

EFFECT OF HOST PLANTS AND PESTICIDES ON PARASITISM OF  
*HAECKELIANA SPERATA* ON *DIAPREPES ABBREVIATUS*

By

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To my parents

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Abstract of Thesis Presented to the Graduate School  
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EFFECT OF HOST PLANTS AND PESTICIDES ON PARASITISM OF  
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Two investigations were conducted to determine the effect of host plants and pesticides on *Haeckeliana sperata* (Hymenoptera: Trichogrammatidae), a gregarious egg-endoparasitoid that was imported to Florida as a biological control agent of *Diaprepes abbreviatus* (Coleoptera: Curculionidae).

The first study hypothesized that the reproductive success of *H. sperata* is affected by the host plants of *D. abbreviatus*. Six host plants with varying degrees of pubescence were used to determine successful parasitism and the effect of leaf trichomes on the searching behavior of *H. sperata*. *Haeckeliana* was able to parasitize *Diaprepes* eggs laid on the six host plants; however, the plants with a high leaf trichome density had a lower percent of parasitism than the plants with smoother leaves. Removal of trichomes from a pubescent biotype of buttonwood, *Conocarpus erectus*, revealed that the presence of leaf trichomes had a negative effect on the overall searching efficiency of *H. sperata*. Searching speed was highly related to parasitism; this parameter could be used to predict the suitability of host plants for the establishment of *H. sperata*. These findings suggest that the reproductive success of *H. sperata* is dependent on the host plant of *D. abbreviatus*.

The second study was designed to evaluate the relative susceptibility of *H. sperata* adults to contact with pesticide residues on leaf surfaces. Five concentrations of seven pesticides were included in the bioassays. All tested pesticides had a negative effect on *Haeckeliana's* survivorship. However, some pesticides caused significantly less harm to this parasitoid. Organophosphate, carbamate and pyrethroid pesticides were highly toxic to *H. sperata*. Dilutions of the organophosphate, carbamate and pyrethroid solutions did not reduce mortality of the parasitoid. Imidacloprid, abamectin, petroleum oil and a phosphonate fungicide allowed *H. sperata* to live longer compared with the previous pesticides, suggesting a certain degree of selectivity. The use of products that have less toxic effects on the introduced parasitoid will increase its chances to parasitize *D. abbreviatus* eggs.

This study proposes the selection of host plants of *D. abbreviatus* and use of selective pesticides to maximize the chances of establishment of *Haeckeliana sperata* in Florida.

## CHAPTER 1 INTRODUCTION

### **Baseline Information about *Diaprepes abbreviatus***

*Diaprepes abbreviatus* (L.) is a root weevil that is native to the Lesser Antilles.

Apparently, it was accidentally introduced to Florida in the mid 1960s (Woodruff 1985). Since its first detection in Apopka (Orange County) in 1964, *Diaprepes* has spread throughout the central and southern part of the Florida peninsula. *Diaprepes* is now considered established in 23 counties, infesting more than 100,000 acres of citrus groves and many other agricultural, ornamental and wild plants (Nguyen et al. 2003, Weissling et al. 2004). The introduction of this pest to Florida has increased the production costs of many crops, and severely hindered the growth of Florida's agricultural markets. Estimates show that this pest has increased production costs over 70 million dollars annually for the citrus industry in Florida (Stanley 1996, Muraro 2000). Moreover, the infestation of this weevil has spread to Texas and California (Grafton-Cardwell 2004, CDFA 2006), resulting in drastic measures to restrict the introduction of ornamental plants from Florida (TDA 2001).

The key pest status of *D. abbreviatus* is partly ascribable to its polyphagy. The list of host plants of *D. abbreviatus* includes more than 270 plant species from 60 plant families (Simpson et al. 1996, Mannion et al. 2003). A large portion of *Diaprepes* host plants are important agricultural crops such as: citrus (all varieties), papaya, sugarcane, peanut, sorghum, sweet potato, cassava, loquat, guava, mango, avocado, banana and corn. Some of the other reported hosts are widely used by the ornamental plant industry, and they include native plants, or are invasive species (i.e., Brazilian pepper tree), all of great ecological significance (Simpson et al. 1996). The wide host range of *D. abbreviatus* has facilitated the spread of this weevil, resulting

in a significant threat to many tropical and subtropical ecosystems. Moreover, the broad variation in the host plants of *D. abbreviatus* represents a big challenge for developing efficient and sustainable management tactics.

