FORAGING AND FECUNDITY OF *Larra bicolor* (HYMENOPTERA: SPHECIDAE) A PARASITOID OF *Scapeaeriscus* MOLE CRICKETS.

By

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To my loving parents and family who have always accepted and encouraged my fascination with insects
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FORAGING AND FECUNDITY OF *Larra bicolor* (HYMENOPTERA: SPHECIDAE)  
A PARASITOID OF *Scapteriscus* MOLE CRICKETS

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Studies on important aspects of the biology and behavior of *Larra bicolor* F. (Hymenoptera: Sphecidae) such as nectar source preferences, host foraging patterns, fecundity and ovary morphology are presented. In July, August and September as many adult wasps were observed on the native plant *Chamaecrista fasciculata* (Michx.) Greene as were observed on the non-native control plant *Spermacoce verticillata* L. In the following months most wasps were observed on the control plant and very few if any on the native species which had deteriorated later in the year. Possibilities for using these two plants in habitat management are discussed.

Studies on the foraging patterns of *Larra bicolor* revealed that the number of parasitized mole crickets was found to be larger in high density host patches. Additionally, greater numbers of parasitized mole crickets were found in pitfall traps that were closer to a plot of *Spermacoce verticillata*. This indicated that *Larra bicolor* females are more abundant near nectar sources. Implications for biological control and population dynamics are discussed. Fecundity studies revealed that female wasps lived an average of 23.5 days and produced an average of 56.05 eggs during their lifetime. Eggs were produced at an average rate of 2.44 eggs per day. The number of eggs that females produce was positively correlated to their size (weight). The number of eggs that they produce is positively linearly related to their lifespan. Characterization of *Larra*
*bicolor*’s ovaries revealed that ovaries and ovarioles averaged 9.68 mm and 9.23 mm in length, respectively. They carried an average of 7.60 mature eggs and an average of 69.0 developing oocytes. Additionally, ovary length, ovariole length and egg load all corresponded positively with female size and eggload correlated negatively with egg length. Observations of superparasitized mole crickets indicated that mole cricket hosts cannot support more than one larval parasite. Superparasitism by *Larra bicolor* yields two possible outcomes, either both larvae die or only one larva survives while the others perish. The effect of superparasitism on biological control and wasp populations is discussed.
CHAPTER 1  
INTRODUCTION AND LITERATURE REVIEW  

Introduction to the Mole Cricket Problem in Florida  

Mole crickets belonging to the genus *Scapteriscus* (Orthoptera: Gryllotalpidae) have become the most destructive turf- and pasture-grass pests in Florida and the southeastern United States (Hudson 1988, Frank 1990). Hospitable environmental conditions in the region, such as abundant food resources, warm temperatures, sandy soil, and the lack of specialist natural enemies, have allowed *Scapteriscus* populations to grow and spread rapidly (Castner 1988b, Frank 1994). Bahiagrass (*Paspalum notatum* Flügge), commonly grown on beef cattle ranches, and Bermudagrass (*Cynodon* spp.), used to sod golf course fairways, are especially susceptible to mole cricket damage (Frank 1999). Mole crickets not only destroy turf-grass, they also injure vegetable seedlings and ornamental plants by feeding on roots and shearing off stems at ground level (Frank 1990, 1999). Lastly, areas heavily populated by mole crickets can attract insectivorous mammals, such as raccoons, moles and armadillos whose foraging activity may cause additional damage to the local vegetation (Frank 1999, 2003).

In Florida alone, mole crickets are responsible for the destruction of several hundred thousand acres of turf each year. In 1986, it was estimated that ranchers, golf courses and sod farmers lost a total of $45 million as a direct consequence of turf damage caused by mole crickets. Moreover, in 1996, turf-related industries reportedly spent approximately $18 million on insecticides or other control treatments (Frank 1999). Because turf-grass is crucial to many economically important industries in the southeastern United States, particularly Florida, there is a need for mole cricket control methods that are effective, economical and environmentally safe.
History of the Mole Cricket Invasion

Three species of *Scapteriscus* mole crickets arrived into the southern United States between 1899 and 1926 (Walker 1981). It is believed that the short-winged mole cricket, *S. abbreviatus* Scudder, the southern mole cricket, *S. borellii* Giglio-Tos, and the tawny mole cricket, *S. vicinus* Scudder, were inadvertently transported from Argentina and/or Uruguay in soil which was used as ballast for cargo ships (Walker 1981, Castner 1984, Frank 1994, Parkman 1996, Frank 1999). Current distributions of the southern mole cricket and the tawny mole cricket extend throughout the southern Coastal Plain from eastern Texas to North Carolina (Walker 1981, Frank 1990, Parkman 1996, Henne 2001). Isolated populations of *S. borellii* have also been reported in Arizona (Nickle 1988, Parkman 1996, Henne 2001) and California (Frank 1994, Henne 2001). The distribution of the short-winged mole cricket remains limited to coastal regions in southern Florida, northeast Florida and southern Georgia (Castner 1988a).

*Scapteriscus abbreviatus’* failure to expand its range further most likely stems from its inability to fly (Castner 1988a, Frank 1990, 1999).

Mole Cricket Basic Biology and Behavior

Mole crickets are so named because they tunnel just below the surface of the soil using a pair of enlarged muscular forelegs. Their forelimbs are able to cut through soil rapidly because they bear heavy blade-like projections known as dactyls (Smith 1893, Frank 1994, 1999). The number and arrangement of dactyls are useful indicators for distinguishing species (Frank 2003). Mole crickets are omnivorous; therefore, they readily feed on both plant and animal material (Matheny 1981). They generally injure grasses and seedling vegetation by feeding aboveground on the foliage and stems, or belowground on the roots and tubers. Their burrowing activities also harm plants by dislodging the roots causing the plants to desiccate (Frank 1998). Southern mole crickets are primarily carnivores; thus, they typically inflict less herbivory damage than either
tawny or short-winged mole crickets (Matheny 1981, Brandenburg 2002). Southern mole crickets, however, tend to be more active diggers. Consequently, the damage that they inflict upon vegetation largely results from their tunneling behavior (Brandenburg 2002). Although they are major pests in southeastern Florida, there is generally less concern for the harm caused by short-winged mole crickets because of their limited distribution (Castner 1988a).

**Benefits of Mole Cricket Biological Control**

Recurrent application of toxic chemicals, such as chlordane or soil fumigants, has been the prevailing mole cricket control strategy in Florida since the 1940s (Frank 1990, 1999, Lewis 1997). However, the treatment of cattle grazing land with pesticides presents a problem because the insecticidal toxins contained within such treatments may also pose a risk to livestock (Adjei 2000). Furthermore, a 1994 survey of Florida pest species exhibiting resistance to commonly used insecticides identified *Scapteriscus* mole crickets as displaying signs of resistance to chlordane (Leibee 1995). A ban on the agricultural use of chlordane in the United States and a general impetus toward reducing or eliminating the use of chemical insecticides necessitates the implementation of self-sustaining area-wide biological control measures (Leibee 1995, Lewis 1997). Establishment of effective biological control methods, which are capable of restricting pest mole cricket populations to acceptable levels, would ultimately benefit turf-grass growers by decreasing their losses due to damage by mole crickets as well as reducing their overall maintenance costs. In addition, biological control of these pests will help curtail the release of potentially harmful chemicals into the environment (Frank 1999).

**History of *Larra bicolor’s* Introduction into Florida**

for combating an invasive species of mole cricket, *Gryllotalpa orientalis* Burmeister. After unsuccessful attempts at relocating *L. bicolor* F. from Brazil and *L. amplipennis* F. Smith from the Philippines, a population of *L. polita* F. Smith, acquired from the Philippines, was finally established in Hawaii by 1925 (Frank 1995, 1999). Correspondingly, a population of *L. bicolor* had been established in Puerto Rico by 1941 in an attempt to control the “Changa”, *Scapteriscus didactylus* Latreille, which had become a serious agricultural pest on the island (Wolcott 1941, Frank 1995).

In the 1940s, a single attempt to import *L. bicolor* into Florida from Puerto Rico failed (Frank 1994, 1995). With the foundation of the University of Florida’s mole cricket research program in 1979, a renewed effort to introduce *L. bicolor* into Florida was organized (Frank 1994, 1995, 1999). In 1981, large numbers of female wasps were captured in Puerto Rico and released in Ft. Lauderdale, Gainesville, and Tampa. In 1982 and 1983, more wasps were released in Bradenton, Ft. Lauderdale and Lakeland. Despite multiple releases at various locations over three years, Ft. Lauderdale became the only release site where *L. bicolor* managed to survive (Sailer 1985, Frank 1990, 1995).

F. D. Bennett hypothesized that establishment of *L. bicolor* was unsuccessful in northern Florida because the stock, which originated from Brazil, was unable to tolerate the colder winters of the higher latitudes (Castner 1988a). Therefore, researchers collected more living specimens of *L. bicolor* from Santa Cruz de la Sierra, Bolivia, and released them in Alachua County, Florida, from 1988-1989 (Frank 1990, 1995 Bennett 1991). By 1993, a population of *L. bicolor* had been established in the Gainesville metropolitan area; by 2002, the wasps had dispersed at least 30 km to the west and northeast and 280 km northwest and south from their initial release site (Frank 2003).
Basic Biology and Behavior of *Larra bicolor*


Adults primarily feed on nectar. Nectar provides the wasps with sugars and other vital nutrients (Smith 1935, Castner 1988b, Pruett 1991). Although these wasps are known to feed from the flowers of various plants, field tests and observations indicate that they are particularly responsive to *Spermacoce verticillata* L. (Rubiaceae) (Williams 1928, Castner 1988a, Bennett 1991, Pruett 1991). The wasp’s preference for *S. verticillata* most likely stems from the fact that the flowers possess shallow corollas which are accessible to *L. bicolor*’s comparatively short tongue (Arévalo-Rodríguez 2005). It is believed that female wasps are observed on flowers less often than males because females spend most of their time hunting for mole crickets (Castner 1988b).

