th>
<th>5% Larval damage sites during L. sayi</th>
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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);
4 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.

ACKNOWLEDGMENTS

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SECONDARY PREDATION ON THE HORSEHAIR WORM
GORDIUS ROBUSTUS (NEMATOMORPHA: GORDIIDAE)

Philip A. Cochran

ABSTRACT

The gut contents of a brown trout (Salmo trutta) included horsehair worms (Gordius robustus, Nematomorpha: Gordiida) emerging from a camel cricket (Ceuthophilus sp., Orthoptera: Gryllacrididae). This provides more evidence of secondary ingestion than most previous reports of predation on horsehair worms, but it also illustrates the difficulty of distinguishing in practice between direct and secondary predation.

The term “secondary ingestion” has been used when food items in a predator’s gut were not eaten directly by that predator but were consumed instead by one of its prey (e.g., Neill and Allen 1956). The same term may be appropriately applied to parasites in the gut of a predator that are released from the body of one of its prey, at least when those parasites do not survive the digestive process.

The life cycles of horsehair worms (Phylum Nematomorpha) include a juvenile stage parasitic within terrestrial insects and a free-living aquatic adult stage (Poinar 2001, Hanelt and Janovy, Jr. 2003). In at least some species, terrestrial hosts infected by maturing horsehair worms are more likely than uninfected individuals to enter water (Thomas et al. 2002). The slow-moving adult worms do not feed and sometimes are found in clumps of from several to many individuals (Cochran et al. 2004).

Predation on horsehair worms was reviewed by Cochran et al. (1999). Subsequently, Poinar (2001) reported that an adult Gordius was brought to nestlings by a bird in Chile. Kinziger et al. (2002) provided additional accounts of predation in Minnesota and Missouri but overlooked an earlier mention of an unidentified horsehair worm in the gut of a hellbender (Cryptobranchus alleganiensis) from the latter state (Peterson et al. 1989). Schmidt-Rhaesa et al. (2003) and Martin and Cochran (2005) reported additional cases of horsehair worms recovered among the gut contents of trout.

Most previously reported cases of predation on horsehair worms involved fish, and most were interpreted to have resulted from fish having preyed directly upon free-living adult worms. However, Cochran et al. (1999) observed that many fish tested in laboratory trials rejected adult horsehair worms, and they recognized the possibility that at least some horsehair worms found among the gut contents of fish may have been ingested secondarily (i.e., before having emerged from their invertebrate hosts). Ponton et al. (2006) staged secondary predation in the laboratory by generalist predators (fishes and ranid frogs) consuming crickets infected by horsehair worms. Depending on the predator, 18-35% of the worms were able to escape by wriggling out through the predator’s mouth, nose, or gills, but most of them were presumably digested.

There is some evidence for secondary predation on horsehair worms in the field. Bolek’s (2000) report of a dog regurgitating a Gordius robustus suggests the possibility that it had eaten an insect containing the worm. Bolek and Coggins

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(2002) found *G. difficilis* within carabid beetles among the gut contents of two green frogs (*Rana clamitans*). The purpose of this note is to describe another apparent case of secondary predation on a horsehair worm.

On 31 August 2006, at approximately 20:00, I collected a brown trout (*Salmo trutta*) by angling in Gilmore Creek below the spillway at the south end of the Saint Mary’s University Campus in Winona, Winona County, Minnesota. The trout measured 32 cm in total length. In addition to fragments of a dragonfly nymph, the trout contained in its stomach a partially digested camel cricket (Orthoptera: Gryllacrididae: *Ceuthophilus* sp.), from which a living male horsehair worm (*Gordius robustus* Leidy) had partially emerged. Two female *G. robustus* extended from the stomach into the intestine, which also contained snails. At some point after the gut contents of the trout were initially examined and preserved in ethanol, a second male *G. robustus* partially emerged from the camel cricket. The camel cricket and *G. robustus* have been placed in the Milwaukee Public Museum invertebrate collection.

The gut contents described above are consistent with a scenario by which the trout consumed the camel cricket just prior to the emergence of the male *G. robustus*. It is also possible that the female worms emerged from the same cricket. Thomas et al. (2002) determined that 5 of 41 crickets infected by *Paragordius tricuspidatus* contained more than one worm. Hanelt and Janovy, Jr. (2004) observed in a laboratory study the successful maturation of multiple *P. varius* within individual crickets. They did not indicate whether these worms were smaller than single worms that matured within separate hosts, but the female worms in the present study were notably short (120 and 123 mm).

Although direct and secondary predation would seem to be mutually exclusive events, an intermediate scenario is possible. A predator might consume both horsehair worm and its insect host during the time that the worm is emerging, as observed in the laboratory by Ponton et al. (2006). Indeed, the emergence itself might draw the attention of the predator, and natural selection might therefore favor rapid emergence and separation from the host. Hanelt and Janovy, Jr. (2004) reported that *P. varius* in laboratory studies began emerging from their hosts within two seconds of the hosts being placed in water and that most worms had exited completely within 90 seconds. However, they also stated that worms formed mating aggregations even while emerging from hosts. Thomas et al. (2002) stated that emergence from a host could be immediate or could take several minutes after the host entered the water, and Ponton et al. (2006) stated that emergence may take as long as 10 minutes.

Neill and Allen (1956) discussed the difficulty of interpreting items that are resistant to digestion and that persist for relatively long times in digestive tracts. For example, vertebrate prey may be digested much more quickly than the invertebrates they themselves have consumed, and it might be wrongly concluded that the invertebrates were preyed upon directly. In the case of insect hosts containing horsehair worms, differences in digestibility are possibly not as extreme, and it might be less likely to find secondarily ingested horsehair worms in the absence of at least some remains of their invertebrate hosts. However, given recent advances in the culture of horsehair worms in captivity (Hanelt and Janovy, Jr. 2004), it would be desirable to test this possibility via laboratory experiments.

*Gordius robustus* has not been previously reported to parasitize camel crickets but has been reported from other orthopterans (Schmidt-Rhaesa et al. 2003). The camel cricket *Ceuthophilus stygius* is parasitized by *Chordodes morgani* in Kentucky (Studier et al. 1991).

Although *Gordius robustus* has been reported recently at several locations in Minnesota (Martin and Cochran 2005), this is the first collection from Gilmore Creek. An earlier report of *G. robustus* from Gilmore Creek (Cochran
et al. 1999) was revised to *G. difficilis* by Cochran et al. (2004), and Martin and Cochran (2005) found the latter species to be more common in cold spring-fed streams in southeast Minnesota. Indeed, on 30 June 2006, while angling in the same pool where the trout containing the *G. robustus* was collected during the present study, I collected two separate *G. difficilis* that became entangled in the treble hook of my lure while it was being retrieved. Moreover, Martin and Cochran (2005) listed several prior collections of *G. difficilis* among the gut contents of brown trout collected in Gilmore Creek. Two of the trout were captured in the same pool as the trout that contained a *G. robustus* during the present study (19 July 2003 and 18 June 2004). The two *Gordius* species can be distinguished by differences in diameter, color, and, in males, the presence or absence of a parabolic fringe of hairlike processes anterior to the cloaca (Bolek and Coggins 2002, Martin and Cochran 2005).

Additional specimens of *G. robustus* were collected farther downstream on the Saint Mary’s University campus on 22 September 2006 and 30 October 2007. The worm collected on the latter date, an adult female, was of special interest because it was found moving in a terrestrial environment, a steep shaded bank approximately 1 meter above the waterline. No host was evident in the immediate vicinity.

**ACKNOWLEDGMENTS**

I thank Stephen Schmitt for assistance with samples from Gilmore Creek.