### **Biology of the *Diaprepes* root weevil**

The adult weevils are long lived ( $\approx 147$  days for females and  $\approx 135$  days for males; Wolcott 1936) and can be found throughout the year in south Florida. Their seasonality in the central portion of Florida is different, showing a primary emergence period from May to October and November. McCoy et al. (2003) found that the magnitude of adult emergence is related to soil moisture. This study concluded that *D. abbreviatus* emergence can be delayed by moisture deficits and that soil moisture could have a positive relationship with adult emergence. Adults prefer to feed on young, tender leaves, leaving irregular notches. They may also feed on old leaves or to a limited degree on fruits, as seen on papayas and young citrus fruit (Woodruff 1968, Knapp et al. 2000). *D. abbreviatus* adults form aggregations of equal sex ratio on new foliage of their host plants, where they can remain for weeks. Even though adults have considerable intrasexual variation in body size, both sexes are considered monomorphic and mate in crowded aggregations of up to 100 individuals per  $0.5\text{m}^2$  (Harari and Landolt 1997, Harari and Brockmann 1999, Harari et al. 1999, 2000, 2003). Evidence of the nature of the formation of aggregations is contradictory. Schroeder (1981) suggested that both females and males produce pheromones to attract the opposite sex to aggregation sites. In contrast, Beavers et al. (1982) suggested that odor of young citrus foliage is the main cue affecting aggregation and that the subsequently produced frass served as a secondary attractant at the aggregation site. Later on, Hariari and Landolt (1997) reported that females were more attracted to damaged food than to males or frass of either sex, and that males were similarly attracted to females, frass of either sex,

or damaged food. Evidence suggests that the nature of the formation of aggregations of *D. abbreviatus* is complex and could be mediated by the interaction of several cues of which damaged food probably plays a primary role.

The female oviposits egg masses between two adjacent leaves. The egg masses are deposited in a gelatinous cement that seals the leaves together and provides protection for the eggs (Nigg et al. 2001, Jacas et al. 2005). Females lay eggs in clusters of 30 to 265 eggs and can deposit approximately 5000 eggs over their life time (Wolcott 1936). Simpson et al. (1996) suggested that *D. abbreviatus* shows little preference for oviposition sites. Weevils confined in artificial settings will oviposit in anything that has two surfaces close together (i.e. strips of wax paper, a piece of wrinkled cloth). However, in a natural environment, *D. abbreviatus* females seem to prefer some host plants and certain plant structures for oviposition. In a two year field study, Peña et al. (2007) compared the oviposition patterns in two host plants of *D. abbreviatus* and found that silver buttonwood, *Conocarpus erectus* L. var. *sericeus* Fors., is more favorable to oviposition than dahoon holly, *Ilex cassine* L. Their findings suggest that the egg masses are aggregated within canopy strata when they are deposited in silver buttonwood plants. Lapointe (2001) reported that *Diaprepes* prefer to oviposit eggs on mature expanded citrus leaves, providing further evidence that suggests females do have preference for oviposition sites. The preference of *D. abbreviatus* towards certain oviposition sites suggests that some plants could serve as egg and larval reservoirs and thus play an important role on the population dynamics of this weevil.

Upon emergence, the neonate larvae escape from the sealed leaves and drop to the soil surface. The timing of the period between egg hatch and permanence in the soil surface has been studied by various researchers as it represents a period of exposure to predation. Jones and

Schroeder (1983) reported that a considerable period of time (48h average) often elapsed from egg hatch to larval escape from the sealed leaves. The pattern of neonate drop was studied in detail by Stuart et al. (2003). This study revealed that neonate drop could occur during all hours of the light and dark phases, but agreed with Jones and Schroeder (1983) in that there is a peak of neonate drop during the second period of the light phase. However, it is unclear to what extent these temporal patterns relate to environmental conditions such as rainfall, which could have a large impact on the ability of neonate larvae to burrow into the soil (Jones and Schroeder 1983). These researchers demonstrated that neonate larvae failed to penetrate dry soil. In addition, they found that neonate larvae might frequently remain exposed on the soil surface for relatively long periods (up to 180 minutes) before penetrating the surface of moist soil.