Adult female wasps locate mole cricket tunnels, known as galleries, by searching along the ground. Once a gallery is located, she digs down into the soil and forces the inhabiting mole cricket to the surface (Smith 1935, Bohart 1976, Castner 1988b, Bennett 1991). Once the cricket surfaces, the wasp chases it down and pounces on it. The female positions herself above the cricket’s pronotum, perpendicular to the longitudinal axis of the host; she then bends her
abdomen underneath and administers repeated stings to the ventral base of the cricket’s prothorax, mesothorax, and cervix (Smith 1935, Steiner 1984, Castner 1988b). As soon as the paralyzing effect from her stings immobilizes the cricket, the wasp attaches a single egg to the cricket’s ventral thorax, between its first and second pair of legs (Bohart 1976, Castner 1988b). When paralysis subsides, the cricket immediately burrows into the ground and resumes its normal activity (Smith 1935, Bohart 1976, Steiner 1984, Castner 1988b).

Eggs begin to hatch in 6-8 days. First instars remain attached to the external surface of their hosts. The larvae feed on their host’s hemolymph by puncturing the cuticle and inserting their head and mouthparts into the mole cricket’s body cavity (Smith 1923, Castner 1988b). Full larval development consists of five instars (Cushman 1935, Smith 1935, Castner 1988b). On completion of the fourth instar, the larvae kill their hosts. Fifth-instar larvae feed on the muscular and soft internal tissues of the cricket’s carcass, leaving behind an indicative pile of sclerotized remains (Smith 1935, Caster 1988b). When the grub reaches maturity, it rests for a short time before constructing an ovate cocoon from silk and soil particles (Smith 1923, Castner 1988b). Adult wasps begin to eclose 6-8 weeks later. Complete development and metamorphosis takes approximately 2 months at a temperature of $26^\circ \pm 2^\circ$ C (Castner 1988b).

**Validation of *Larra bicolor* as an Effective Classical Biological Control Agent**

Successful mole cricket control programs employing *Larra* wasps in Hawaii and Puerto Rico suggest that *L. bicolor* could be used as an effective biological control agent of *Scapteriscus* mole crickets in Florida (Frank 1999). Preliminary estimates suggest that *L. bicolor* parasitizes roughly 70% of the mole crickets inhabiting areas where the wasps are abundant (Frank, pers. comm. 2004). Furthermore, *L. bicolor* is a specialist on *Scapteriscus* spp. and shows no indication of harming the native northern mole cricket, *Neocurtilla hexadactyla* Perty or any

**Project Objectives**

Biological control ultimately reduces the costs associated with managing these destructive pests because it establishes a permanent stable control system which requires little or no effort to maintain. Moreover, biological control circumvents many of the drawbacks often associated with the use of chemical pesticides, such as pest resistance and environmental pollution. Unfortunately too many biological control programs fall short of accomplishing their goals due to the lack of information concerning the basic biology and behavior of the selected control species. In the case of *L. bicolor*, significant deficiencies remain in our understanding of the wasp’s feeding preferences, foraging behavior and fecundity. The main idea behind this project was to resolve these deficiencies in order to acquire a more complete understanding of *Larra’s* biology and behavior. The first objective was to evaluate the viability of native plants as food sources for *L. bicolor* by comparing the number of adult wasps attracted to the native plant species with the number attracted to the adventive species *Spermacoce verticillata*. The second objective was to determine the distance *L. bicolor* females will travel from a food source to forage for suitable hosts. This was accomplished by measuring parasitism levels in mole crickets collected at increasing distances from a large patch of *S. verticillata* plants, the wasp’s preferred food source. The third objective was to measure the potential fecundity and oviposition rate in female wasps by captive rearing females and counting the number of eggs they produced each day. The fourth and final objective was to characterize the structure of *L. bicolor*’s ovaries by taking digital photographs of the organs and quantifying important features using image analyzing software.
CHAPTER 2
EVALUATION OF NATIVE PLANT SPECIES FOR VIABILITY AS NECTAR SOURCES AND ATTRACTANTS

Introduction

There is growing concern among ecologists, conservationists and land managers regarding the economic and ecological costs associated with the introduction of non-indigenous plant species (Gordon 1998, Curnutt 2000). The geography and subtropical climate of Florida make it particularly vulnerable to invasions by exotic species. Furthermore, periodic natural disturbances in Florida, such as hurricanes and fires, often allow adventive species to become established in areas where they would normally be excluded by biogeographical barriers or other ecological constraints (Gordon 1998, Volin 2004).

One of the principal challenges to implementing state-wide distribution and biological control of mole crickets using Larra bicolor, is that the adult wasp’s preferred food plant, Spermacoce verticillata, is generally considered non-indigenous and a potential nuisance in Florida. This plant is native to the Caribbean, Central and South America. However, its current distribution also includes south Florida, the Keys and occasional patches as far north as Alachua County in north-central Florida (Wunderlin 1998). Exactly how this plant’s range was expanded into the continental United States remains unclear. It seems likely that S. verticillata could have been distributed to other locations by storms or other natural means (Arévalo-Rodríguez 2003). Although this plant is considered non-invasive in Florida, many turf growers and farmers are still reluctant to grow S. verticillata despite the fact that it readily attracts many beneficial insects such as L. bicolor.

The availability of adult food resources can be a critical factor for the success of Hymenoptera parasitoids (Baggen 1998, Ceballo 2004, Rogers 2004). Plant nectar is one of the most widely distributed and easily obtainable food sources for wasps and other insects (Baggen
In the 1920s, *S. verticillata* was reported as being an important nectar source for *L. bicolor* (Williams 1928). Many synovigenic parasitoids, including *L. bicolor*, rely on nectar, and pollen to provide the essential nutrients required for the metabolic demands of flight activity and ovogenesis (Leius 1960, 1967a, Takasu 1995, Lewis 1998, Eliopoulos 2003). Adult feeding positively affects parasitoid fitness parameters such as longevity, fecundity, and activity levels (Lewis 1998, Baggen 1998, Eliopoulos 2003, Rogers 2004). Acquiring more nourishment allows them to sustain longer periods of activity and provision eggs more quickly than nutritionally-deprived individuals (Takasu 1995, Lewis 1998, Wäckers 2004, Wanner 2006). In addition, the physiological condition of female parasitoids influences the likelihood that they will search for food or for hosts (Lewis 1998, Fadamiro 2001, Rogers 2004). Well-fed parasitoids should spend more time searching for hosts instead of foraging for food. Consequently, higher levels of parasitism are expected to occur in areas where rich nutrient resources and suitable host species are both readily available (Leius 1967a, Lewis 1998, Rogers 2004, Vattala 2006).

Recent studies lend support to this concept by suggesting that the availability of high-quality nectar and pollen sources attracts greater numbers of natural enemies to the target area which leads to an increase in their efficiency as biological controls. Suitable nectar resources may also facilitate the introduction and establishment of new parasitoid species (Lewis 1998, Rogers 2004, Wanner 2006). However, the mere availability of flowering plants may not be sufficient to guarantee a suitable nectar supply for adult parasitoids such as *L. bicolor* (Wäckers 2004). Current efforts have concentrated on modifying agro-ecological systems using select host plants which are known to be preferentially attractive to particular parasitoid species (Arévalo-Rodríguez 2003, Wäckers 2004). This concept can be readily applied to agro-ecosystems where effective biological control of pest mole crickets is desired. Using preferred host-plant species in
habitat management systems could be crucial to *L. bicolor*’s success at controlling pest mole crickets.

The primary objective of this study was to identify and evaluate the viability of native species of flowering plants as suitable nectar sources for *L. bicolor*. Integrating harmless native plant species into our mole cricket biological control strategy ensures that the strained ecological balance in Florida is preserved. Growing and maintaining preferred native flowering plants should increase local populations of *L. bicolor* which will result in higher mole cricket mortality rates. This “environmentally friendly” alternative to planting *S. verticillata* is expected to win approval from cattle ranchers, golf course superintendents and sod farmers.

**Materials and Methods**

Four experimental native plant species were selected for evaluation: partridge pea, *Chamaecrista fasciculata* (Michx.) Greene [Fabaceae], golden rod, *Solidago fistulosa* Michx. [Asteraceae], woodland false buttonweed, *Spermacoce remota* Lamarck [Rubiaceae] and prostrate false buttonweed, *Spermacoce prostrata* Aubl. [Rubiaceae] (Bell & Taylor 1982, Taylor 1992, Wunderlin 1998, Garland 2005 pers. comm.). *Chamaecrista fasciculata* and *S. fistulosa* were chosen on the basis of historical observations of *L. bicolor* feeding from these plants in the field (Smith 1935, Hudson 2001, Arévalo-Rodríguez 2005 pers. comm). *Spermacoce remota* and *S. prostrata* were included because they are indigenous *Spermacoce* species which occur throughout Florida (Wunderlin 1998).

In December 2005, a combination of seed stock and wild-collected seedlings of each plant species, *C. fasciculata, S. fistulosa, S. remota, S. prostrata* and *S. verticillata* (control), were collected from various locations around the Gainesville metropolitan area. From these seeds and seedlings, >40 specimens of each plant species were potted in commercial potting soil and grown up in a greenhouse behind the Entomology and Nematology building at University of Florida in
Gainesville, FL. Immediately following planting, the seeds and seedlings were watered regularly and fertilized once per month with a solution of “Peter’s All Purpose Plant Food” 20-20-20 (N-P₂O₅-K₂O) (United Industries, St. Louis, MO).