**LITERATURE CITED**


ABUNDANCE OF RICE ROOT APHID AMONG SELECTED PLANT SPECIES AND ON PLANTS GROWN WITH DIFFERENT SOIL-SURFACE MEDIA

Louis S. Hesler\textsuperscript{1} and S. Dean Kindler\textsuperscript{2}

**ABSTRACT**

The rice root aphid, *Rhopalosiphum rufiabdominalis* (Sasaki), is distributed worldwide and colonizes a wide range of plants. However, relatively little is known about the suitability of different host plants, optimal rearing techniques, and the aphid’s impact on plant fitness. To improve understanding of these factors, laboratory experiments were conducted to compare the abundance of rice root aphid on plants grown using three different soil-surface media and among selected monocotyledonous and dicotyledonous plants. Rice root aphid was more abundant on plants grown with a sandy soil surface than a surface with fine wood chips or only bare non-sandy soil. Rice root aphid was more abundant on ‘Elbon’ rye than on ‘Bart 38,’ ‘Dart,’ ‘Fletcher’ and ‘Ramona 50’ wheat. More winged rice root aphids were produced on Elbon rye than on Dart wheat, but the number of winged aphids on Elbon rye did not differ from that on other wheat lines. Rice root aphid was more abundant on Elbon rye and ‘TAM 110’ wheat than on ‘Marmin,’ ‘Marshall’ and ‘Sharp’ wheat. Additional observations with monocotyledonous plants showed that abundance of rice root aphid on ‘Kivu 85’ triticale was comparable to that on Elbon rye. Rice root aphid did not reproduce on potato or soybean, although winged adults persisted up to 24 days on caged potato plants. The implications of differential abundance of rice root aphid on plants are discussed in regard to colony rearing, future experiments and possible pest management considerations.

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and abundance on small-grain crops, tests of direct impact by rice root aphid on plant fitness are lacking.

A critical first step in evaluating the effect of a potential pest aphid on plant fitness is to determine the range of host plants that are capable of supporting large populations of the aphid. Once identified, these plants may then be used as rearing hosts and as test plants for evaluating any impacts of aphid infestations (Blackman 1990). The identification of suitable rearing plants is important, as pretest conditions in which aphids are held may directly affect experimental outcomes (Smith et al. 1994). For instance, the use of a particular plant species or line for rearing and subsequent impact testing may predispose test aphids to feed on it and lead to exaggerated estimates of impact.

It is also important to determine particular conditions that support suitable numbers of aphids on rearing plants or experimental plants or that facilitate the execution of experiments (Blackman 1990, Tsai and Liu 1998). For instance in laboratory experiments with the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), a thin layer of light-colored quartzite sand serves as a useful contrasting surface for tracking this generally dark colored aphid during infestation and evaluative counting, when it may become dislodged from test plants and fall onto the soil surface of experimental arenas (Hesler and Tharp 2005). As bird cherry-oat aphid and rice root aphid closely resemble one another morphologically (Richards 1960, Pike et al. 1990), light-colored sand may also be a useful soil surface in experiments with rice root aphid, even though a dark soil mix is suitable for rearing and for experiments (Paliwal 1980, Kindler et al. 2004). Anecdotal observations have also indicated that population growth of rice root aphid may be facilitated by the placement of a thin layer of fine wood chips on the soil surface around the base of rearing plants (Kindler et al. 2004), but a layer of wood chips may also hinder tracking the aphids during infestation and evaluative counting. Thus, the population growth of rice root aphid needs comparison among different soil-surface treatments, such as sand and wood chips, to determine their utility in future experiments. The objective of this research is to determine the suitability of selected plants and soil media for rice root aphid by measuring the abundance of rice root aphid over time when placed on selected monocotyledonous and dicotyledonous plants.

**MATERIALS AND METHODS**

**Aphids.** Rice root aphids used in the experiments were obtained from a virus-free, multiclonal stock colony maintained on ‘Elbon’ rye plants (Kindler et al. 2004) growing in a 15.2-cm diameter pot covered with a cylindrical cellulose nitrate cage (Hesler and Tharp 2005). The colony was maintained in a growth chamber (Controlled Environments Inc., Pembina, ND, USA) under constant conditions (13 h light at 19°C, 11 h dark at 18°C) at the USDA North Central Agricultural Research Laboratory (NCARL), Brookings, South Dakota. A non-viruliferous colony of rice root aphid was established by collecting numerous individuals from a winter wheat field near Brookings in autumn 1999, placing them on sachets of Parafilm® (American National Can Co., Greenwich, CT, U.S.A.) containing 20% sucrose solution, removing neonate offspring, and transferring them to noninfested rye plants (Hesler and Tharp 2005). This procedure was repeated about once per year with colony aphids, and occasionally leaf tissue was tested serologically (Agdia, Elkhart, IN, U.S.A.) to ensure that colony plants were free of BYDV. The integrity of the colony was also checked weekly by examining a few hundred individuals to ensure no contamination by morphologically similar species such as bird cherry-oat aphid. Winged aphids became present in cages 3 weeks after initial infestation and aggregated on the inner surface of each cylindrical cage. The colony was perpetuated by regularly infesting one-week-old rye plants with winged rice
root aphids obtained from caged plants infested 3 to 4 weeks earlier (Kindler et al. 2004). Voucher specimens of the aphids are deposited at NCARL.

**Abundance of rice root aphid on Elbon rye using different soil-surface media.** Experimental plants were prepared by germinating seeds between layers of moist paper towels held in plastic containers in the dark at 20°C (Hesler and Tharp 2005). After 24 to 48 h, 25 to 40 individual seedlings exhibiting uniform root and coleoptile growth were planted into a 2:1:1 mixture of Vienna soil (fine-loamy, mixed Calcic Hapludolls), perlite, and coarsely ground coconut shells (Coir, J. R. Johnson Supply Inc., Roseville, MN, U.S.A.). Pots were thinned to 20 seedlings 5 or 6 days later.

Three types of soil-surface media treatments were tested, and they were applied to pots when rye plants were 7-d old. Treatments consisted of adding volumes of 5 oz. of soil mixture, 8 oz. of fine cedar-wood chips, or 5 oz. of light-colored quartzite sand to each test pot and spreading each soil treatment over the soil surface and around the base of test plants. After treatments were applied, the modified soil surface in each pot was sprayed lightly with water. Then, each pot of 20 seedlings was infested with 28 winged rice root aphids selected randomly from the cylindrical cages that had covered colony plants infested 3 to 4 weeks earlier. Infested plants were immediately caged and placed into a growth chamber (13 h light at 19°C, 11 h dark at 18°C) as a randomized complete block design with four replications.

Abundance of rice root aphids was measured 24 d later when plants were about 40-cm tall with 3 to 4 leaves and had dense aphid populations around their basal half. Aphids were counted immediately from plants clipped at the soil surface of each quadrant in an experimental pot (4 plants total). Winged aphids on the cut plants were more likely to escape and were counted first. The numbers of all aphids on each set of 4 plants was summed for each treatment replicate. Additional plants from the pots were gently dug to confirm that rice root aphids had not infested roots, consistent with our previous, unpublished observations. As a second measure of abundance, the number of winged aphids on the inner surface of each treatment cage was also counted. The four-plant counts and counts of winged aphids from cages were subjected to separate analyses of variance (PROC ANOVA; SAS Institute 2002), and treatment means were separated by Tukey’s HSD method. A significance level of $\alpha = 0.05$ was used for statistical tests.

**Abundance of rice root aphid on selected monocotyledonous plants.** The abundance of rice root aphid on monocot plants was evaluated in two separate, quantitative experiments and a third experiment in which abundance was observed but not quantified. The first experiment compared rice root aphid abundance on Elbon rye to that on wheat lines ‘Fletcher,’ ‘Dart,’ ‘Baart 38,’ and ‘Ramona 50.’ These lines express symptoms of BYDV infection (Oswald and Houston 1953; NGRP 2007a, b, c) and were evaluated as possible hosts of rice root aphid for studies on virus transmission and yield effects. A second experiment compared abundance of rice root aphid on Elbon rye to that on two spring-wheat lines, ‘Marshall’ and ‘Sharp,’ and two winter- wheat lines, ‘Marmin’ and ‘TAM 110.’ Marshall, Sharp, Marmin, and TAM 110 are lines that are regionally adapted to the northern Great Plains. Each of these two experiments was conducted identically to the soil-media experiment. In addition to these quantitative tests, abundance of rice root aphids was also evaluated qualitatively on ‘Kivu 85’ triticale, which in preliminary tests appeared to support large populations of rice root aphids. Sets of triticale plants were infested and maintained in the same manner as the quantitative experiments with wheat and rye. A set of Elbon colony plants was infested at the same time and placed into a separate chamber (13 h light at 19°C, 11 h dark at 18°C). After 4 weeks, aphid abundance on triticale and rye was observed in situ.
Abundance of rice root aphid on selected dicotyledonous plants. Potato and soybean were evaluated as hosts of rice root aphid in separate experiments. In the first experiment, three potato plants per pot were used. To achieve this, three cuttings of potato tuber (each from a different tuber) were planted about 2 inches deep in a 15.2-cm diameter pot filled with a modified soil mix. The mix was modified to enhance potato growth by adding an equal volume of sand to the original soil mix used with monocots. One of three common, locally available lines of potato (‘Irish Cobbler,’ ‘Norkotah Russet,’ or ‘Red Pontiac’) was planted per pot. A separate control treatment of Elbon rye (20 plants per pot) was also included. Potato plants were three-weeks old and rye plants were one-week old when they were infested on the same date with 28 winged rice root aphids. Infested treatment plants were placed into a growth chamber (13 h light at 19°C, 11 h dark at 18°C) according to a randomized complete block design with four replications. After 24 days, aphids were counted on the potato plants in each pot and from 4 randomly selected rye plants per pot. In a second experiment, seeds of soybean line ‘91B91’ were planted in 15.2-cm diameter pot with soil mix and thinned to two to three plants two weeks later. Each of three pots of soybean was infested with 28 winged rice root aphids, immediately caged, and placed into a growth chamber (16 h light at 22°C, 8 h dark at 19°C). A set of three Elbon colony plants was infested at the same time and placed into a separate chamber (13 h light at 19°C, 11 h dark at 18°C). After 2 weeks, aphid abundance on soybean and rye were observed in situ.