After penetrating the soil surface, larvae burrow down to the root system where they begin feeding on fibrous roots. As they grow, the larvae move to feed on larger structural roots, where they create deep grooves as they consume the outer bark and the cambium layer (McCoy et al. 2007). This stage causes major crop damage by debilitating the root system, making it more vulnerable to infection by soil born pests such as *Phytophthora* spp. and nematodes, and less tolerant to water stress. The larval development period may include up to 16 instars and varies widely even between the individuals of a single mass. Under laboratory conditions the duration of the larval period of *D. abbreviatus* obtained from field-collected females was of 132-297 days (Beavers and Selheime 1975). However, in the field the larval period could last up to one year. Moreover, there are reports stating that *D. abbreviatus* can enter a quiescent period inside a soil chamber for up to 388 days (Wolcott 1936). If no control measures are taken, a few large larvae can cause a mature healthy tree to become non-productive.

### **Identification of *Diaprepes abbreviatus*.**

It is important to differentiate *D. abbreviatus* from other Florida root weevils that inhabit similar ecological niches in and have a similar life cycle. *Diaprepes* belongs to the subfamily Entiminae (Coleoptera: Curculionidae) which groups the “broad-nosed” weevils that possess a short and stout rostrum (Marvaldi 1997, Anderson 2002). This is a large and widely distributed group that recognizes 124 genera in 23 tribes in North America (Anderson 2002). At least 24 genera and 36 species of weevils that belong to the subfamily Entiminae are known to be present in Florida (O’Brien and Wibmer 1982, Peck and Thomas 1998). However, only a few species share ecological niches with *Diaprepes* in Florida and inflict damage to plants that could be mistakenly attributed to *Diaprepes*. Even though the adults of this weevil are easily recognizable by morphological characters (see Appendix A), some developmental stages (i.e., eggs) show great similarities and could be easily confused from one species to another. The most relevant weevil species inhabiting Florida that also share the same environment and deposit eggs in between two leaves as *Diaprepes* include: *Pachnaeus litus* (Germar), *P. opalus* (Oliver), *Artipus floridanus* Horn, *Lachnopus floridanus* Horn, *L. argus* (Rieche) and *L. hispidus* (Gyllenhal). The first four species are native to Florida and the last two are adventive species that are doubtfully established in Florida (Peck and Thomas 1998, Anderson 2002). These species, together with *D. abbreviatus*, form a complex of economically damaging citrus root weevils. In most cases, the use of adequate cultural practices should be enough for managing citrus root weevils except for *D. abbreviatus* (McCoy et al. 2007). When a grove is infested with *D. abbreviatus*, additional management practices are required.

## Management of the *Diaprepes* root weevil

Throughout the last few decades several strategies have been implemented for the control of *D. abbreviatus*. A synopsis of the main tactics proposed for management for *D. abbreviatus* in citrus is shown in figure 1. The first step in any management program of *D. abbreviatus* is to

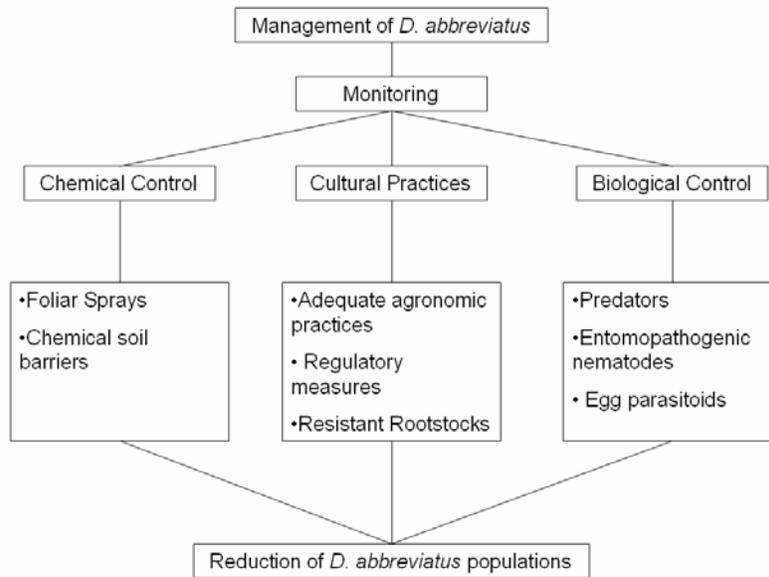


Figure 1-1. General scheme of the main strategies proposed for management of *D. abbreviatus* in citrus.