In March 2006, 15 plots that were ½ m × 1 m in size were established at two separate sites around the Gainesville metropolitan area: The University of Florida’s Beef Research Unit (BRU) and Horse Teaching Unit (HTU). Each site contained three groups of 5 treatment plots arranged in randomized complete blocks. Blocks were spaced > 30 m apart. Individual treatment plots were laid out in a side-by-side parallel array with 4 m of spacing between each plot. To prevent weeds and grasses from taking over the plots, each plot was covered with a 50 cm × 100 cm × 0.015 cm sheet of black polyethylene plastic sheeting. Plants were grown through four evenly spaced 15 cm diam. holes, cut through the plastic. Each plot was bordered by 1.0 cm diam. yellow, nylon rope which was supported by wooden stakes positioned at the corners.

Observations of adult wasps began in July 2006, when the wasps became sufficiently abundant, and continued through November 2006. Wasp counts were conducted semi-weekly around 1:00 PM at the HTU and 2:00 PM at the BRU (peak activity time). Both male and female wasps observed visiting the plants in each individual plot were counted for 1 min. A timer was used to standardize the amount of time spent at each plot. Plant condition, time of day, local temperature and basic weather conditions (sunny, partly cloudy, overcast or rainy) were also recorded for each count day.

Data were analyzed by multilevel analysis of variance (ANOVA) to determine main effects from farm, month, treatment, and their interactions using Proc GLM of the Statistical Analysis System (SAS) 9.1 (SAS Institute, Cary, NC). Count values were log + 1 transformed to achieve normality. Means and standard errors for numbers of males, females and total wasps observed on
each plant were calculated for each month. Within each month, data were pooled across farms and treatment effects were analyzed by one-way ANOVA followed by mean comparisons using Duncan’s Multiple Range tests (MRT). Analysis was performed on log-transformed data, but untransformed means and standard errors are presented in the tables.

**Results**

Multi-level Analysis of Variance (ANOVA), indicated significant (P< 0.05) effects on males, females, and total wasps from treatment, month and treatment × month interactions, but not from farm (P< 0.01). During the month of July, the greatest number of male wasps was observed on the native *C. fasciculata* and the control plant *S. verticillata*, attracting averages of 22.67 ± 11.07 and 15.00 ± 5.60 males respectively (Table 2-1). The highest average number of females was found on *C. fasciculata*, which attracted an average of 14.83 ± 8.45 female wasps (Table 2-2). The next largest number of female wasps was observed on the control plants, *S. verticillata*, which had an average of 2.17 ± 1.25 females. The native *S. fistulosa* plants attracted an average of 0.5 ± 0.5 females but no males. No wasps were observed visiting either of the other two native plant species (*S. prostrata* and *S. remota*) in July. Patterns observed in abundance of total wasps (males + females) over time (Table 2-3) were similar to those observed for male wasps (Table 2-1).

Similar numbers of male wasps were observed on the control plants, *S. verticillata* and the native *C. fasciculata* plants during the month of August, attracting averages of 30.17 ± 13.96 and 37.33 ± 18.15 male wasps respectively. However, the average number of females seen visiting the two species differed, with the native species attracting significantly more females. Small numbers of male and female wasps were also found on *S. fistulosa*. An average of 0.33 ± 0.21 males was observed on the native *S. prostrata* plants, but no males or females were seen on *S.
remota in August. Average numbers of males and females recorded for S. fistulosa, S. prostrata and S. remota were not statistically different from zero.

Comparable numbers of male and female wasps were observed visiting the control plants and the native C. fasciculata plants in September. Spermacoce verticillata attracted averages of 23.50 ± 6.85 males and 2.17 ± 1.22 females; C. fasciculata attracted averages of 18.67 ± 11.62 males and 2.83 ± 1.05 females. A small number of males, 0.5 ± 0.5, but no females were also seen on the native S. prostrata plants. No wasps were detected on either S. fistulosa or S. remota in September.

During the month of October, the greatest numbers of both male and female wasps were observed on the control plants, which attracted averages of 39.00 ± 22.52 males and 1.67 ± 1.09 females. The only other plant visited by wasps in October was S. fistulosa, which attracted an average of 0.83 ± 0.65 males but no females. The number of males observed on S. fistulosa in October was significantly less than the number of males recorded for the control plant.

The control plant, S. verticillata, continued to categorically outperform the four native plant species in November, attracting an average of 30 ± 23.18 male wasps. In comparison, 2.00 ± 2.00, 2.00 ± 1.30, and 0.10 ± 0.10, males were recorded visiting C. fasciculata, S. fistulosa, and S. remota, respectively. November was the only month that wasps were seen on S. remota. Despite attracting a large number of males, the relatively low number of females found on the S. verticillata plants was not statistically different from the numbers of females seen on the four native plant species. An average of 1.0 ± 0.63 females was observed on the control plant while C. fasciculata and S. fistulosa averaged 0.40 ± 0.40 and 0.40 ± 0.24 females respectively. No wasps were found on S. prostrata in November.
Discussion

This study investigated the viability of using native plants to attract the mole cricket hunting wasp *L. bicolor*. In the past, patches of non-native *S. verticillata* have been established throughout Florida in efforts to increase and enhance local populations of this beneficial parasitoid. Unfortunately, many turf growers and land owners are reluctant to use non-native plants for habitat management. Therefore, I evaluated the potential of four native species by comparing the numbers of wasps observed on the native plants, to the numbers of wasps found on adjacent *S. verticillata* plants.

The five plant species displayed extensive diversity in their basic phenology, growth rates and number of nectar sites (both floral and extra-floral). Hence, no effort was made to standardize plant size or flower number among species. Attempting to artificially standardize these variables across the different species would have significantly altered their primary structures and number of available nectaries which could negatively affect the plant’s ability to attract *L. bicolor*. Therefore, the different plants were allowed to grow and develop naturally because we wanted to accurately imitate natural growth conditions.

My results indicate that only one of the four native plant species (*C. fasciculata*) attracted a significant number of wasps. In fact, these plants attracted at least as many wasps as the *S. verticillata* control plants during the months of July, August and September. However, during the months of October and November *S. verticillata* continued to attract large numbers of wasps while *C. fasciculata* attracted very few if any. Many wasps were observed on *Spermacoce verticillata* during the final two months of the study because this plant continued to produce flower blossoms, whereas *C. fasciculata* began to display signs of senescence in early October. By November all the *C. fasciculata* plants had withered and died. It is interesting to note that a
few wasps were observed on this plant in November. However, it seems likely that the wasps were simply taking shelter in the plant’s tangle of dried branches.

Contributing to the mortality of *C. fasciculata* was the plant’s apparent sensitivity to cooler temperatures. During the 2nd week of October, the evening temperatures around the Gainesville metropolitan area descended to 10-15°C and by the 4th week, temperatures fell below 10°C (NCDC 2007). Coinciding with the decrease in temperature was a conspicuous deterioration in the condition of *C. fasciculata*. In contrast, the control plants were able to persist well through December that year. Exposure to cooler temperatures in October could have triggered premature mortality in *C. fasciculata* which would explain why these plants ceased to attract *L. bicolor* in the final two months of the study.

There is some ambiguity regarding the lifespan of *C. fasciculata*. According to R. P. Wunderlin’s Guide to the Vascular Plants of Florida, *C. fasciculata* blooms only during spring (March-May) and summer (June-September) (Wunderlin 1998). Richard Weaver from the Division of Plant Industry in Gainesville, FL, believes that *C. fasciculata* plants typically live 22-24 weeks (Weaver 2007 pers. comm). However my plants lived approximately 36 weeks. It is unclear why my plants lived longer than their previously reported lifespan. My plants’ resilience suggests that *C. fasciculata*’s persistence is probably influenced more by temperature and weather conditions rather than a predetermined duration period.

The lack of wasps observed on *S. prostrata* was unexpected given its similarity in appearance to *S. verticillata*. In fact, the two species can only be distinguished by a single characteristic. The calyx lobes of *S. prostrata* have green centers with white margins, whereas the calyx lobes of *S. verticillata* are uniformly green (Wunderlin 1998). The lack of wasps observed on *S. prostrata* could be explained by the fact that *S. prostrata* did not flourish as well
as *S. verticillata*. Although these native plants prospered from March through May, they did not seem to cope well with the higher temperatures and lack of water in the summer months. By the end of June many of the plants appeared stressed and ceased growing or producing flower blossoms. This plant is typically found in wet flatwoods and floodplain forests, indicating that it prefers habitats with moist soils and partial sunlight (Wunderlin 1998). These wildflowers were planted in horse and cow pastures which have drier, sandy soils and receive full sunlight. These conditions probably made it difficult for *S. prostrata* to grow successfully and produce enough floral nectar to attract *L. bicolor*.

*Spermacoce remota*, formerly named *S. assurgens* (Ruiz & Pavón), also failed to attract many wasps. Interestingly, this species was listed by James Castner as a native plant which *L. bicolor* fed from frequently in both the laboratory and the field (Castner 1988a). Castner’s findings differ sharply from my results. The explanation for this discrepancy remains unknown. However, plants that typically attract *L. bicolor* also tend to attract many other species of insects particularly Hymenoptera, Diptera, and Coleoptera. During my surveys I noted a conspicuous lack of other insects visiting this plant. This observation supports the hypothesis that *S. remota* is not an important nectar source for *L. bicolor*.

Another native plant reported by Castner as being attractive to *L. bicolor* is *Chamaesyce hirta* (L.) Millsp. (Castner 1988a). In this instance, my own field observations confirm Castner’s assessment. *Chamaesyce hirta* grows throughout the state of Florida and is commonly found in pastures and disturbed sites. According to Wunderlin, this plant flowers all year (Wunderlin 1998). However, my observations indicate that *L. bicolor* could only be found on this plant during the fall and winter months. Although this species may have potential as a nectar source
for *L. bicolor*, it was excluded from my evaluations, because this plant propagates so rapidly and aggressively that it often becomes a nuisance for turf growers.