RESULTS

Abundance of rice root aphid on Elbon rye using different soil-surface media. Abundance of rice root aphid on Elbon rye varied with soil-surface media \( (F = 1.28, \text{df} = 2, 14, P = 0.0012) \). Plants grown with a sandy soil surface \( (\bar{x} \pm SE = 900.1 \pm 92.8 \text{ per 4 plants}) \) had more aphids than a surface with fine wood chips \( (\bar{x} \pm SE = 494.8 \pm 54.2 \text{ per 4 plants}) \) or only soil \( (\bar{x} \pm SE = 453.8 \pm 53.9 \text{ per 4 plants}) \). In addition, more winged aphids \( (F = 37.59, \text{df} = 2, 14, P < 0.001) \) were collected from cages in the sand treatment \( (\bar{x} \pm SE = 240.6 \pm 23.9) \) than with treatments of wood chips \( (\bar{x} \pm SE = 60.4 \pm 8.9) \) or only soil \( (\bar{x} \pm SE = 52.1 \pm 11.7) \).

Abundance of rice root aphid on selected monocotyledonous plants. In the first experiment (Table 1), rice root aphid was more abundant on Elbon rye than on Bart 38, Dart, Fletcher and Ramona 50 wheat \( (F = 8.10, \text{df} = 4, 12, P = 0.002) \). More winged rice root aphids were collected from cages of Elbon rye than from those of Dart wheat, but the number of winged aphids on Elbon rye did not differ from that of other wheat lines \( (F = 3.70, \text{df} = 4, 12, P = 0.035) \). In the second experiment (Table 1), rice root aphid was more abundant on Elbon rye and TAM 110 wheat than on Marmin, Marshall and Sharp wheat \( (F = 28.13, \text{df} = 4, 12, P < 0.001) \), and more winged aphids were collected from cages of Elbon rye and TAM 110 wheat than from cages of other wheat plants \( (F = 18.67, \text{df} = 4, 12, P < 0.001) \). Additional observations showed that rice root aphid became highly abundant on Kivu 85 triticale, with abundance appearing comparable to that on Elbon rye infested for the same length of time. Stems and lower leaves of rye and triticale were virtually covered with dense colonies of rice root aphids, and winged aphids were abundant on the inner surfaces of cages.

Abundance of rice root aphid on selected dicotyledonous plants. Winged rice root aphids were found on potato and soybean plants within a few hours after being introduced into test cages, and they remained active in test arenas for at least several days. In potato tests, alates were generally observed on plants rather than on the inner surface of cages. After two weeks, however, dead alates began to appear increasingly on the soil surface of the potato test arenas, but several alates survived on potato throughout the 4-week test period. However, no offspring were found on the shoots, roots, or tubers of potato plants.
after 24 days. After 24 days in the potato test, Elbon rye had $1816.8 \pm 466.5$ (x± SE) aphids per 4 plants and $290.8 \pm 170.8$ (x± SE) winged aphids per cage. Most of the winged aphids on soybean died in about one week and no offspring were found on soybean plants, but after 4 weeks Elbon rye was heavily infested with hundreds of rice root aphids per stem and many alates were found on the inner surface of cages.

**DISCUSSION**

The abundance of rice root aphid was greater on plants growing above a sandy soil surface than with surfaces of wood chips or soil mix. Our test was not designed to determine why rice root aphids were more abundant in the sand treatment, and this may be addressed in future studies. Nonetheless, the use of light colored sand could facilitate tracking aphids during infestation and counting, and the use of a sandy surface is recommended especially for laboratory and greenhouse experiments involving infestations of shoots with rice root aphids. The sandy surface could also enhance colony production of rice root aphids on Elbon rye.

Rice root aphid had differential population growth among various plant species and no population growth on potato, which had been reported previously as a host (Essig 1944). The population of rice root aphids in this study was collected from a field of ‘Roughrider’ wheat, and results showed that it was well adapted to rye, wheat and triticale, but not potato or soybean. Previous results have shown that this population of rice root aphid also becomes only moderately abundant on barley and oats and is poorly adapted to rice and other grasses (Kindler et al. 2004), but other North American populations of rice root aphid have been well adapted to barley and oats (Paliwal 1980). Collectively, these results suggest that the population of rice root aphid in this study may represent a biotype based on its differential survival and development on particular host plants (Eastop 1973, Diehl and Bush 1984, Drés and Mallet 2002).

The rice root aphid did not reproduce on potato and soybean, but alates had prolonged survival on potato and limited survival on soybean. The survival of
alates on potato and soybean raises the question of whether rice root aphid may be a vector of plant-disease viruses in these crops, but its ability to transmit viruses to potato and soybean is unknown. Several congeneric aphid species do not colonize potato or soybean, but they are a vector of stylet-borne plant-disease viruses to these crops. For instance, both corn leaf aphid, *Rhopalosiphum maidis* (Fitch), and bird cherry-oat aphid transmit potato leafroll virus to potato (Halbert et al. 2003) and soybean mosaic virus to soybean (Halbert et al. 1981), and *Rhopalosiphum insertum* (Walker) is capable of transmitting potato virus Y to potato (van Hoof 1980).

Rice root aphid may be prevalent in regions of North America where wheat is grown near soybean and potato (Kieckhefer and Gustin 1967, Al-Raeesi et al. 1992, Chapin et al. 2001, Kindler et al. 2004), but it has not been considered significant in the epidemiology of aphid-borne viruses in potato and soybean. There is no record of rice root aphid colonizing soybean, and it composes <<1% of alates captured in traps within soybean fields (Halbert et al. 1981). Alate rice root aphids have not been trapped in Minnesota and North Dakota potato fields (DiFonzo et al. 1997). However, as rice root aphid (as *Cerosipha californica*) has been recorded on potato in California (Essig 1944), and given the prolonged survival of alates on potato in the present study, tests of its ability to transmit viruses to potato may be warranted.

Rice root aphids in this study differed in abundance among wheat lines, with greatest numbers on TAM 110 wheat and with decreased abundance of winged aphids on Dart wheat. The differential abundance of rice root aphid among wheat lines has some implications for rearing and experimentation. First, TAM 110 was the only wheat line with an abundance of rice root aphid comparable to that on Elbon rye. Abundance of rice root aphid on Kivu 85 triticale, a wheat × rye cross, was also comparable to that of Elbon rye. Thus, TAM 110 and Kivu 85 may be equally suitable to Elbon rye as rearing hosts. However, the differential abundance of rice root aphid among wheat and triticale lines suggests that experiments to test for an impact of rice root aphid on the growth and grain yield need to be designed to maintain an equal number of aphids across test plants over time (Lamb and MacKay 1995). From a pest management standpoint, the differential abundance of rice root aphids among wheat lines suggests inherent variation in wheat that could be exploited to develop and eventually deploy lines that limit aphid infestations (Webster 1991).

One objective of this study was to identify wheat lines that support large numbers of rice root aphid and also readily express symptoms of BYDV infection for possible use in studies on virus transmission and yield effects. The wheat lines Baart 38, Dart, Fletcher and Ramona 50 are moderately to extremely susceptible to BYDV (Oswald and Houston 1953; NGRP 2007a, b, c), and rice root aphid was fairly abundant on these lines, although relatively low numbers of aphids were produced on Dart. Thus, Baart 38, Fletcher and Ramona 50 may be useful as colony plants for maintaining rice root aphids and BYDV, and in future experiments to determine the effects of rice root aphid and BYDV on wheat growth and yield.