establish a sampling method to detect infested areas and to monitor groves with established populations. Detection of *D. abbreviatus* has relied mostly on observation of the adult stage. Visual monitoring for adults can be effective when a grove is inspected on a regular basis by looking for the irregular notches on leaves resulting from adult feeding, especially in new flushes. If leaf notching is observed, it is necessary to capture the adults in order to obtain a positive identification. A capture method used by growers and researchers is the “beat” method, which takes advantage of the behavior of the adult weevils that, when disturbed, fall to the ground and pretend to be dead. The adult weevils can be captured by placing an open umbrella

or a cloth between the tree and the ground and then beating the tree. Nigg et al. (2001) studied the distribution and movement of marked adult *Diaprepes* weevils in a citrus grove using the beat method, Tedders traps and Malaise traps. The conclusion of this study was that the beat method is much more accurate in determining adult population levels than either of the traps, and that the Tedders traps, which are widely used for monitoring other weevil species, were inefficient for estimating abundance indices but could be used to signal when weevils first appear in the trees during a season (Nigg et al. 2001). In another study, McCoy et al. (2003) assessed the seasonal life stage abundance of *D. abbreviatus* in irrigated and non-irrigated citrus plantings. They used modified Tedders traps to monitor adults, time-limited visual inspections to the accessible part of the tree canopy to monitor eggs, modified pitfall traps to monitor neonate drop, and removed trees to monitor larvae and pupae present in the soil. In contrast to the findings by Nigg et al. (2001), this study reported that Tedders traps were efficient to measure changes in adult weevil abundance within a season. Furthermore, they reported a close association of adult trap counts, egg mass counts and neonate counts, and therefore proposed that adult captures in modified Tedders traps provided a reliable indicator for estimating the abundance of other life stages (i.e. eggs). Interestingly, Peña et al. (2007) recently reported that *D. abbreviatus* prefers to oviposit on the upper stratum in the canopy of silver buttonwood plants, which suggests that monitoring the most accessible part of the canopy of a host plant might not necessarily give a good predictor of the abundance of eggs in the whole tree, especially at low population densities. The later study proposes sample sizes for monitoring *Diaprepes* root weevil egg infestations in silver buttonwood plants and conclude that sampling procedures for egg masses will depend on the host plant. Several efforts have been made to design an efficient trap to capture adult weevils that could be used to standardize the monitoring

procedures. Some researchers have looked for volatile aggregating or mating pheromones that could be incorporated as an attractant to Tedders or other traps (Lapointe et al. 2004). However, no efficient attractants have been identified yet and the commercial use of these traps remains limited.

The chemical control proposed for *D. abbreviatus* has two components: foliar sprays and chemical soil barriers. Foliar sprays target the peaks of adult emergence with the purpose of reducing egg deposition, thereby limiting the number of neonate larvae entering the soil (McCoy et al. 2007). The products recommended for foliar sprays are often mixed with petroleum oil to improve their residual effect and to affect the bonding characteristics of the secretions that females use to bond eggs to leaves (McCoy et al. 2007). Recommended pesticides for foliar sprays include: carbamates (Sevin XLR, 80S & 4F), organophosphates (Orthene 97, Imidan 70WP), minerals (Kryocide 96WP), pyrethroids (Danitol 2,4EC) and insect growth regulators (Micromite 80WGS) (McCoy et al. 2007). Chemical soil barriers are used to prevent the neonate larvae from burrowing into the soil and reaching the root system. Two pyrethroids (Brigade WSB and Capture 2EC) are recommended as chemical soil barriers that are more effective when applied two weeks after the peak of adult emergence (McCoy et al. 2007). In addition, the presence of *Diaprepes* in a grove induces the application of soil fungicides to control infections of secondary pathogens (i.e. *Phytophthora* spp.) (McCoy et al. 2007).

The cultural practices proposed to manage a grove that is infested with *Diaprepes* are designed mostly to reinforce the host plants ability to overcome larval damage. Using *Phytophthora*-resistant rootstocks as well as keeping optimal soil drainage are proposed for reducing infections in the root system and limiting tree decline (McCoy et al. 2007). Another recommended practice is to apply additional fertilizers to promote new root growth after

*Diaprepes* larvae have inflicted damage to the roots. A control measure that could be considered a cultural practice is the use of kaolin-based particle films. The particle films act as feeding and oviposition deterrents while having a beneficial effect on carbon assimilation, leaf temperature, tree growth, and yield of fruit trees in semiarid and subhumid environments (Glenn et al. 2001, Lapointe et al. 2006). Additional actions have been taken by the regulatory agencies. Locations with known *Diaprepes* infestations have been subject of quarantine programs to prevent the spread of the weevil (CDFA 2006), but these actions have negatively impacted the market of many agricultural commodities in Florida.