Based on my evaluations of four native plants species, I conclude that *C. fasciculata* could be used successfully to enhance local populations of *L. bicolor*, particularly in the summer months. However, due to the decline in *C. fasciculata*’s ability to attract large numbers of wasps during October and November, this plant may be less suitable for use in habitat management systems in regions of Florida which experience cooler temperatures during these months. The prospects for this plant are more promising farther south where *C. fasciculata* persists year-round by continually reseeding itself (Weaver 2007. pers. comm.). In southern Florida, this native wild flower should attract large numbers of wasps throughout the year. In addition to its ability to attract beneficial insects, the plant’s numerous bright yellow flowers make it rather visually pleasing. Its aesthetic appeal would allow this plant to be used in broader contexts such as decorative landscapes and flower gardens. Turf growers and landowners who seek to control pest mole crickets by attracting *L. bicolor* but are concerned about maintaining non-native plants, should be encouraged to grow *C. fasciculata* instead of *S. verticillata*. 
Table 2-1  Number (\( \bar{x} \pm \text{SEM} \)) of adult males observed on each plant species. Values represent counts from all plots (2 locations \( \times \) 3 replications). Means with same letters are not significantly (\( P<0.05 \)) different according to Duncan’s MRT on log-transformed data.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermacoce verticillata</td>
<td>15.00 ± 5.60</td>
<td>A 30.17±13.96</td>
<td>A 23.50 ± 6.85</td>
<td>A 39.00±22.52</td>
<td>A 30.20±23.18</td>
</tr>
<tr>
<td>Chamaecrista fasciculata</td>
<td>22.67±11.07</td>
<td>A 37.33±18.15</td>
<td>A 18.67±11.62</td>
<td>A 0.00</td>
<td>B 2.00 ± 2.00</td>
</tr>
<tr>
<td>Solidago fistulosa</td>
<td>0.00</td>
<td>B 0.67±0.67</td>
<td>B 0.00</td>
<td>B 0.83 ± 0.65</td>
<td>B 2.00 ± 1.30</td>
</tr>
<tr>
<td>Spermacoce prostrata</td>
<td>0.00</td>
<td>B 0.33±0.21</td>
<td>B 0.50±0.50</td>
<td>B 0.00</td>
<td>B 0.00</td>
</tr>
<tr>
<td>Spermacoce remota</td>
<td>0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.10±0.10</td>
</tr>
</tbody>
</table>

Table 2-2  Number (\( \bar{x} \pm \text{SEM} \)) of adult females observed on each plant species. Values represent counts from all plots (2 locations \( \times \) 3 replications) Means with same letters are not significantly (\( P<0.05 \)) different according to Duncan’s MRT on log-transformed data.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermacoce verticillata</td>
<td>2.17±1.25</td>
<td>A 1.33±0.80</td>
<td>A 2.17±1.22</td>
<td>A 1.67±1.09</td>
<td>A 1.0 ± 0.63</td>
</tr>
<tr>
<td>Chamaecrista fasciculata</td>
<td>14.83±8.45</td>
<td>A 10.50±5.48</td>
<td>A 2.83±1.05</td>
<td>A 0.00</td>
<td>B 0.40 ± 0.40</td>
</tr>
<tr>
<td>Solidago fistulosa</td>
<td>0.50±0.50</td>
<td>B 0.50±0.50</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.40 ± 0.24</td>
</tr>
<tr>
<td>Spermacoce prostrata</td>
<td>0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
</tr>
<tr>
<td>Spermacoce remota</td>
<td>0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
</tr>
</tbody>
</table>

Table 2-3.  Number (\( \bar{x} \pm \text{SEM} \)) of total wasps (males + females) observed on each plant species. Values represent counts from all plots (2 locations \( \times \) 3 replications). Means with same letters are not significantly (\( P<0.05 \)) different according to Duncan’s MRT on log-transformed data.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermacoce verticillata</td>
<td>17.17 ± 6.54</td>
<td>A 31.50±14.57</td>
<td>A 25.67 ± 7.86</td>
<td>A 40.67±23.60</td>
<td>A 31.20±23.46</td>
</tr>
<tr>
<td>Chamaecrista fasciculata</td>
<td>37.50±19.25</td>
<td>A 47.83±23.62</td>
<td>A 21.50±12.38</td>
<td>A 0.00</td>
<td>B 2.40 ± 2.40</td>
</tr>
<tr>
<td>Solidago fistulosa</td>
<td>0.50 ± 0.50</td>
<td>B 1.17 ± 1.17</td>
<td>B 0.00</td>
<td>B 0.83 ± 0.65</td>
<td>B 2.40 ± 1.47</td>
</tr>
<tr>
<td>Spermacoce prostrata</td>
<td>0.00</td>
<td>B 0.33±0.21</td>
<td>B 0.50±0.50</td>
<td>B 0.00</td>
<td>B 0.00</td>
</tr>
<tr>
<td>Spermacoce remota</td>
<td>0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.00</td>
<td>B 0.10±0.10</td>
</tr>
</tbody>
</table>
CHAPTER 3
EFFECT OF ADULT HOST PLANTS ON FORAGING PATTERNS AND LOCAL DISTRIBUTION

Introduction

Parasitoids make up more than 10% of all metazoan species. Most belong to three orders of insects: Hymenoptera, Diptera and Coleoptera (Hassell 2000, Borror and Delong 2005). Adult parasitoid females usually oviposit one or more eggs on, in, or near a single invertebrate host (Comins 1996, Hassell 2000). After hatching from their eggs, parasitoid larvae subsist by consuming the tissues of their hosts. All the nutritional resources, necessary to complete larval development and achieve maturity, come from their hosts (Rivero-Lynch 1997, Heimpel 1998, Jervis 2001). The larva’s feeding activity typically weakens and eventually kills the host. Unlike typical predators, which consume their prey immediately, parasitoid larvae must delay killing their hosts until the parasitoid larvae are fully developed (Rivero-Lynch 1997, Heimpel 1998, Hassell 2000, Jervis 2001).

In addition to their importance as biological controls, parasitoids are also useful models for studying ecological theories of competition and predator-prey distribution (Mattiacci 1999, Hassell 2000, Darrouzet-Nardi 2006). Competition involves the struggle to obtain the necessary resources for survival and reproduction (Price 1997, Molles 2002). Synovigenic hymenopteran parasitoids, such as Larra bicolor, require two essential resources in order to survive and reproduce successfully: suitable hosts and food (Lewis 1998). Typically food and hosts are arrayed in uneven or patchy distributions. In addition they are often not simultaneously present at the same general location (Desouhant 2005, Wanner 2006). A divergent distribution of food and hosts is typical for agricultural and ornamental landscapes because these methods produce a mosaic of simple monocultures bordered by more complex habitats. Furthermore, foraging
distances for predators and parasitoids tend to be greater in simple monocultures compared with complex landscapes (Tscharntke 2004).

When adult female parasitoids are challenged with environments where hosts and food are found in different locations, the optimal decision to search for hosts or food strongly depends on the energy reserves of each female and the probability of pinpointing new hosts (Baggen 1998, Lewis 1998). When a female’s energy reserves are high she should search for more hosts. Conversely, when her energy reserves are low, she should disrupt host foraging in order to locate food. At extreme probabilities of finding food, female parasitoids should continue to search for hosts (Desouhant 2005). Consequently, landscape features in spatially patchy environments, such as the presence of flowering plants, can considerably influence the population and behavioral dynamics of natural enemies and their prey (Comins 1992).

Female parasitoids generally travel from one resource patch to another by flight (Desouhant 2005). Insect flight is very energy-demanding (Desouhant 2005, Wanner 2006). During flight, an insect’s metabolic rate increases 50-100 fold compared with its metabolism at rest (Wanner 2006). Due to their substantial energy requirements, adult wasps must locate food frequently to avoid starvation (Lewis 1998, Lavandero 2005). This implies that they must periodically interrupt their host foraging activities to find food, but flying long distances from host patches to search for food is costly in terms of energy loss and mortality risks (Baggen 1998, Lewis 1998). To minimize these costs, adult parasitoids should travel shorter distances between host patches and food sources (Wanner 2006).

Resource availability may be more important than habitat area to population survival and biodiversity (Tscharntke 2004). Applied studies have revealed that the presence of food plants affects habitat preferences by parasitoids by attracting and retaining these natural enemies to the
target areas (Takasu 1995, Lewis 1998). Additionally, the presence of food sources have been correlated with increased levels of parasitism (Mattiacci 1999, Rogers 2004). Conversely, parasitoid populations and parasitism rates in target patches without food were significantly reduced (Lewis 1998). Feeding experience in the field also appears to improve foraging efficiency since parasitoids tend to spend more time searching for hosts near the food source (Takasu 1995, Lewis 1998, Rogers 2004).

Parasitoid foraging activity is an important behavioral characteristic for species used in biological control (Wanner 2006). Female foraging and oviposition decisions dictate their dispersal and spatial distribution within landscapes. Their decisions may, in turn, be based on the distribution patterns of resources such as food and hosts (Chow 2000). Parasitoid females foraging in low-quality resource patches can increase their odds of encountering a higher-quality patch by dispersing (King 2005). However, migration between patches can have a detrimental effect on population stability because life history traits are often correlated with other factors imposing reproductive constraints on dispersers (Comins 1992, Desouhant 2003). Therefore population dynamic models emphasize the importance of density dependence and low levels of dispersal (Darrouzt-Nardi 2006).

Habitat quality is important in biological control because the lack of resources can trigger dispersal by parasitoids, thereby reducing their effectiveness at managing pests (King 2005, Wanner 2006). Moreover, the effects of habitat arrangement can be even more pronounced in simpler agricultural landscapes (Tscharntke 2004). Currently it is not well understood how habitat manipulations will effect the local distributions of L. bicolor females. For example, it is unclear how far female wasps will travel from nectar sources while hunting for suitable hosts. The second objective of this project addresses these uncertainties by measuring the total number
and the percentage of mole crickets parasitized by *L. bicolor* collected in a series of pitfall traps located at increasing distances from a rich nectar source. Ultimately, information regarding the host foraging range of the wasp will provide turf growers with useful guidelines for establishing patches of nectar source plants. Moreover, insight gained regarding seasonal changes in the levels of parasitism could help maximize the efficiency of other control treatments.