**ACKNOWLEDGMENTS**

We are grateful to Eric Beckendorf, David Mills, Keith Mirkes, Cecil Tharp, Emily Alesch, Will Allgaier, Jillian Bakker, Phil Olinger, Josh Pedro, and Ryan Rubbelke for technical assistance. Norm Elliott, Billy Fuller, Eric Beckendorf, and Lauren Hesler graciously reviewed drafts of this paper. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement.
LITERATURE CITED


CAN EMERALD ASH BORER, *AGRIILUS PLANIPENNIS* (COLEOPTERA: BUPRESTIDAE), EMERGE FROM LOGS TWO SUMMERS AFTER INFESTED TREES ARE CUT?

Toby R. Petrice1 and Robert A. Haack1

ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious invasive pest of ash trees (*Fraxinus* spp.) in North America. Much of EAB’s range expansion has been attributed to human-assisted movement of infested items such as ash logs and firewood. It is unclear the amount of time that logs cut from live EAB-infested ash trees should be restricted from movement until they are no longer capable of producing viable EAB adults. In March and April 2004, we cut log sections from EAB-infested green ash (*F. pennsylvanica* Marsh) trees in Ann Arbor, Washtenaw County, Michigan. Log sections (mean length = 24.8 cm; diam. = 11.6 cm) were stood upright on one cut end and stored beneath a hardwood forest canopy. Adult EAB were allowed to freely emerge from log sections during summer 2004. When logs were dissected in November 2004 to January 2005, approximately one half of the total EAB life stages that were present in the logs were dead, while the other half either emerged as adults in summer 2004 or were live prepupae. Also, adults emerged from a subset of these log sections when reared in the laboratory in January to February 2005. These data suggest that EAB adults can emerge from logs for two successive emergence periods after infested ash trees have been cut.

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious invasive pest of ash trees (*Fraxinus* spp.) and, as of May 2008, it was established in the US states of Illinois, Indiana, Maryland, Michigan, Ohio, Pennsylvania, and West Virginia; and the Canadian province of Ontario (Haack 2006; http://www.emeraldashborer.info). EAB is native to Asia and was first discovered in North America in the Detroit metropolitan area of Michigan in 2002 (Yu 1992, Haack et al. 2002, Poland and McCullough 2006). Most of the range expansion of EAB has been attributed to inadvertent human-assisted movement of infested ash nursery stock, logs, and firewood. A federal quarantine was imposed to limit human-assisted dispersal of EAB by regulating movement of these articles (Federal Register 2003).

EAB larvae develop through four instars as they feed in the phloem of ash trees. When fourth instar larvae have completed feeding, they excavate pupation chambers in the outer sapwood or bark of ash trees (Cappaert et al. 2005, Wei et al. 2007). In southern Michigan, most EAB larvae overwinter as prepupae in their pupation chambers after they have developed from eggs laid in early summer of that same year. However, some EAB larvae do not complete larval development the same year they eclosed from eggs and overwinter as larvae in the phloem of ash trees (Cappaert et al. 2005). Some of these larvae complete feeding the following spring and emerge as adults later that same summer. However, a percentage of these larvae do not complete feeding and become prepupae until late summer or fall and overwinter a second time before emerging as adults. Thus, logs cut in mid-summer may have both newly initiated and fully developed larvae.

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EAB prefer to oviposit on live ash trees, but they will occasionally oviposit on freshly cut ash logs (Anulewicz et al. 2008). However, larvae that develop from eggs laid on cut logs rarely complete their development (Anulewicz et al. 2008; T.M. Poland and D.G. McCullough, pers. comm.). Therefore, it is unclear the length of time logs and firewood cut from EAB infested trees should be restricted from movement to assure the absence of live EAB life stages. We present information here from a recent study (Petrice and Haack 2006b) where data were generated suggesting that EAB adults can emerge from log sections two successive emergence-periods after infested trees are cut.

In April 2004, before adult EAB emerged, we cut 136 logs from green ash, *F. pennsylvanica* Marsh, trees that contained live EAB life stages in Ann Arbor, Washtenaw County, MI. Logs were cut approximately 50-cm long and averaged 12 cm in diameter. In May and June 2004, prior to EAB adult emergence, each log was cut into two equal sections, with one section treated with an insecticide and one section untreated to serve as a control (see Petrice and Haack 2006b for more details). Log sections averaged (mean ± SE) 24.8 ± 0.3 cm long and 11.6 ± 0.2 cm in diam. All exit holes present on log sections from previous years (2003 and earlier) were marked with white caulk. Data presented here represent only the control log sections. Log sections were stood upright on one cut end and placed under a hardwood-forest canopy during June to October 2004 and EAB adults were allowed to freely emerge during this period. During November 2004 to January 2005, 126 log sections were brought back to the laboratory and dissected. We recorded the number of EAB adults that had emerged from the sample log sections during summer 2004 based on the presence of new exit holes at the time of dissection. We also recorded the number of dead EAB adults, pupae, prepupae (larvae that have completed feeding and excavated pupation chambers in the bark or wood), and larvae present in log sections; and the number of live EAB prepupae in each log section. Live prepupae, which were apparently undamaged during dissection, were reared in petri dishes at 24°C. Their final stage of development at death (e.g., prepupae, pupae) or whether they successfully developed to adults was recorded. On 4 January 2005, the 10 remaining log sections that had been cut in April 2004 were brought into the laboratory and placed in cardboard tubes to monitor for adult emergence.

Log dissections revealed that 30.9 ± 2.2 % of the total EAB in log sections had emerged as adults during summer 2004, 22.4 ± 1.9% were live prepupae, 17.0 ± 1.6% died as larvae, 9.6 ± 0.9 % died as prepupae, 0.1 ± 0.1 % died as pupae , and 20.1 ± 1.8% died as callow adults. Furthermore, 18% of the 151 live prepupae that were dissected from log sections in November 2004-January 2005 and reared in petri dishes developed to adults, while 17% died as pupae and 65% died as prepupae. A total of 8 adults emerged in February 2005 from 5 of the 10 log sections that were cut in April 2004 and reared in the laboratory during January-February 2005 (Table 1).

The logs for this study were only moderately infested with EAB and, therefore, competition for food among larvae was low. This likely enhanced EAB survival as compared to logs that would have been heavily infested. We suspect adults that emerged in summer 2004 were fourth instars or prepupae at the time logs were cut in April 2004. While adults that emerged in the laboratory in 2005 were likely younger instars in April 2004. These earlier-instar larvae would have had to continue developing during summer 2004, with adult emergence occurring in late summer 2004 or early summer 2005. Development may have been protracted because of log moisture loss and cooler temperatures that resulted from the log sections being stored in the shade. Alternatively, given that some EAB larvae may require a two-year life cycle (Cappaert et al. 2005; Wei et al. 2007), it is equally likely that live prepupae we found in log sections in November 2004-January 2005 and adults that emerged in February 2005 may have needed two seasons to complete development regardless of when logs were cut from infested trees. We assume all EAB individuals that emerged in
2004 and 2005 had resulted from eggs laid in 2003 or possibly one year earlier. Although highly unlikely, it could be argued that the EAB adults that emerged in 2005 resulted from eggs laid on the log sections during summer 2004 because the log sections were exposed to natural attack. As mentioned above, EAB oviposition has been recorded on cut logs but is evidently rare (Anulewicz et al. 2008; T.M. Poland and D.G. McCullough, pers. comm.).

Surprisingly, even though the log sections were cut to very short lengths (25 cm) within 1-2 months after they were initially cut from trees in March and April, they still contained live EAB prepupae in November 2004-January 2005 that were able to develop into adults in the laboratory. Petrice and Haack (2006a) found that when firewood logs cut from EAB-infested trees remained uncovered outdoors in either the sun or shade, EAB survival was reduced the following summer compared to logs stored under tarps. This difference was likely a result of logs desiccating more when they were exposed to ambient conditions. Therefore it would be assumed that desiccation would have greatly reduced survival of EAB in the short, small-diameter log sections used in the current study. If log sections in the present study would have remained outdoors an additional 4-5 months until the 2005 summer emergence, it is likely that further desiccation would have lowered EAB survival even more. Nevertheless, our data show that EAB adults can be reared from short, small-diameter logs during two successive emergence periods after logs are cut from infested trees. Based on these results, ash logs and firewood potentially infested with EAB should be held at least two summers after trees are cut to allow EAB adults time to emerge prior to any log movement if the objective is to prevent human-assisted movement of EAB. Nevertheless, current EAB quarantine regulations prohibit movement of all hardwood firewood and ash logs to areas outside of EAB quarantine zones unless they have been treated with an approved method (Federal Register 2003).