The other major component of the management programs of *D. abbreviatus* is biological control. Several efforts have been made to identify natural sources of mortality of *Diaprepes* in Florida and some Caribbean islands. There are reports of several predators of *Diaprepes* adults. These range from *Bufo marinus* (Dexter 1932), to stink bugs (Mead 1976) and spiders (Anonymous 2005). No adult parasitoids of *Diaprepes* have ever been reported (Peña unpublished). Several parasitoids (eg., *Micronotus* spp., *Cenosoma* spp., *Oestrophasia* spp.) have been collected from other species of citrus weevils, such as *Artipus* (Bullock 1984, Kovarik and Reitz 2005) in Florida and from *Pachnaeus* in Cuba (Hernandez and Perez 1981, Grillo and Alvarez 1984). Therefore, the incognita still exists of the existence of an adult parasitoid of the *Diaprepes* root weevil.

Various predators, entomogenous nematodes, entomopathogenic microorganisms and egg parasitoids have been found affecting *Diaprepes* eggs and/or larvae. In 1982, Whitcomb et al. identified and evaluated several predators of *D. abbreviatus* first instar larvae present at the High Acres Orange Grove, Forest City, Florida. Nine species of ants (Hymenoptera: Formicidae) were the dominant predators responsible for removing an average of 47% of the neonate larvae

crawling on the soil surface during the evaluation periods (intervals of 20 minutes). In addition, a few predation events by one spider species (*Corythalia canosa* Walckenaer) and earwig juveniles (*Labidura* sp.) were reported. The nine ant species in decreasing order of percent predation included: *Pheidole dentate* Mayr, *Pheidole floridana* Emery, *Tetramorium simillimum* Roger, *Paratrechina bourbonica* Forel, *Pheidole morrissi* Forel, *Solenopsis invicta* Buren, *Conomyrma flavopecta* (M.R. Smith), *Pheidole moerens* Wheeler, and *Paratrechina vividula* (Nylander). The first four species were considered prominent predators because of the consistency of appearance in the testing arenas and their high predation rates. *P. morrissi* and *S. invicta* occurred rarely but had high rates of predation. Similarly, Stuart et al. (2003) evaluated neonate predation by ants in three citrus groves of Central Florida at Lake Alfred and Alturas (Polk County), and Southport (Osceola County). Eight species of predatory ants removed an average of 11.9% of the neonate larvae crawling on the soil surface during the evaluation periods. Ant species in decreasing order of percent predation included: *S. invicta*, *P. moerens*, *Dorymyrmex reginacula* (Trager), *Brachymyrmex obscurior* Forel, *Dorymyrmex bureni* (Trager), *Cardiocondyla emeryi* Forel, *P. bourbonica* and *P. morrissi*. Interestingly, three out the four dominant predatory ant species found by Whitcomb et al. (1982) in Forest City were not detected in the later study. Moreover, *S. invicta* and *P. moerens* were considered minor predators in Forest City whereas in the later study they were consider major predator species. The results of these two studies suggest that ants are important predators of *D. abbreviatus*, but also that either the ant fauna among sites is highly variable or that the ant composition is undergoing changes probably caused by the introduction of invasive ant species such as *S. invicta* (Stuart et al. 2003). An interesting defense mechanism used by *Diaprepes* neonate larvae against ants was described by Pavis et al. (1992). Observations made by these researchers revealed that a common

predatory ant in the island of Guadeloupe, *Solenopsis marginata* (F.), was repelled by the first instar larva of *D. abbreviatus*. Further studies revealed that first instar larvae of *D. abbreviatus* secrete an ant-repellent chemical that limit the magnitude of predation by this ant species.

Whether Florida's populations of *D. abbreviatus* secrete ant-repellents or if the repellents affect the ant species present in Florida is unknown.

Three species of lady beetles (Coleoptera: Coccinellidae) were reported as potential predators of *Diaprepes* eggs and neonate larvae (Stuart et al. 2002). *Cycloneda sanguinea* (L.), *Harmonia axyridis* Pallas and *Olla v-nigrum* Mulsant are all generalist predators that consume various citrus pests. Bioassays conducted with these three species demonstrated that *Diaprepes* eggs and neonate larvae could become part of their diet. However, there is no information on the contribution of this species to the biological control of *D. abbreviatus* under natural conditions.