**Materials and Methods**

In August 2005, a plot 1 m × 3 m in size, containing 16 *S. verticillata* plants, was established at a private horse farm (Duncan Farm), located about 10 km north of the town of Hampton in north-central Florida. The plants were grown and maintained using the same methods outlined in Chapter 2. To prevent weeds and grasses from crowding the *Spermacoce* plants, the soil surface of the plot was covered with a sheet of black polyethylene plastic 1 m × 3 m × 0.015 cm in size. The plants grew through two rows of evenly spaced 15 cm diam. holes cut through the plastic. The plot was surrounded with barbed wire to discourage the horses from destroying the plants. A linear array of 10 pitfall traps, spaced at 20 m intervals, extended out from the long edge of the plot.

Each trap was constructed from four, 3 m sections of split 7.62 cm PVC pipe laid out at approximately 90° angles in a cross-shaped pattern (Lawrence 1982). All the pipes were capped at the far end and buried at ground level. Buried where the pipes intersect, were 20 L plastic buckets, with 4 large holes bored into the sides to accommodate the 4 sections of pipe. Smaller inner buckets filled with moist sand were placed inside the 20 L buckets. Shorter 10.5 cm lengths of thin-walled 7.62 cm PVC pipe were fitted into the larger pipes and extended into the 20 L buckets. These “extender tubes” were removable so that the smaller buckets could be easily removed and cleaned out. Mole crickets, trapped in the pipes would make their way along until they dropped off the end of the extender tubes into the inner bucket.
All traps were monitored 9 times from October-December 2005 and sixteen times from August-December 2006. Mole crickets caught by the traps, were taken back to the University of Florida’s Entomology and Nematology building in Gainesville, FL, and inspected under a stereomicroscope for the presence of *L. bicolor* larvae or eggs. Numbers of *S. borellii* and *S. vicinus* mole crickets caught, their corresponding pronotal lengths and presence of parasites were recorded for each trap. Parasitoid larval instars were also noted.

Average monthly means and standard errors were calculated for total cricket numbers and percent parasitism. Yearly means and standard errors for numbers of mole crickets, parasites, and percent parasitism were calculated for all distances from the *S. verticillata* plot, Statistical Analysis System (SAS) 9.1 (SAS Institute, Cary, NC). Correlation coefficients (*r*) were calculated between all variables to determine any relationships between them. Using the Proc Reg procedure of (SAS) 9.1, regression analysis determined the exact linear pattern of each significant (*P*< 0.05) relationship.

**Results**

The average monthly mole cricket catch increased by 42% from 2005 to 2006. The highest levels of parasitism for both years were observed in the months of November and December (Table 3-1). Due to wide variation between samples, the numbers of mole crickets and parasitoids recorded from each trap were averaged over yearly periods. The average numbers of mole crickets and parasitoids recorded for 2005 ranged from $2.56 \pm 1.23$ to $7.67 \pm 2.03$ crickets and $0.11 \pm 0.11$ to $2.00 \pm 0.90$ parasitoids per sample. In 2006, the average numbers ranged from $4.44 \pm 0.88$ to $20.56 \pm 4.84$ crickets and $0.50 \pm 0.18$ to $2.56 \pm 0.58$ parasitoids per sample. Correlations between the number of crickets caught in the traps and the number of associated parasitoids were highly significant (*r*=0.84, *P*< 0.003, *N*=10) for 2005 and (*r*=0.84, *P*=0.002, *N*=10) for 2006.
Numbers of parasitoids present at a particular location increased positively with the numbers of mole crickets also present in 2005 ($0.2874X - 0.1614$) and in 2006 ($0.1104X + 0.2731$) (Figure 3-1). The relationship was highly significant for 2005 ($R^2=0.7114$, $P<0.003$) and 2006 ($R^2=0.718$, $P<0.003$). In fact, the relationship was nearly identical for both years. Correlation analysis indicated a negative correlation between distance from the plants and the number of parasitized mole crickets per trap for 2005 ($r=-0.54$, $P<0.11$, $N=10$) and 2006 ($r=-0.64$, $P<0.05$, $N=10$). The correlation was stronger in 2006 than in 2005. Regression analysis (Figure 3-2) shows a general decline in the number of parasitized mole crickets as their distance from the host plants increased in 2005 ($-0.0056X + 1.7556$) and 2006 ($-0.0067X + 2.0042$). The relationship was weaker for 2005 ($R^2=0.29$, $P<0.11$) compared to 2006 ($R^2=0.41$, $P<0.05$). These results suggest that the probability of finding parasitized mole crickets diminishes with greater distance from *L. bicolor*’s food source.

**Discussion**

Previous studies found strong correlations between nearby flowering vegetation and greater parasitism ratios (Lewis 1998, Rogers 2004, Lee 2006, Wanner 2006). In the case of the mole cricket parasitoid, *L. bicolor*, it was not known how a nearby nectar source would affect local parasitism levels or the wasp’s distribution patterns. These effects were examined by measuring the proportion of mole crickets which were parasitized by *L. bicolor*. Crickets were collected with a series of pitfall traps, positioned at increasing distances (up to 200m) from a patch of *S. verticillata* plants, the preferred host plant of *L. bicolor*.

The data indicate that the mole cricket population in the area was not evenly distributed. This was not surprising given that most insects tend to exhibit clumped distributions (Price 1997). Parasites also exhibited a clumped distribution which correlates with the distribution of mole crickets. These results (Figure 3-1) suggest a strong positive relationship between the
number of mole crickets caught and the number of associated parasites. This indicates that
greater numbers of parasitized mole crickets can be found in areas with higher cricket population
concentrations.

Additional analysis revealed that the percentages of parasitized mole crickets caught at
each location were highly variable and did not significantly correlate with cricket abundance or
distance from the nectar source. These findings imply that *L. bicolor* females are not
concentrating their efforts in areas where mole crickets are more prevalent, rather their foraging
patterns appear to be more random. When females forage randomly, the probability of
encountering suitable hosts is dependent on the density of mole crickets inhabiting the foraging
area. The likelihood of encountering hosts increases in high density patches and deceases in low
density patches. Consequently, more parasites should be present in high density host patches.

Furthermore if females are foraging randomly, the percentage of mole crickets parasitized
in each patch should vary considerably depending on the number of individuals present and the
success rate of the wasps. If *L. bicolor* females were concentrating their search efforts on host-
rich patches, parasitism levels would correlate more closely with host density. However, the
results did not support this, so the hypothesis that *L. bicolor* females focus their host searching
activity on high density patches can be rejected.

The data also indicated that the distribution of parasitized mole crickets was affected by
their distance from the nectar producing plants. The second set of results (Figure 3-2) shows that
the number of parasitized mole crickets declines significantly as their distance from the wasp’s
host plants increases. As previously mentioned, no significant correlation exists between trap
distance and the number of mole crickets captured. Therefore I reject the hypothesis that the
decline in parasitism levels, relative to distance, was due to a corresponding decrease in mole
cricket abundance. These results suggest that *L. bicolor*’s host searching patterns are not completely random, but rather they spend significantly more time searching for hosts close to familiar nectar sources.

By integrating the conclusions from both sets of results, we recognize that parasitism levels are influenced by two aspects of mole cricket population demographics; the population’s regional density and its proximity to high-quality nectar sources. Both factors have important implications concerning habitat management and biological control. These results suggest that the most effective use of habitat management would be to focus on areas that are inhabited by dense populations of mole crickets. Nectar source patches established in close proximity (< 100 m) to these areas could increase and enhance the resident *L. bicolor* population, thereby maximizing the number of mole crickets encountered by the wasps. This should result in a rise in parasitism levels and a proportional reduction in the mole cricket populations.

**Table 3-1** Number (x̄ ± SEM) of mole crickets caught in pitfall traps and percent parasitized by *Larva bicolor*. Values represent monthly sampling means (2005: Oct N=2, Nov N=5, Dec N=2; 2006: Aug N=1, Sept N=4, Oct N=4, Nov N=6, Dec N=1).

<table>
<thead>
<tr>
<th>month</th>
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<tr>
<td></td>
<td>Total Crickets</td>
<td>Crickets/Trap</td>
<td>% Parasitism</td>
<td>Total Crickets</td>
<td>Crickets/Trap</td>
<td>% Parasitism</td>
</tr>
<tr>
<td>August</td>
<td>57.0 ± 0.0</td>
<td>5.7 ± 0.0</td>
<td>5.3 ± 0.0</td>
<td>5.7 ± 0.0</td>
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</tr>
<tr>
<td>September</td>
<td>48.3 ± 7.4</td>
<td>4.8 ± 0.7</td>
<td>5.8 ± 2.2</td>
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<td></td>
</tr>
<tr>
<td>October</td>
<td>35.0 ± 13.0</td>
<td>3.5 ± 1.3</td>
<td>6.4 ± 1.9</td>
<td>92.5 ± 53.8</td>
<td>9.3 ± 5.4</td>
<td>9.8 ± 2.2</td>
</tr>
<tr>
<td>November</td>
<td>42.8 ± 10.1</td>
<td>4.3 ± 1.0</td>
<td>31.5 ± 6.4</td>
<td>118.8 ± 31.8</td>
<td>11.9 ± 3.2</td>
<td>18.7 ± 1.5</td>
</tr>
<tr>
<td>December</td>
<td>57.0 ± 44.0</td>
<td>5.7 ± 4.4</td>
<td>26.9 ± 6.4</td>
<td>70.0 ± 0.0</td>
<td>7.0 ± 0.0</td>
<td>17.1 ± 0.0</td>
</tr>
</tbody>
</table>
Figure 3-1 Average number of parasites present as a function of the number of mole crickets captured in 10 pitfall traps placed at 20 m intervals extending out from a patch of nectar source plants. (A) Numbers recorded for 2005. (B) Numbers recorded for 2006.