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Table 1. Length and diameter of ash log sections that were cut from EAB-infested trees in Washtenaw County, MI in April 2004, and number of EAB adults that emerged from each log section during summer 2004 while log sections were stored outdoors and during February 2005 after log sections were brought indoors in January 2005.

<table>
<thead>
<tr>
<th>Tree cutting date</th>
<th>Log dimensions (cm)</th>
<th>Number of adults that emerged</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Length</td>
<td>Diameter</td>
</tr>
<tr>
<td>3 April 2004</td>
<td>22.0</td>
<td>13.2</td>
</tr>
<tr>
<td>3 April 2004</td>
<td>23.2</td>
<td>15.7</td>
</tr>
<tr>
<td>3 April 2004</td>
<td>20.8</td>
<td>17.4</td>
</tr>
<tr>
<td>29 April 2004</td>
<td>29.5</td>
<td>10.7</td>
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<td>23.7</td>
<td>12.5</td>
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<td>11.4</td>
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LITERATURE CITED


ERYTHRODIPLAX UMBRATA (ODONATA: LIBELLULIDAE): NEW FOR MICHIGAN

Julie A. Craves1, 2 and Darrin S. O’Brien2

ABSTRACT

Two band-winged dragonlets, Erythrodiplax umbrata (Linnaeus), collected in Wayne County, Michigan on 6 October 2007 represent the first records for this genus and species in the state, as well as the northernmost record for the species. They were found during a period in which many individuals were seen or photographed in Ohio, which prior to 2006, had only two records.

Erythrodiplax (Brauer) is a primarily Neotropical genus of 56 species (Gar- rison et al. 2006), six of which occur regularly in North America north of Mexico (Dunkle 2000). Erythrodiplax umbrata (Linnaeus), the band-winged dragonlet, is found in South America south to Argentina, Central America, Mexico, the Greater Antilles, and the southern United States. Prior to 2006, there were only four records outside the southern U.S., all represented by specimens. One was taken at Cedar Bog, Champaign County, Ohio on 11 June 1934 (Borror 1935). Two teneral individuals were collected in Indiana by B. E. Montgomery on 1 September 1934, a male in Gibson County, and a female in Pike County (Borror 1935). Kansas has two records, a teneral male collected by G. F. Hevel on 11 July 1964 in Labette County and a female taken on 8 June 1999 in Sedgwick County by R. J. Beckemeyer (Beckemeyer 2004).

On 11 August 2006, a male E. umbrata was photographed at Armleder Park, Cincinnati, Hamilton County, Ohio, which remained present until at least 23 August (Abbott 2007, Hull 2007). In 2007, Ohio had a spate of records for this species. One was photographed on 29 August at Headlands Dunes State Nature Preserve, Lake County and two were seen at this location on 10 September (Rosche 2007). Two adult males were found and photographed on 4 September at Frohring Meadows, Geauga County and another was photographed there on 18 September (Rosche 2007). An adult male was found on 14 September at the Leroy Wetlands, Lake County. Multiple individuals, including juveniles, were observed at this site through 22 October; the peak number was at least 20 juveniles on 26 September (Rosche 2007, J. Pogacnik, pers. comm.). A male was photographed at Cuyahoga Valley National Park, Cuyahoga County on 8 October and two teneral individuals on 17 October (Gardella 2007a, L. Gardella, pers. comm., contra Rosche 2007). None of these individuals were collected.

Bearing the recent Ohio findings in mind, on 6 October we took advantage of unseasonably warm (30°C) and sunny weather to do a final survey of adult odonates at the Detroit River International Wildlife Refuge, Humbug Marsh Unit, located along the lower Detroit River, in Wayne County, Michigan. Part of this unit is an 18 ha brownfield owned by Wayne County. The only surface water on the brownfield site were rainwater puddles unintentionally created by construction equipment earlier in the summer. These puddles were restricted to a 3 ha section approximately 300 m from the Detroit River.

Immediately upon entering the site, JAC spotted a male E. umbrata at an 8 × 4 m puddle (42°06'53"N, 83°11'38"W). As we attempted to photograph
it, a second male flew in and the two chased each other. For about 15 min, they alternated between the original puddle and another of similar size approximately 20 m away. Although they were wary and difficult to approach, JAC was eventually able to capture both. Voucher specimens have been deposited into the Univ. Michigan Museum of Zoology, Insect Division, and have been cataloged by the Michigan Odonata Survey.

This location is roughly 40 km farther north than the northernmost record for Ohio (at Headlands Dunes State Nature Preserve) and over 220 km farther north than the previous northernmost specimen, the one taken by Borror in 1934 outside of Columbus (Borror 1935).

DISCUSSION

Erythrodiplax umbrata inhabits marshy ponds, pools, and lakes, often temporary water (Dunkle 2000, Abbott 2005, Garrison et al. 2006). The Michigan dragonlets were in < 3-month-old depressions created by earth-moving equipment. All the 2007 Ohio records were found in similar pools and puddles of recent vintage. The Frohring Meadows park was under construction and the dragonlets there, as well as the one at Headland Dunes, were found in “simple scrapes” (L. Rosche, pers. comm.). Leroy Wetlands is a newly created wetland complex and the site containing the dragonlets had held water for < 2 months (Pogacnik 2007). The Cuyahoga Valley National Park site is a mitigated wetland and the dragonlet was in what was described by the observer as a “mud puddle” (Gardella 2007b).

These northern records of E. umbrata constitute a substantial northern range expansion for this species. Hickling et al. (2005) documented a northward shift in the range margins in 34 of 37 species of non-migratory British Odonata between 1960-1970 and 1985-1995. Catling (1996) reported that the range of Enallagma civile (Hagen), (Odonata: Libellulidae), had moved north by at least 200 km in southern Ontario between 1959 and 1996. Authors of both these papers noted that these range shifts could be associated with global climate change. More short-term climatic events might also help explain the recent northward movements of E. umbrata. For much of 2006, Texas and Oklahoma, core areas of the range of E. umbrata in the U.S., experienced severe to extreme drought (NWSGPC 2008) with 2007 the driest year in the 112-year record in the southeastern U.S. (NCDC 2008a). The drought was coupled with above-average temperatures in 2006-2007 over the south and southeast (NCDC 2008b). These conditions may have pushed E. umbrata north in search of breeding sites as the shallow ponds and puddles dried up over much of their range or above-average temperatures created unsuitable thermal conditions in surviving aquatic environments.

The presence of teneral E. umbrata in northeast Ohio suggests they were able to breed in the temporary ponds near which they were found. The two Michigan males on 6 October were fully pruinose adults. No E. umbrata were present at the Michigan site in over a dozen previous weekly visits or one subsequent visit and no nymphs were found during larval sampling in the puddles on 13 October. These puddles will be checked again in 2008, although they are likely to be destroyed early in the spring season.

ACKNOWLEDGMENTS

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recent Ohio records: Lou Gardella, Bob Glotzhober, John Pogacnik, and Larry Rosche. Additional photographers that documented the Ohio records include William Hull, Sally Isacco, Linda Gilbert, and Judy Semroc.

LITERATURE CITED


EFFICACY OF MORPHOLOGICAL CHARACTERS FOR DISTINGUISHING NYMPHS OF *EPITHECA CYNOSURA* AND *EPITHECA SPINIGERA* (ODONATA: CORDULIIDAE) IN WISCONSIN

Robert B. DuBois¹*, Kenneth J. Tennessen², and Matthew S. Berg³

ABSTRACT

Attempts to distinguish exuviae and last-instar nymphs of *Epitheca cynosura* (Say) and *Epitheca spinigera* (Selys) (Odonata: Corduliidae) using lateral spine characters have proven to be unreliable, and recent use of setae counts on only one side of the prementum or one labial palp have led to confusion because these structures often hold unequal numbers of setae on the two sides of the same specimen. Based on exuviae of 67 reared *E. cynosura* and 55 reared *E. spinigera* from lakes throughout Wisconsin, we tested the efficacy of previously used character states for distinguishing these species and searched for new characters to improve the reliability of regional keys. The most reliable diagnostic character was the combined number of setae on both sides of the prementum and on both labial palps (≤ 35 – *E. cynosura*; ≥ 36 – *E. spinigera*), which correctly determined 96% of our specimens. For the small percentage of specimens that lie in the region of overlap in total setae number, we found that total exuviae length, cerci ÷ epiproct ratios of females, tubercle distance ÷ epiproct ratios of males, and the shape of the dorsal hook on segment 8 could be used to strengthen determinations.