Another predator of *Diaprepes* neonate larvae is the striped earwig *Labidura riparia* (Pallas) (Tryon 1986). This species was the dominant predator in fields where ants are suppressed by application of pesticides, showing a high predation rate (up to 20 neonate larvae per minute) during the first three hours after the sunset (Tryon 1986).

As described in the previous paragraphs, all Florida's inhabitant predatory natural enemies are generalists, may largely contribute to the biological control of *D. abbreviatus*, and some of them could be good candidates to develop conservation biocontrol programs.

*Diaprepes* also has subterranean natural enemies. Beavers et al. (1983) surveyed several citrus producing areas and found that *Diaprepes* larvae were naturally attacked by four entomopathogenic fungi including: *Metarrhizium anisopliae* (Metschnikoff), *Beauveria bassiana* (Balsamo), *Paecilomyces lilacinus* (Thom) and *Aspergillus ochraceus* Wilhelm. Quintela and McCoy (1997) evaluated the pathogenicity of *M. anisopliae* and *B. bassiana*, in

combination with sublethal doses of imidacloprid, to first instar larvae of *D. abbreviatus*.

Interestingly, they found that the combination of these two fungi with imidacloprid as contact or oral treatments resulted in a synergistic increase in both mortality and mycosis of first instar larvae of *D. abbreviatus*.

Besides the identification of entomopathogenic fungi, Beavers et al. (1983) found two species of entomogenous nematodes (*Steinernema feltiae* (Filipjev) and *Heterorhabditis* sp. Poinar) infecting *Diaprepes* larvae in several citrus producing areas in Florida. Further research revealed that nematodes had a large potential for control of *Diaprepes* in part because they can suppress all larval instars (Schroeder 1987). Shapiro and McCoy (2000) conducted a laboratory comparison of nine species and 17 strains of entomopathogenic nematodes for virulence toward larvae of *D. abbreviatus*. The nematodes that were evaluated included six strains of *H. bacteriophora* Poinar, two strains of *H. indica* Poinar, Karunakar and David, two strains of *H. marelatus* Lui and Berry, a single strain of *H. megidis* Poinar, Jackson and Klein, one strain of *H. zealandica* Poinar, one strain of *S. riobrave* Cabanillas, Poinar and Raulston, three strains of *S. feltiae* (Filipjev), and one strain of *S. glaseri* (Steiner). From all the nematodes tested, *S. riobrave* caused greatest larval mortality. In contrast to these results, Shapiro et al. (1999) found that *H. indica* caused equal or greater mortality than *S. riobrave*. Commercial formulation of *S. riobrave* (Bio Vector) and *H. indica* (Grubstake 100) are currently applied in citrus groves. *S. carpocapsae* was commercially available for several years but later studies revealed a low efficacy to control *D. abbreviatus* larvae in the field (Duncan et al. 1996). The poor efficacy was attributed to the host searching behavior of this nematode, which is that of an ambusher (Shapiro and McCoy 2000). Nematodes that have high-host searching and dispersal abilities, and that can persist in the soil, are good candidates to be used as biological control agents for *D. abbreviatus*.

(Shapiro and McCoy 2000). Unfortunately, most of the species that have been applied commercially against *D. abbreviatus* have a low persistence in the soil (Duncan and McCoy 1996). For this reason, Shapiro and McCoy (2000) suggested that another nematode, *S. glaseri*, even with lower levels of virulence than *S. riobrave*, but with higher dispersal, host finding abilities and good potential of persistence, could be a more effective long term solution to suppress *D. abbreviatus* larvae. Even though selecting an appropriate nematode strain is vital for the successful control of *D. abbreviatus* larvae, recent studies have revealed that physical properties the soil will influence the ability of different nematode species to disperse and search for hosts (Jenkins et al. 2007)

The effect of other entomopathogenic microorganisms on *D. abbreviatus* has been evaluated. Weathersbee et al. (2002) reported a reduction in the survival of neonate larvae exposed to *Bacillus thuringiensis* subsp. *tenebrionis* incorporated into a rearing diet, and through soil applications in potted citrus plants. Later, in 2003, Hunter and Lapointe reported the infection of *D. abbreviatus* cell cultures by an iridovirus. However, the use of these microorganisms to control *D. abbreviatus* has been limited and the extent to which they could contribute to control *D. abbreviatus* remains unknown.