Figure 3-2 Average number of parasites present as a function of distance from the adult wasps preferred food source. (A) Numbers of parasitoids recorded in 2005. (B) Numbers of parasitoids recorded in 2006.
CHAPTER 4
POTENTIAL FECUNDITY AND OVIPOSITION RATE

Introduction

Fecundity and oviposition behavior are fundamental aspects of an insect’s lifehistory, ecology, and population dynamics. Fecundity refers to the number of eggs or offspring (for species that bear live young) produced by individual females (Minkenberg 1992, Molles 2002). This life history trait is particularly important for parasitoids because of their frequent application in biological control (Mills 2000). Parasitoids that have relatively high fecundity tend to be more successful at reducing pests because they have the ability to reproduce rapidly in direct response to increases in pest abundance (Ceballo 2004). Hence, a clear understanding of the reproductive strategies of parasitoid natural enemies is necessary for improving the success and efficiency of these insects as biological controls (Eliopoulos 2003, Zhang 2004).

Reproductive success of female parasitoids is determined by the number of eggs that they oviposit in their lifetime and the survivorship of the offspring (Minkenberg 1992). A parasitoid’s overall reproductive performance can be characterized by three elements which are related to their total reproductive output: maximum fecundity, potential fecundity and realized fecundity. Maximum fecundity represents the greatest number of eggs that an individual female can produce under optimal conditions. Potential fecundity denotes the average number of eggs that a population of females can generate under optimal conditions. Realized fecundity is the average egg output by the corresponding population under a specific set of field conditions (Mills 2000).

Maximum fecundity is a genetically determined characteristic which may vary among species but does not vary on average within a species. A population’s potential fecundity is reduced from the species’ maximum by factors influencing the quality and quantity of resources acquired during juvenile development. Realized fecundity of a population is further diminished
below its potential fecundity by factors which affect the longevity of the females, oogenesis and oviposition behavior (Mills 2000). A female’s total egg output is limited by a combination of factors, such as the number of mature eggs that she can produce during her lifetime, the number of suitable hosts she can locate, and her oviposition rate.

Due to the importance of fecundity as a life history and population characteristic, it is surprising that there is a substantial lack of data on potential fecundity and even fewer estimates of realized fecundity (Leather 1998, Mills 2000). Many estimates of insect fecundity are calculated from easily measured parameters like female body size, egg load or number of ovarioles. Although such related parameters may produce accurate assessments of fecundity, these associations can be invalidated by other interfering factors which were overlooked (Leather 1998, Mills 2000). For instance, adult weight may be used to estimate the total number of eggs contained within an individual female, but this quantity may have little bearing on the actual number of eggs she deposits (Mills 2000). Consequently, estimates of both potential and realized fecundity based on such shortcuts should be regarded with skepticism (Leather 1998).

The best method for attaining a better understanding of a parasitoid’s potential and realized fecundity is to measure its lifetime reproductive rate directly, by counting the number of eggs each female lays beginning the day she ecloses until her death (Sweetman 1936). Although the values obtained by these methods represent total fecundity under ideal conditions, the biology and behavior of most parasitoids make obtaining fecundity data from field populations extremely difficult. The aforementioned situation is analogous for the mole cricket hunting parasitoid *L. bicolor*. Although *L. bicolor* has been used for almost 20 years to combat *Scapteriscus* mole crickets in Florida, significant uncertainty remains regarding the mean number of eggs these wasps lay daily or their total lifetime reproductive output. The objective of this study was to
measure *L. bicolor’s* potential fecundity and daily oviposition rate. This study will also help to elucidate egg-laying patterns within days and across days throughout the wasp’s lifetime. *Larra’s* reproductive output will be measured by allowing captive-reared females to oviposit on an abundance of host mole crickets. By dividing potential fecundity with the developmental success ratio for the eggs, we will be able to more accurately estimate *L. bicolor’s* practical impact on mole cricket populations.

**Material and Methods**

Beginning in the fall of 2006, *Scapteriscus* mole crickets parasitized by *L. bicolor* were collected in pitfall traps. The parasitoids were allowed to grow and develop on their mole cricket hosts. In order to prevent the crickets from preying on one another, single crickets were housed in separate vials filled with moist sterilized sand. To keep the mole crickets alive long enough for the larvae to complete their development, the crickets were fed “FRM Cricket & Worm Feed” (Flint River Mills Inc. Bainbridge, GA, USA) twice per week and water was added to the sand as needed. Environmental conditions were standardized by keeping the vials containing parasitized crickets in a ‘Florida Reach-In’ environmental chamber (Walker 1993) (27°C, humidity 55%, 16/8 hr L/D).

Once the wasp larvae constructed their cocoons, they were carefully removed from the vials. Cocoons were sorted and separated based on size in order to keep the sexes separate. The large cocoons were presumed to house females and small cocoons contained males. Female and male cocoons were placed into separate clear polystyrene observation boxes filled halfway with moist sterilized sand. The observation boxes were also kept in a Florida Reach-In chamber (27°C, humidity 55%, 12 hr L/D) and monitored daily for adult activity.

Upon eclosing, adult wasps were removed from their plastic observation boxes and placed into a plastic shell vial. Wasps were first weighed inside the plastic vials then released into
oviposition arenas. Single females were placed in an arena with one or more males. Each oviposition arena was made by placing 2.5 cm of moist sand on the bottom of a 30×30×30 cm screened insect cage. A small potted *S. verticillata* plant and an artificial nectary were also placed inside each cage to provide food for the adult wasps. Artificial nectaries were constructed from small glass vials filled with a 50/50 mixture of honey and 20 % sucrose solution topped with an absorbent cotton wick. All oviposition arenas were kept in a temperature-regulated (85° C) greenhouse behind the University of Florida’s Entomology and Nematology building in Gainesville, FL.

Seven *Scapteriscus* mole crickets were added to each oviposition arena. Typically 4-5 *S. vicinus* and 2-3 *S. abbreviatus* were offered to each female. Adult *S. vicinus* were collected from the field using sound traps. Adult *S. abbreviatus* were acquired from the Department of Entomology and Nematology’s mole cricket rearing facility. Before being offered to the females, field caught mole crickets were thoroughly inspected to ensure that no previous *L. bicolor* eggs or larvae were present. Crickets were left in the cages for approximately 24 hr. Because *Larra* wasps are diurnal, their feeding, oviposition and flight activities would ceased ≈2 hr before sunset. Therefore, all crickets were removed from each cage around 6:00 pm and checked for eggs. Eggs were carefully detached from crickets using forceps and the crickets were placed back into clean vials with moist sand and food. Previously parasitized crickets were allowed to recover for several days before being offered to the wasps again. Parasitized crickets were always replaced by fresh *Scaptericus* specimens. This cycle was repeated daily until the female wasps died.

Analyses were performed using Statistical Analysis System (SAS) 9.1 (SAS Institute, Cary, NC). Means and standard errors were calculated for the following variables: wasp weight,
lifespan (days), total number of eggs and eggs per day. Correlation coefficients (r) were calculated between each pair of variables to determine if any relationships existed between them. Regression analysis was used to determine the exact pattern linear equation of each significant (P< 0.05) relationship.

Results

The relative size of each female wasp was quantified by its weight. Often hind tibia length is used to quantify inter-specific size variation in insects. However, it was established that female weight corresponded very strongly with tibia length for this species (data not shown) (r=0.89, P<0.0005, N=10). Average, minimum and maximum values for wasp weight, lifespan (days), lifetime fecundity, and oviposition rate (eggs/day) are shown on table 4-1. The average lifespan for this sample of females was 23.5 ± 1.87 days. The sample consisted of six females that stayed alive for ≥30 days, seven that lived 20-29 days, six that lived 10-19 days and 1 that survived <10 days. The shortest and longest lived individuals ranged from 8 to 40 days respectively, a difference of 32 days. The females produced an average 56.05 ± 4.38 eggs during their lifetime. Their minimum and maximum lifetime output ranged from 17 to 91 eggs respectively. The average daily oviposition rate for this group of females was 2.44 ± 0.14 eggs per day with a range difference of 10 eggs per day. Their average daily oviposition rate ranged from 1.32 to 3.77 eggs per day (data not shown).

Correlation analysis revealed a significant positive relationship between female weight and oviposition rate (r=0.67, P<0.006, N=15). There was also a strong correlation between female lifespan and lifetime fecundity (r=0.80, P<0.001, N=20). Daily oviposition rate (y) increases linearly (y= 0.0121x + 1.2027) as female weight (size) (x) increases (R²=0.4576, P<0.006 N=15) (Figure 4-1). This result indicates that larger females are capable of ovipositing successfully more often than smaller females. Lifetime fecundity of L. bicolor (y) is linearly
related (y=1.8751+11.985) to the number of days that the female survives (x) (R²=0.643, P< 0.001, N=20) (Figure 4-2). This result suggests that females which survive longer will produce larger clutches than short-lived individuals.

**Discussion**

Fecundity and reproductive success are key facets of female parasitoid biology. Despite *L. bicolor*’s importance for biological control of *Scapteriscus* mole crickets, very little was known regarding its reproductive capabilities and limitations. By rearing 20 adult females and maintaining them in a controlled environment, I was able to observe and measure their oviposition rates and total egg outputs. Although a few individuals were relatively short-lived, the majority (about 2/3) of the wasps lived more than 20 days. Based on these results, I conclude that *L. bicolor* survives for at least 3-4 weeks as adults.