Despite numerous revisions (Muttkowski 1911, 1915; Davis 1933; Kormondy 1959; Tennessen 1973), the difficult genus *Epitheca* (Odonata: Corduliidae) has caused much confusion in North America (May 1995). This genus is often referred to as *Tetragoneuria* by workers who relegate *Epitheca princeps* Hagen to the genus *Epicordulia* (see Walker 1966). Confusion about this genus has encompassed both the naming of species, with only half of the 20 names that have been referred to the genus still widely accepted today, and discriminating among species in both the adult (Donnelly 1992, 2001; Needham et al. 2000) and nympha stages, of which the latter are our current focus. Four currently accepted species of *Epitheca* are known in Wisconsin, our focal region of study. *E. princeps* and *E. canis* (McLachlan) are readily distinguished as last-instar nymphs and exuviae. However, efforts to distinguish between the last-instar nymphs and exuviae of *E. cynosura* (Say) and *E. spinigera* (Selys) have had a long and vexing history.

Referring to *E. cynosura* and *E. spinigera*, Walker (1913) remarked that, “A careful comparison was made between the exuviae of these two species, but no differences could be detected between them, except that in spinigera the lateral abdominal appendages average slightly longer than those of cynosura. This difference, however, does not appear to be constant.” In contrast

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to Needham’s (1901) use of the divergence of the lateral spines of abdominal segment 9 to separate these species. Walker (1913) reported considerable variation in this character among individuals of the same species. Despite this finding, Needham and Heywood (1929) used divergence of abdominal segment 9 lateral spines as the single diagnostic character for separating *E. cynosura* from *E. spinigera* in their key to nymphs of *Tetragoneuria*. Garman (1927) found the nymphs of the species of *Tetragoneuria* in Connecticut to be so similar that he did not attempt to construct a key for their separation. In their key to nymphs of North American Anisoptera, Wright and Peterson (1944) noted that very little dependence can be placed on nymphal determinations in the genus *Tetragoneuria*. However, Needham and Westfall (1955) again used the divergence of the lateral spines on abdominal segment 9 in their key to separate the group that included *E. cynosura* from the group that included *E. spinigera*.

Kormondy (1959) conducted the first in-depth study of the taxonomy and systematics of *Tetragoneuria* that included the nymphs. He initiated the possible utility of counts of palpal and premental setae to distinguish nymphs of *E. cynosura* and *E. spinigera*, and noted that last instar nymphs of *E. spinigera* averaged larger than those of *E. cynosura*. However, he concluded that considerable variation in both counts of raptorial setae and in last-instar size made them unreliable characters for taxonomic purposes. In his summary remarks about these species, he stated that “no dependable morphological characters serve to separate individual last-instar larvae...” He noted that as populations, they differed significantly in a number of ways including the larger mean size of *E. spinigera*, the much higher growth rate of *E. spinigera* from the 11th to 12th instar, and the earlier seasonal appearance of *E. spinigera*.

Walker and Corbet (1975) used the number of premental setae (usually 10 or less for *E. cynosura*; usually 11 or more for *E. spinigera*) as the sole character to separate these species. In their diagnosis under *E. spinigera*, they reiterated that the relative length and direction of the lateral spines on segment 9 was not reliable, that overlap could be expected in both palpal and premental setae counts, and that *E. spinigera* averaged larger than *E. cynosura*, but only in part of the former’s range. In their on-line key, Bright and O’Brien (1998) separated the species using the number of premental setae (usually not less than 11 for *E. spinigera*; usually not more than 10 for *E. cynosura*), followed by the number of palpal setae (usually 7-8 for *E. spinigera*; usually 6 for *E. cynosura*). Needham et al. (2000) similarly used the number of premental setae (usually 11-12), followed by the number of palpal setae (usually 7) to separate *E. spinigera* from the group containing *E. cynosura* (premental setae usually 8-10; palpal setae usually 5-6). We tried to use these keys to distinguish last-instar nymphs and exuviae of *E. cynosura* and *E. spinigera* from waters in Wisconsin, but were uncertain about many determinations. Many specimens belonging to one of these species had 11 or more setae on one side of the prementum, but 10 or fewer setae on the other side, or at least 7 setae on one labial palp, but only 6 setae on the other. For some specimens, counting premental setae led to a different determination than counting palpal setae (e.g., 10 or fewer setae on both sides of the prementum indicating *E. cynosura*, but 7 or more setae on each palp indicating *E. spinigera*).

To make firmer recommendations for separating exuviae and last-instar nymphs of these species, we reared specimens of both species from numerous lakes in Wisconsin to form a database on which a number of morphological analyses could be performed. Our objectives were to determine if either premental or palpal setae counts, or a combination of the two, would reliably distinguish these species in Wisconsin, and to search for and evaluate other characters that could be used to separate them.
MATERIALS AND METHODS

We used only reared exuviae in our analyses to be certain of their identity. Nymphs were collected after molting to F-0 and were reared to emergence in aquaria. Teneral adults were maintained alive in small cages for several days after emergence, then were soaked overnight in acetone, dried, and stored in standard Odonata envelopes. Exuviae were placed in individual vials of 70% ethanol. Each adult/exuvia association was given a unique accession number immediately after emergence to preclude any possibility of confusing the specimens.

Our dataset was comprised of exuviae of 55 *E. spinigera* and exuviae of 67 *E. cynosura*. All of the exuviae except four were reared by one of the authors, and the first author verified all determinations and made all counts and measurements. Not all exuviae were used in all analyses. Two exuviae of *E. cynosura* and one exuvia of *E. spinigera* lacked essential mouth parts and could not be used for raptorial setae counts. Three exuviae of *E. cynosura* and one exuvia of *E. spinigera* were not measured for total length because the heads were detached or missing. One exuvia of *E. spinigera* had a twisted epiproct and was not used in analyses involving that character. Exuviae of 6 *E. spinigera* and 1 *E. cynosura* were omitted from analyses involving abdomen dimensions, and exuviae of 3 male *E. spinigera* and 1 male *E. cynosura* were omitted from analyses involving dimensions of the cerci and epiproct. All specimens are housed either in the Odonata Collection of the Wisconsin Department of Natural Resources (WDNR) in Superior, or in the private collection of KJT in Wautoma, Wisconsin.

Abdominal segments are designated by the letter “S” and the segment number (e.g., S9 = abdominal segment 9). Counts and measurements were done under magnification using either an ocular micrometer or millimeter rule, and all measurements are reported in mm. Counts of setae on both labial palps and on both sides of the prementum were done in dorsal view with the prementum pulled outward with a teasing needle. The most medially located premental setae were small, and care was taken to count all of them. Total lengths of exuviae were measured in dorsal view with a millimeter rule. We assessed the direction in which the tip of the dorsal hook on S8 pointed in lateral view relative to the long axis of the body. The left lateral spine on S9 was measured for width at the base and length in strict dorsal view with an ocular micrometer. Also measured in dorsal view were the lengths of both of the lateral spines on S8, the lengths of the margins of S8 including the spines, and the lengths of both cerci and the epiproct. For the males, the length of the epiproct from the base to the distal margin of the ante-apical tubercles was also measured. The abdomen was measured in ventral view from the base of S1 to the apex of S10 to determine maximum width, and across the width of S6 with the sclerite lightly depressed to determine maximum width.

Statistical analyses were performed using SigmaStat statistical software (SPSS 1997) with alpha set at 0.05. We used one way ANOVA (F), or Kruskal-Wallis one way ANOVA on ranks (H), to test for differences in setae numbers and total exuviae lengths among lakes and years. Pearson product moment correlation (r) was used to examine the strength of correlation between setae numbers and total exuviae lengths. The Chi-square test (χ²) was used to examine differences between species in the shape of the left lateral spine on S9 in dorsal view: categories were straight (1), and slightly incurved (2). Mann-Whitney Rank Sum tests (T) were used to examine differences between species in abdomen shape ratios. Statistical tests were not applied to other key characters because our goal was to assess the performance of character states in potential key couplets, not to determine statistical significance.