As seen throughout this review, there are vast numbers of chemical, cultural and biological tools for the management of *D. abbreviatus*. However, despite the wide array of proposed management tactics, no long term sustainable solution has been found to reduce the weevil populations to tolerable levels. Without specialist natural enemies and with many host plants available, *Diaprepes* has continued to spread throughout Florida and other states causing more economical and ecological damage. Classical biological control with egg parasitoids is viewed as a possible long term solution to regulate weevil populations (Peña et al. 2001, 2004). For this

reason, and being that this topic is the main framework of my study, a more complete review of biological control of *D. abbreviatus* with egg parasitoids follows.

### **Classical Biological Control of *D. abbreviatus* with Egg Parasitoids**

Several efforts have been made to identify and introduce classical biological control agents of *D. abbreviatus* in an attempt to reduce weevil populations to tolerable levels. Attempts to introduce egg parasitoids of *D. abbreviatus* to Florida began in the 1970s, with the introduction of *Quadrastichus (Tetrastichus) haitiensis* (Gahan) (Hymenoptera: Eulophidae) (Schauff 1987). This endoparasitoid was found as the most common parasitoid of citrus weevils in Jamaica, and was also present in the Dominican Republic, Puerto Rico, Andros Island and Cuba (Van Whervin 1968, Schauff 1987). Unfortunately, this first attempt to establish *Q. haitiensis* in Florida failed. Two factors were proposed by Beavers and Selhime (1975) to explain the failure on the establishment of this parasitoid. The first factor is the cold winter temperatures in Florida that will limit the development of this tropical parasitoid. The second explanation was the lack of synchrony between the life cycles of *Q. haitiensis* and *D. abbreviatus* in Florida. However, Sutton et al. (1972) proposed that intensive applications of carbaryl during the time of introduction had contributed to limit the possibilities of establishment of *Q. haitiensis*.

In the late 1990's, efforts were re-initiated to introduce hymenopteran egg parasitoids from the Caribbean islands into Florida (Peña et al. 2001). Throughout 1997, ten Florida citrus groves and one grove located in Puerto Rico were routinely monitored to study egg parasitism. No egg parasitism was recorded in Florida, while the parasitism in Puerto Rico ranged from 12-68%. The primary parasitoids found in Puerto Rico were *Q. haitiensis* and *Aprostocetus vaquitarum* (Wolcott) (Hymenoptera: Eulophidae) (Hall et al. 2001). In 1998, *Q. haitiensis* was collected

from *Diaprepes* eggs in Puerto Rico and brought for the second time into Florida. Field releases in citrus groves and ornamental fields were initiated in 2000, resulting in the successful establishment of *Q. haitiensis* in the southern part of the state (Peña et al. 2004). This parasitoid has become a permanent mortality factor parasitizing 12-55% of *Diaprepes* eggs in south Florida (Peña et al. 2004, 2006).

Before the second introduction of *Q. haitiensis* another parasitic wasp, *Ceratogramma etiennei* Delaware (Hymenoptera: Trichogrammatidae), was introduced to Florida in 1997 and released in 1998 at locations with known infestations of *Diaprepes* (Peña et al. 2001). Release sites included citrus groves, ornamental fields and natural habitats. This gregarious endoparasitoid was discovered in the island of Guadeloupe and was regarded as highly specific (Etienne et al. 1990). Amalin et al. (2005) studied certain aspects of the biology of this parasitoid and suggested that characteristics of the host plant of *D. abbreviatus* such as the leaf thickness, leaf pubescence, and plant strata, could have an effect on the parasitism by *C. etiennei*. This parasitoid was recovered in Florida one year after its release in lime (*Citrus aurantifolia* Swingle) and pygmy palms (*Phoenix roebelenii* O'Brien), but was not recovered in the subsequent years (Peña et al. 2004). The reasons for its disappearance are unknown (Peña et al. 2004).

In 2000, a third egg parasitoid *Aprostocetus vaquitarum* [= *A. gala*=*Tetrastichus gala* (Walker)] (Hymenoptera: Eulophidae) was introduced into Florida from the Dominican Republic where it is a primary parasitoid of *D. abbreviatus* (Jacas et al. 2005). *A. vaquitarium* deposit their eggs in close contact with those of its host by introducing their ovipositor through the sealed leaves (Jacas et al. 2005). After eclosion, larvae of *A. vaquitarium* feed externally on several eggs of *D. abbreviatus* to complete their preimaginal development. They pupate within

the sealed leaves and eventually emerge from them. After screening under quarantine conditions, adult wasps obtained from laboratory culture were released from 2000 through 2003 in several Florida counties. *A. vaquitarum* is now considered to be established in parts of southern Florida where parasitism levels of 70-90% have been achieved (Peña et al. 2004).