My results show two significant relationships related to female fecundity. The first relationship indicates that larger females are ovipositing more frequently than smaller females. The precise reason for this inequality is unclear. Perhaps larger females have the ability to mature eggs faster than smaller females. This situation appears plausible if we assume that all females lay eggs which are roughly the same size and the process of yolk provisioning is nutrient-limited and not rate-limited. Additionally, venom is physiologically expensive to manufacture and is probably depleted rapidly because these wasps administer multiple stings to their prey. Large females should be equipped with proportionally bigger crops than small females. If large females are ingesting greater volumes of nectar, they potentially consume more nutrients than lesser females. Greater quantities of nutrients would allow large females to be able to provision eggs and replenish their venom supply faster than small females. As a result, large females will be ready to oviposit more often than small females.
The second relationship suggests that females that survive for longer periods of time produce a greater number of eggs than shorter-lived females. The idea that total egg output is determined by longevity is not surprising given that *L. bicolor* females are synovigenic and therefore only capable of producing a finite number of eggs per day (typically 2-3). In addition, the average egg rate for my sample of females (2.44 eggs per day) showed only minor variation (SEM=0.14) which indicates that there is little population variation in the daily oviposition rate of this species. Therefore I hypothesize that the main factor affecting the lifetime fecundity of *L. bicolor* females is the duration of their lifespan.

These results have significant implications for managing this species as a biological control of pest mole crickets. At present, there is ample evidence suggesting that parasitoid fitness parameters, such as longevity and fecundity are positively affected by adult feeding (Baggen 1998, Eliopoulos 2003, Ceballo 2004, Rogers 2004). Hence, habitat management could be used to maximize *L. bicolor*’s ability to parasitize pest mole crickets. By providing these insects with plentiful nutrient resources such as nectar, we may be able to boost the cricket killing capabilities of local wasp populations by increasing female longevity. This could substantially reduce local mole cricket populations and minimize the damage that they cause.

Table 4-1 Summary statistics for experimental variables. Values represent mean ± SEM, minimum and maximum for wasp weight (N=15), lifespan (N=20), lifetime fecundity (N=20), and oviposition rate (N=20).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
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<tbody>
<tr>
<td>Wasp Weight (mg)</td>
<td>107.43 ± 8.71</td>
<td>58</td>
<td>185</td>
</tr>
<tr>
<td>Lifespan (days)</td>
<td>23.5 ± 1.87</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Lifetime Egg Output</td>
<td>56.05 ± 4.38</td>
<td>17</td>
<td>91</td>
</tr>
<tr>
<td>Daily Egg Output</td>
<td>2.44 ± 0.14</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 4-1  Average number of eggs oviposited per day as a function of female weight (N=15).

Figure 4-2  Total lifetime egg production as a function of female lifespan (N=20).
CHAPTER 5
CHARACTERIZATION OF OVARIAL ULTRA-STRUCTURE AND QUANTIFICATION OF INTRASPECIFIC VARIATION

Introduction

Parasitoid females lay their eggs on, in or near invertebrate hosts. After hatching from the eggs, parasitoid larvae must feed on the host’s tissues in order to obtain the nutritional resources required to reach maturity. Larval feeding activity typically weakens and eventually kills their hosts (Rivero-Lynch 1997, Heimpel 1998, Jervis 2001). The larvae from many parasitoid wasps, such as *Larra bicolor*, live as koinobionts (Castner 1983, 1989). Koinobiont parasitoid larvae feed and develop on active hosts. In this type of host-parasitoid association, the parasitized hosts continue to grow and develop (as immatures) or even reproduce (as adults) before to being killed by the parasitoid (Jervis 2001).

Koinobiont parasitoid wasps can be categorized as either pro-ovigenic or synovigenic based on their method of egg production (Flanders 1950, Heimpel 1998, Jervis 2001). Before oviposition, pro-ovigenic parasitoids already possess a fixed number of mature eggs. The egg capacity of pro-ovigenic parasitoids is determined by the amount of nutrients acquired by the larval stages. Conversely, synovigenic parasitoids, such as *L. bicolor*, continue to provision and produce mature eggs throughout their reproductive life (Flanders 1950 Jervis 2001). Synovigenic species typically obtain the nutrients necessary for egg production from feeding on nectar, honeydew or host tissues (Heimpel 1998, Jervis 2001, Eliopoulos 2003). Synovigenic koinobiont parasitoids are especially valuable as biological controls because they can attack the adult or immature stages of a host. This enables these parasitoids to develop successfully under a greater range of host population structures (Ceballo 2004).

The reproductive strategies and capabilities of parasitoids are constrained by their evolutionary history and anatomy (i.e., morphology of their reproductive organs) (Jervis 2001,
Ohl 2002). Hymenopteran ovaries are formed from multiple, elongated tube-like, functional units called ovarioles. Each ovariole contains a progressive series of developing oocytes which are individually enclosed in epithelial follicles (Nation 2002). All Hymenoptera bear meroistic polytrophic ovarioles; meaning, each developing oocyte possesses a cluster of associated nurse cells (meroistic). Nurse cells are located either within the same follicle as the oocyte or in an adjacent follicle (polytrophic) (Nation 2002, Simiiczyjew 2002, Martins 2004).

Ovarioles are subdivided into three basic structural regions: the terminal filament, the germarium, and the vitellarium. The terminal filament, located at the proximal tip of the ovariole, acts as an attachment point that links the ovarioles to supporting connective tissue (Martins 2004). The germarium is the small region adjacent to the terminal filament where the process of oocyte production (ovigenesis) occurs. The remaining bulk of the ovariole consists of the vitellarium. Oocyte development, yolk uptake and vitelligenesis take place in this region (Nation 2002, Martins 2004).

Maximum rate of ovigenesis influences the lifetime reproductive output of synovigenic parasitoids (Rosenheim 2000, Eliopoulos 2003). Ovigenesis is a nutrient-limited process and adult wasps require nutrients for both metabolic maintenance and reproduction. As a result, egg maturation rate and reproductive output are often directly related to the amount of nutritional resources acquired by the female (Jervis 2001, Ceballo 2004).

Another ovarial characteristic which also affects egg maturation rate and realized fecundity is egg load (Rosenheim 2000, Ceballo 2004). Egg load is the maximum number of mature eggs that a female wasp can store in her ovaries. Egg load is the end result of a dynamic process involving input from egg production and output from oviposition (Minkenberg 1992). Egg load influences behaviors such as searching efficiency and motivation to oviposit (Eliopoulos 2003).
Wasps have been shown to maintain relatively constant egg loads by adjusting their egg maturation rates (Rivero-Lynch 1997, Jervis 2003). Optimal fecundity models predict that clutch size should correlate positively with egg load and the rate of egg production (Minkenberg 1992).

Many studies that examine parasitoid reproduction tend to focus mainly on their reproductive output and behavior. As a consequence, associations with the insect’s internal anatomy are often overlooked. Despite *L. bicolor*’s importance as a biological control of *Scapteriscus* mole crickets, very little information was available on the anatomy of its reproductive system. The objective of this study was to characterize *L. bicolor*’s ovary morphology and measure the variability of each trait. Knowledge of this beneficial parasitoid’s reproductive anatomy, combined with information regarding its potential fecundity, will improve our understanding of this insect’s overall reproductive capabilities and limitations.

**Materials and Methods**

Female wasps were collected from the University of Florida’s Beef Research Unit (BRU) and immediately transported back to the lab where they were chilled in a refrigerator. Once the wasps were immobilized, the length of their right hind tibia was measured. Next, their abdomens were severed at the petiole, using small spring scissors. Ovaries were removed from the loose abdomens by grasping the last abdominal segment with a forceps and gently pulling it away from the rest of the segments; bringing along with it the wasp’s entire reproductive tract. Once removed, the reproductive organs were placed on a 60×15 mm Petri dish filled with ice cold 1% phosphate buffer saline (PBS) solution (pH 7.0). The ovaries were quickly separated from the rest of the wasp’s reproductive organs and rinsed in a new Petri dish with fresh PBS. After rinsing, the ovaries were put into a small vial filled with 30% glycerol in PBS and placed in the refrigerator to equilibrate overnight. The following day the ovaries were moved to a watch-glass filled with neutral red staining solution. After 2 min the ovaries were removed from the stain and
washed for 5 min in a 5 ml beaker of ice cold PBS. After washing, the ovarioles were carefully separated from one another and each ovary was detached from the common oviduct. Next, each ovary was temporarily slide-mounted in 30% glycerol solution.

After slide-mounting, the ovaries were photographed using a stereomicroscope outfitted with the Auto-Montage® digital-photography system (Leica Microsytems, CA). Photographs were taken at 10× magnification. Graphics editing software (Photoshop 5.5®) was used to remove noise and enhance the quality of the images. Auto-Montage® image processing software was used to scale the images and make measurements of the different ovarial components.

Means, standard errors and ranges were calculated (Proc means) for tibia length, ovary length, ovariole length, mature egg number, mature egg length, oocyte number, largest oocyte length and smallest oocyte length. To determine any relationships between each pairs of variables, correlation coefficients were computed using Statistical Analysis System (SAS) 9.1 (SAS Institute, Cary, NC).

Results

All wasps had two ovaries (right and left) and each ovary consisted of three ovarioles. No variation in the basic structure of the ovaries was observed. Various parts of the ovaries such as, the germarium, oocytes, nurse cells and mature eggs, stain differently. For instance, no color could be detected within the germarium. Undeveloped oocytes stained weakly and therefore appeared pink. More intense staining of nurse cells caused them to look reddish. Mature eggs typically appeared pallid and showed little indication of significant staining.

Female tibia length averaged 3.01 ± 0.08 mm, with minimum and maximum values of 2.70 mm and 3.45 mm respectively (Table 5.1). Ovaries had an average length of 9.68 ± 0.37 mm with the smallest ovary measuring 7.70 mm and the largest ovary measuring 11.53 mm. Average ovariole length for the wasps was 9.23 ± 0.38 mm. The shortest ovariole measured 7.21 mm and
the longest ovariole measured 11.10 mm. Average egg load was 7.60 ± 0.63 eggs. The lowest number of eggs recorded from a single female was 4 and the most was 10. The average length for mature eggs was 1.63 ± 0.03 mm. The smallest egg measured 1.50 mm and the largest egg measured 1.80 mm. Females possessed an average of 69.0 ± 3.30 developing oocytes. The lowest number of oocytes counted was 54 and the highest number counted was 83.