Material examined – WISCONSIN: BAYFIELD CO.: Sawdust Lake, 18 April and 5 May 2005, UTM 15 632537E 5159021N (all coordinates NAD83/WGS84), RBD (exuviae of 11 reared *E. cynosura* and exuviae of 3 reared *E. spinigera*);

**RESULTS**

Combining the data on total number of palpal + premental setae (= total setae) for the two species resulted in a bimodal curve (Fig. 1). Overlap occurred from 34 through 37 total setae, although few individuals were represented in this range. Approximately 95% of the *E. cynosura* had 35 or fewer total setae, whereas 96% of the *E. spinigera* had 36 or more total setae (Table 1). For *E. cynosura*, total setae counts did not differ among five lakes having the largest sample sizes ($H = 2.616$, $df = 4$, $P = 0.624$), and total setae number was not correlated with total length of exuviae ($r = -0.0673$, $N = 65$, $P = 0.594$). However, for *E. spinigera*, total setae counts differed significantly among four lakes having the largest sample sizes ($H = 9.427$, $df = 3$, $P = 0.024$), and total setae number was positively correlated with total length of exuviae ($r = 0.381$, $N = 54$, $P = 0.005$).

Numbers of premental setae also resulted in a bimodal distribution, but substantial overlap occurred from 20 to 23 setae, with most overlap at 22 setae (Table 1). For *E. cynosura*, 86% of exuviae had 21 or fewer premental setae, with most exuviae having 19 to 21 setae. For *E. spinigera*, 96% of exuviae had 22 or more premental setae, with most exuviae having 22 to 25 setae. Exuviae of both species often had unequal numbers of setae on the two sides of their prementum; this occurred on 51% of *E. cynosura* and 56% of *E. spinigera*.

Substantial overlap in numbers of palpal setae between species occurred from 13 through 15 setae and the single highest percentage of both species had 14 setae (Table 1). Fifty nine percent of *E. cynosura* had 13 or fewer palpal setae, while only 4% of *E. spinigera* had that number. Twenty six percent of *E. spinigera* had 15 or more palpal setae, while only 3% of *E. cynosura* had that number. Exuviae of both species sometimes had unequal numbers of setae on their two labial palps; this occurred on 34% of *E. cynosura* and 22% of *E. spinigera*. 
Figure 1. Numbers of total raptorial setae (left + right palpals plus left + right prementals) possessed by reared exuviae of *Epithea cynosura* and *E. spinigera* from Wisconsin.
Table 1. Frequency of exuviae with number of raptorial setae (left + right palpalps, left + right prementals, and total) in reared *Epitheca cynosura* (n = 65) and *E. spinigera* (n = 54) from Wisconsin.

<table>
<thead>
<tr>
<th>Palpals</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. cynosura</em></td>
<td>18</td>
<td>20</td>
<td>25</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>E. spinigera</em></td>
<td>0</td>
<td>2</td>
<td>38</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prementals</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. cynosura</em></td>
<td>1</td>
<td>3</td>
<td>14</td>
<td>23</td>
<td>15</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. spinigera</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>17</td>
<td>12</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>≤31</th>
<th>32</th>
<th>33</th>
<th>34</th>
<th>35</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
<th>≥40</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. cynosura</em></td>
<td>6</td>
<td>11</td>
<td>18</td>
<td>17</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. spinigera</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>16</td>
<td>10</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>

Exuviae of *E. spinigera* averaged 22.9 mm in total length, while exuviae of *E. cynosura* averaged 21.9 mm in total length, but the overlap between species was substantial. Differences in mean exuviae length among lakes were evident for both species that could have been attributable to lake effects, inter-annual effects, or both. For *E. cynosura* total exuvia length was significantly different among four lakes sampled in 2005 (*H* = 13.195, df = 3, *P* = 0.004). Significant differences among lakes sampled in 2007 were found also for *E. spinigera* (*F* = 13.221, df = 3, *P* < 0.001). Our data allowed for only one direct test of an inter-annual effect on total exuviae length, at a pond at Memory Lake Campground in Burnett County, where *E. spinigera* were sampled in 2005, 2006, and 2007. Here we did not find evidence of an effect attributable to year (*H* = 1.566, df = 2, *P* = 0.457).

Female exuviae of *E. spinigera* had longer cerci than did female exuviae of *E. cynosura*. This was most clearly seen in the ratio obtained by dividing the mean length of both cerci by the length of the epiproct. Despite some overlap, the cerci ÷ epiproct ratio was < 0.75 for all female *E. cynosura* and ≥ 0.75 for 90% of female *E. spinigera* (Table 2). We did not find a difference between species in the cerci ÷ epiproct ratio for male exuviae (*T* = 821.5, N = 65, *P* = 0.188).

The position of the ante-apical tubercles on the epiproct of male exuviae, expressed as the ratio of the distance from the base of the epiproct to the distal margin of the tubercle ÷ the total length of the epiproct, differed between species. The tubercles of *E. cynosura* were located more distally on the epiproct than those of *E. spinigera* (Table 2). However, the difference between species was slight and there was some overlap. Nearly all (98%) *E. cynosura* had a tubercle distance from base ÷ epiproct length ratio ≥ 0.66, whereas 73% of *E. spinigera* had values < 0.66.

The shape of the dorsal hook on S8, with respect to the direction in which the tip was pointed in strict lateral view, sometimes differed between species (Fig. 2). Often (55% of *E. cynosura*; 36% of *E. spinigera*), the tip of the dorsal hook on S8 pointed straight rearward in line with the long axis of the body. In these cases, the character would have had no diagnostic value. However, among the remainder, the tip of the hook pointed either slightly downward (ventrally – Fig. 2a) or slightly upward (dorsally – Fig. 2b) relative to a long-axis body line.
Table 2. Mean character ratios of reared exuviae of *Epitheca cynosura* and *E. spinigera* from Wisconsin (SE in parentheses).

<table>
<thead>
<tr>
<th>Species</th>
<th>Cerci/epiproct</th>
<th>Base to tubercle/epiproct</th>
<th>Spine of 8/Spine of 9</th>
<th>Abdomen W at base/L</th>
<th>Abdomen W at S6/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. cynosura</em></td>
<td>0.656 (0.00746)</td>
<td>0.708 (0.00381)</td>
<td>0.181 (0.00253)</td>
<td>0.298 (0.00458)</td>
<td>0.685 (0.00275)</td>
</tr>
<tr>
<td><em>E. spinigera</em></td>
<td>0.783 (0.00701)</td>
<td>0.645 (0.00623)</td>
<td>0.163 (0.00211)</td>
<td>0.301 (0.00533)</td>
<td>0.692 (0.00416)</td>
</tr>
</tbody>
</table>

Figure 2. Dorsum of abdomen in lateral view of exuviae of *Epitheca spinigera* (a) and *E. cynosura* (b); arrows indicate direction of S8 hook relative to long axis of body (represented by horizontal line).
All cases in which the tip pointed slightly upward were *E. cynosura*, and 93% of cases in which the hook pointed slightly downward were *E. spinigera*.

The lateral spines on S8, relative to the length of the lateral edge of the segment including the spine, averaged longer on *E. cynosura*, but the mean difference in this ratio between species was slight (Table 2) and there was substantial overlap. The slenderess of the left lateral spine on S9, expressed as the ratio of width at the base \( \div \) length, did not differ between species (Table 2). The shape of the left lateral spine on S9 (straight vs. slightly incurved) also did not differ between species \( (\chi^2 = 1.002, \text{ df} = 1, P = 0.317) \).

The shape of the abdomen (ratio of the maximum width at S6 \( \div \) total abdomen length) did not differ between exuviae of the two species \( (T = 3028.0, P = 0.229; \text{ Table 2}) \). The maximum width of the abdomen at S6 was greater for *E. spinigera* (avg. = 8.6 mm) than for *E. cynosura* (avg. = 8.1 mm); however, this difference was attributable to the larger mean size of *E. spinigera*, not to a difference in shape, and there was substantial overlap in the character.