Correlation analysis determined that several of the traits were related. Tibia length corresponded positively with ovary length (r=0.67, P<0.04, N=10), ovariole length (r=0.68, P<0.03, N=10) and egg load (r=0.68, P<0.03, N=10). Ovary length closely corresponded with egg load (r=0.76, P=0.01, N=10). Ovariole length also corresponded strongly with egg load (r=0.81, P<0.004, N=10). Lastly, egg load corresponded negatively with average egg length (r=-0.70, P<0.03, N=10).

**Discussion**

To date, information regarding the structural organization of *Larra bicolor*’s internal reproductive organs is especially limited. It is important to be familiar with the basic anatomy of parasitoids because the physical characteristics of their reproductive systems can be key determinants of their reproductive capabilities (Jervis 2001, Ohl 2002). Ovarial traits including ovariole number, numbers of mature eggs, and mature egg size closely correlate with the habits of female parasitoids (Iwata 1955, Jervis 2001). In the case of some parasitoids such as ichneumonids (Hymenoptera) and tachinids (Diptera), fecundity strongly correlates with the number of ovarioles per ovary (Price 1997). However, the number of ovarioles per ovary does not vary between individuals of *L. bicolor*. This makes it more difficult to estimate *Larra*’s potential fecundity because their fixed ovariole number cannot account for observed variations in egg output (see Chapter 4).
My results show several significant correlations between variables. The first set of correlations suggest that the magnitude of certain traits, such as the average length of ovaries and ovarioles and the number of mature eggs carried by females, are at least partially determined by the size of the wasp. This is understandable, considering that mature egg size and largest ooctye size (data not shown) displayed relatively minor variation (SEM= ±0.03) and (SEM= ±0.04) respectively. These correlations also help to explain the differences in the number of mature eggs carried by the females. We can assume that if mature eggs have a relatively constant volume and abdomen size is proportional to tibia length, than large females will be able to hold more eggs because their abdomens have higher storage capacities then small females. Furthermore, females that are carrying more mature eggs will have increased opportunities to oviposit than females carrying fewer eggs.

Another significant correlation indicates that egg load varies inversely to the size of the eggs. This result suggests that a parental investment trade-off exists within the context of available egg storage space. Therefore females must either produce fewer large eggs or a greater number of small eggs. Evolutionarily, both strategies have potential to increase female fitness levels. Producing larger eggs increases the probability that the larvae which hatch will be healthy and have better survival rates. Producing greater numbers of eggs increases the probability that at least some the resulting larvae will live to adulthood, despite lower survival rates. Currently it is uncertain what factors are important for determining egg size and number for *L. bicolor*. A comprehensive analysis of r vs K strategy for parasitoids is beyond the scope of this thesis, but would be an interesting subject for future work.
Figure 5-1  *L. bicolor* ovary stained with neutral red and photographed at 10× original size using the Auto-montage® imaging system attached to a stereomicroscope. Ovary was slide-mounted in 30% glycerol.

Table 5-1. Summary statistics for ovarial traits. Values represent $\bar{x} \pm$ SEM, minimum and maximum for tibia length (N=10), Ovary length (N=10), ovariole length (N=60), mature egg number (N=10), egg length (N=76), and number of oocytes (N=10).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibia Length (mm)</td>
<td>3.01 ± 0.08</td>
<td>2.70</td>
<td>3.45</td>
</tr>
<tr>
<td>Ovary Length (mm)</td>
<td>9.68 ± 0.37</td>
<td>7.70</td>
<td>11.53</td>
</tr>
<tr>
<td>Ovariole Length (mm)</td>
<td>9.23 ± 0.38</td>
<td>7.21</td>
<td>11.10</td>
</tr>
<tr>
<td>Mature Eggs</td>
<td>7.60 ± 0.63</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Egg Length (mm)</td>
<td>1.63 ± 0.03</td>
<td>1.50</td>
<td>1.80</td>
</tr>
<tr>
<td>Developing Oocytes</td>
<td>69.0 ± 3.30</td>
<td>54</td>
<td>83</td>
</tr>
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</table>
CHAPTER 6
SUPERPARSITISM AND DEVELOPMENTAL SUCCESS OF SUPERNUMERARY LARVAE

Introduction

Host selection has major implications for parasitoid fitness. Generally, it is advantageous for females to select the highest quality hosts for their offspring (Keasar 2006). Acceptance of hosts often depends upon the host’s physical and physiological characteristics. One important characteristic is whether the host has been previously parasitized (Plantegenest 2004). For many species of solitary wasps, including *Larra bicolor*, only one larva is able to develop successfully per host. The presence of supernumerary larvae leads to competition (Plantegenest 2004, Darrouzet 2007). Typically, older larvae out-compete and eventually eliminate younger rivals (Plantegenest 2004).

There are two types of superparasitism. Conspecific-superparasitism occurs when a female oviposits on a host already parasitized by another female of the species. Self-superparasitism occurs if a female oviposits on a host which she had previously parasitized (Jaramillo 2007, Darrouzet 2007). Generally, parasitoids are reluctant to superparasitize. The factors involved in a parasitoid’s motivation to superparasitize could be mediated by environmental conditions such as low host availability or by the physiological condition of the female, such as a large egg load or nearing the end of her reproductive period (Keasar 2006, Jaramillo 2006). Despite female host-discriminating abilities and individual fitness disadvantages, superparasitism is commonly observed in nature and in the laboratory (Plantegenest 2004, Jaramillo 2006).

Superparasitism by female *Larra spp.* was first described for *Larra analis* F. by Charles Smith in 1935. Smith (1935) recorded the fate of four mole crickets (*Neocurtilla hexadactyla*) carrying two eggs each. According to his description, two of the crickets died before the larvae hatched from the eggs. For the other two crickets, both larvae developed to maturity, although
they were smaller than average (Smith 1935). *L. bicolor* females typically remove previous eggs when they encounter them. However, *Scapteriscus* mole crickets occasionally become superparasitized (Castner 1988a and pers. field observs.). The following is a brief description of the fate of *Scapteriscus* mole crickets and their *L. bicolor* supernumerary parasites.

**Materials and Methods**

Superparasitized mole crickets were encountered during experiments on *L. bicolor* fecundity (see chapter 4). Mole crickets found to possess two or more eggs were placed individually into vials filled with moist clean sand. Vials were then placed in a ‘Florida Reach-In’ environmental chamber (Walker 1993) (27°C, humidity 55%, 16/8 hr L/D). Crickets were fed “FRM Cricket & Worm Feed” (Flint River Mills, Bainbridge, GA) at least twice per week, and water was added to the substrate as needed. Cricket condition and parasite development were monitored by removing the crickets or peering through the walls of the vial. Efforts were made not to disturb the crickets and parasites frequently because this seemed to adversely effect their survival.

**Results and Discussion**

Superparasitized mole crickets appeared to behave normally. The number and general location of the eggs were recorded. Results show some variation in these characteristics. Ten crickets were found with two eggs positioned on opposite sides of the mole cricket. Nine crickets were found with two eggs positioned on the same side and adjacently (Fig 6-1A). One cricket was found with three eggs, two on one side and one opposite (Fig 6-1B). Because the crickets were exposed to single females, these are all examples of self-superparasitism. However, it could not be determined whether the eggs were attached at the same time or the eggs resulted from separate attacks.
The larvae that hatched from each egg remained attached to the mole crickets in the same location as the egg (Fig 6-1A & B). Adjacent larvae made no attempts to distance themselves from one another. Despite each egg being laid within 24 hrs, larvae displayed disparity in their developmental rates (Fig 6-2B). Contrary to earlier conjecture regarding fighting between conspecific larvae (Castner 1988a), no indications of injury or death as a result of physical conflict were observed. The principal level of competition appears to be that of nutrient acquisition.

Figure 6-1  Photographs showing the different numbers and locations of *L. bicolor* eggs. A) two eggs adjacent B) three eggs.

Figure 6-2  Photographs showing the development of supernumerary *L. bicolor* larvae. A) larvae opposite sides B) larvae same side.
Twenty instances of supernumerary larvae and their mole cricket hosts were monitored and recorded. For the 10 instances where mole crickets were found with two eggs on opposite sides, half of the time, only one larva per cricket was able to develop to maturity. For the rest, both larvae and their host crickets died. Larvae exhibited a 25% survival ratio. Of the nine crickets found with two adjacent eggs, in four instances, one larva out of two managed to complete its development. The remaining five crickets and their parasites died. In this case larvae exhibited a mere 22.2% survival ratio. Only one larva developed fully on the lone cricket found with three eggs. The total percent survival for all the larvae was 24.4%. This is a much lower proportion compared with their normal 97.6 % survival ratio (Cabrera-Mireles 2002).

These results indicate that a single mole cricket cannot successfully support the complete development of more than one *L. bicolor* larva. Furthermore, superparasitism does not contribute to increasing the death rate of mole crickets. One larva is sufficient to kill a mole cricket. More importantly, the drastic reduction in larval survival suggests that superparasitism lowers the reproductive fitness of females. By lowering larval survival ratios, superparasitism should negatively affect future adult parasitoid populations.
LIST OF REFERENCES


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BIOGRAPHICAL SKETCH

Scott Linus Portman was born on February 21, 1972 in St. Louis, Missouri. He earned his Bachelor of Science in Biology from Southeast Missouri State University in 1996. After graduation he began a job at Incyte Genomics Inc, St. Louis, MO, and worked on a project to map the human genome. After the project was terminated in 1999, he was supervising technician in the DiAntonio Lab at the Washington University School of Medicine, St. Louis, MO. Scott spent four years working with Aaron DiAntonio on the development and functioning of nerve synapses at the Drosophila neuron-muscular junction. Scott always held a certain fascination for insects, so in 2004 he was accepted into the Department of Entomology and Nematology’s graduate program at the University of Florida. After completing his master of science at the University of Florida, Scott will begin working toward his Ph.D. at Pennsylvania State University, under the sponsorship of Dr. James Marden.