**DISCUSSION**

Raptorial setae counts reliably distinguished between reared exuviae of *E. cynosura* and *E. spinigera*, but only if all premental setae, or preferably, total setae (all premental + all palpal setae) were counted. An optimal couplet using total setae counts \( (*E. cynosura* \leq 35; *E. spinigera* \geq 36) \) was most effective, and would have correctly determined 96% of the reared exuviae in our sample, with no substantial overlap at any single number of total setae. An optimal couplet using just premental setae counts \( (*E. cynosura* \leq 21; *E. spinigera* \geq 22) \) would have correctly determined 91% of the reared exuviae in our sample, with substantial overlap occurring only at 22 setae. The increase in efficacy gained by use of total setae counts over just premental setae counts is worthwhile and easily attained because palpal setae are easy to count, and the labial palps are exposed and readily visible when the setae on the prementum are counted. Palpal setae counts alone were unreliable for distinguishing between these species because there was considerable overlap at 14 setae, and the most reasonable diagnostic couplet using palpal setae \( (*E. cynosura* \leq 13; *E. spinigera* \geq 14) \) would erroneously determine 42% of *E. cynosura* as *E. spinigera*.

When setae counts are used, we urge that setae on both sides of the prementum and on both labial palps be counted because we frequently found unequal numbers of these setae on the two sides of exuviae of both species. Counting setae on both sides of both structures provides results that are less ambiguous and less confusing than employing counts on only one side, as has been the current practice (Walker and Corbet 1975, Bright and O’Brien 1999, Needham et al. 2000). For example, premental setae counts were used in these keys as either the sole character, or the primary character, to separate *E. spinigera* (11 or more setae on one side) from *E. cynosura*, or the group containing *E. cynosura* (10 or fewer setae on one side). Based on our data, this character would erroneously determine 14% of *E. cynosura* that have at least 11 setae on each side of the prementum, and would leave ambiguous another 25% of *E. cynosura* that have 11 setae on one side of the prementum. Thus, the character would correctly and unambiguously determine only 62% of *E. cynosura*. Adding palpal setae counts to the couplet as a secondary character (Bright and O’Brien 1999, Needham et al. 2000) did not reduce the confusion because a substantial proportion of both species have unequal numbers of palpal setae on their two sides.

We do not generally support use of total exuviae length as a diagnostic character to distinguish these species even though *E. spinigera* is often somewhat larger. Difficulties could occur because the area of overlap in length is extensive, and some lakes have small *E. spinigera*. Kormondy (1959) noted a north-south cline of last-instar size in Michigan, with smaller individuals in the southern
part of the state. Exuviae ≥ 24 mm in total length in Wisconsin are at least 12 times more likely to be *E. spinigera* than *E. cynosura*. However, only 38% of *E. spinigera* in our sample attained that size. If total setae counts of a last-instar nymph or exuviae are in the center of the region of overlap between species (35 or 36 setae), then a total length ≥ 24 mm would strongly indicate *E. spinigera*. The findings that total lengths of exuviae of both species varied among lakes, and that total lengths of *E. spinigera* were positively correlated with total setae counts, suggest that separating these species using setae counts will be most challenging with small *E. spinigera* that sometimes occur in certain lakes. Our inability to demonstrate an inter-annual effect on total exuviae length for *E. spinigera* over three years at just one site does not necessarily mean that this character does not vary among years in either species at other sites.

The findings that the cerci ÷ epiproct ratio differed significantly between female exuviae of these species was not unexpected because adult female *E. spinigera* have considerably longer cerci than do adult female *E. cynosura*. This ratio could have limited utility as an ancillary diagnostic character to separate female exuviae and last instar nymphs of these species (cerci at least 3/4 the length of the epiproct = *E. spinigera*; cerci less than 3/4 the length of the epiproct = *E. cynosura*). Although there is relatively little overlap between species, the cerci ÷ epiproct ratio has drawbacks that would reduce its diagnostic value in most circumstances including: 1) most specimens of both species are quite close to the 0.75 ratio cutoff value, requiring precise measurements to be made, 2) the two cerci on a single specimen may differ slightly in length, requiring that both be measured and averaged, which takes additional time, 3) the character can be applied to specimens of only one gender, and 4) reliability in excess of 95% can be achieved using the simpler character of total setae counts alone. However, in cases where last-instar nymphs and exuviae have total setae counts of 35 or 36, and total lengths that are < 24 mm, calculating the cerci ÷ epiproct ratios of females could aid in making determinations.

The finding that the position of the ante-apical tubercles on the epiprocts of male exuviae differs somewhat between species also has limited diagnostic value, because as with the previous character, the difference was slight (thus requiring exacting measurements), there was some overlap, the character can be applied to only part of the population, and a better diagnostic character exists. As with the cerci ÷ epiproct ratios of females, the tubercles ÷ epiproct ratios of males (< 0.66 = *E. spinigera*; ≥ 0.66 = *E. cynosura*) could be helpful to weigh into the identification process in cases where last-instar male nymphs and exuviae have total setae counts of 35 or 36, and total lengths < 24 mm.

We hesitate to recommend use of dorsal hook and lateral spine characters to separate these species, even though some significant differences were found, because substantial variation occurred in these characters that could have been environmentally induced. The direction in which the tip of the dorsal hook on S8 points could cautiously be used as an ancillary character to reinforce determinations made using other characters. In the approximately 54% of the exuviae of the two species in our sample in which the tip of this hook did not point straight rearward in line with the long axis of the body, an upward pointing tip strongly indicated *E. cynosura* and a downward pointing tip suggested *E. spinigera*, though somewhat less strongly. Although the lateral spine on S8 averaged significantly longer relative to the length of the segment margin on *E. cynosura*, the difference between the species was slight and there was considerable overlap, which reduced the diagnostic value of the character. Our assessments of the shape and dimensions of the left lateral spine on S9 led us to the same conclusion reached by Walker (1913) and Kormondy (1959), that there are no useful diagnostic attributes associated with this spine.

Contrary to our expectations, the shape of the abdomen was highly variable for each species, and it did not differ between species. Part of the reason...
for this variation could have been associated with the difficulty, because of the various contorted shapes of exuviae, of depressing the sclerite on the venter of segment 6 in a consistent way to determine maximum width, and of obtaining a consistent value for total abdomen length.

RECOMMENDATIONS

Use of total setae counts (E. cynosura ≤ 35; E. spinigera ≥ 36) is the most effective way to separate exuviae and last-instar nymphs of these species. These counts correctly determined 96% of the reared exuviae in our sample, with no substantial overlap at any single number of total setae. For specimens in the center of the area of slight overlap (35 or 36 total setae), several ancillary characters can be used to reinforce determinations including: total length (if ≥ 24 mm – E. spinigera), cerci ÷ epiproct ratio for females (E. cynosura < 0.75; E. spinigera ≥ 0.75), distance to ante-apical tubercle ÷ epiproct for males (E. spinigera < 0.66; E. cynosura ≥ 0.66), and the direction the tip of the dorsal hook on segment 8 projects in lateral view (slightly upward – E. cynosura; slightly downward – E. spinigera). These recommendations are intended for application only with last-instar nymphs and exuviae and only within the region we sampled (Wisconsin). Extrapolation of these recommendations with younger nymphs or outside this region should be undertaken cautiously.

ACKNOWLEDGMENTS

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LITERATURE CITED


A NEW STATE RECORD FOR *OLIXON BANKSII* (HYMENOPTERA: RHOPALOSOMATIDAE) IN MISSOURI.

Diane L. Wood¹ ² and M. Anthony Maupin¹

The cosmopolitan family Rhopalosomatidae is comprised of four genera and 37 species (Townes 1977, Goulet and Huber 1993, Fernandez and Sarmiento-M 2002, Lohrmann and Ohl 2007). It is represented in America north of Mexico by three genera, each with one species: *Olixon banksii* Brues, *Rhopalosoma nearticum* Brues, *Liosphex varius* Townes (Town 1977, Goulet and Huber 1993, MacGowan 1998). Rhopalosomatids have been reported only as ectoparasitoids of immature crickets (Goulet and Huber 1993).

*Olixon banksii* occurs primarily in the eastern United States (Ramsdell and Taylor 2006). It is rarely collected (Maes et al. 1993, McGown 1998, Krauth 2000), most often in pitfall traps (Ramsdell and Taylor 2006).

During an insect inventory of southeast Missouri, four males and three females of *O. banksii* were collected in pitfall traps placed in stands of *Arundinaria gigantea* (Walt.) Muhl. (Gleason and Cronquist 1991) in Bollinger, Cape Girardeau, and New Madrid counties during July 2006 and July 2007. All were brachypterous and had only mesothoracic wings. This is the first known recorded collection of *O. banksii* from Missouri.

ACKNOWLEDGEMENTS

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LITERATURE CITED


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