As a result of the first three introductions of egg parasitoids into Florida, the two eulophid parasitoids (*Q. haitiensis* and *A. vaquitarum*) have successfully established in the southern portion of the Florida peninsula where they have become an important mortality factor of *Diaprepes* eggs (Peña et al. 2004, 2006). However, no parasitoids are established in the central portion of the state where the weevil attacks citrus orchards. The inability of these parasitoids to establish in central Florida has been attributed in part to the parasitoids sensitivity to the ambient temperatures registered in Central Florida. Castillo et al. (2006) and Ulmer et al. (2006a) found that the lower temperature thresholds for completing the life cycle of *Q. haitiensis* and *A. vaquitarum* are 16°C and 15.8 °C, respectively. These reports suggest that both parasitoids can fulfill their thermal requirements in south Florida where the minimum ambient temperatures fluctuate between 15-17°C, whereas they can not survive in central Florida where the minimum temperatures fluctuate between 10-13 °C.

Two new parasitoids, *Fidiobia dominica* Evans and Peña (Hymenoptera: Platygasteridae) and *Haeckeliana sperata* Pinto (Hymenoptera: Trichogrammatidae) have been introduced in an attempt to get them established in central Florida. *F. dominica* is a highly specific, solitary endoparasitoid of *Diaprepes* spp., that was collected in the island of Dominica during 2003. *Haeckeliana sperata* is a gregarious endoparasitoid of *Diaprepes* sp., that was collected for the first time in Dominica during 2003. Both parasitoids were recently described as new species (Evans and Peña 2005, Pinto 2005) which explains the limited information available on many

aspects of their biology, physiology, and ecology. Jacas et al. (2007) studied the thermal requirements for the two species. Their findings suggest that both parasitoids have a greater thermal plasticity than the parasitoid already established in south Florida, which could increase their chances of establishment in the central portion of the state. However, Lapointe et al. (2007) reported that *Diaprepes* eggs were absent during long periods of time (up to 141 days) during the winter in a citrus grove with known infestation in central Florida. If the lack of *Diaprepes* egg masses during long periods of the winter is a generalized condition throughout central Florida, the establishment of these parasitoids will be limited (Jacas et al. 2007). However, the observations made by Lapointe et al. (2007) were done exclusively in citrus plants and the possibility of finding eggs in other host plants has been overlooked. Moreover, the presence of alternate host eggs could potentially allow these parasitoids to reproduce during periods of absence of *Diaprepes* eggs (Jacas et al. 2007). As mentioned before, other weevil species such as species from the genera *Pachnaeus*, *Artipus* and *Lachnopus* share the same environment and deposit eggs in the same manner as *Diaprepes*. These parasitoids, if they establish, would provide welcome mortality factors to help reduce populations of the *Diaprepes* root weevil.

In addition to compatibility of these two parasitoids to Florida's climate, other important factors could influence parasitoid fitness in their new environment. An important biotic factor that could influence parasitoid fitness is the host plant species of *D. abbreviatus*. Botrell and Barbosa (1998) reported that many factors of the host plant can affect the colonization, phenology, and effectiveness of natural enemies used in biological control. Plants employ many physical and chemical defenses against insect herbivores that are not necessarily compatible with natural enemies. Physical defenses may include structural traits such as surface waxes, leaf trichomes, and/or spines whereas chemical defenses are often secondary metabolites that act as

repellents, toxins and/or digestibility reducers (Southwood 1986). Plant defense mechanisms may directly or indirectly inhibit natural enemies. Direct plant effects on parasitoids may involve simple mechanisms, such as reduced parasitoid searching efficiency caused by trichomes (Botrell and Barbosa 1998). Indirect host plant effects on insect natural enemies are more complex. For example, some insects sequester chemical compounds from their host plant, which renders them unpalatable to natural enemies, as seen the danaid butterflies that specialize on host plants containing cardenolides (Bowers 1990).

The effect of host plants on natural enemies has been studied in several systems. An interesting case is the effect of some alfalfa cultivars on the parasitism of *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae) by *Anagrus nigriventris* Girault (Hymenoptera: Mymaridae) (Lovinger et al. 2000). Some alfalfa cultivars with glandular trichomes were released for resistance to *E. fabae*. However, these cultivars had a negative impact on the searching behavior its primary natural enemy, *A. nigriventris*. Their results showed that specific plant characteristics (i.e. glandular trichomes) reduced the effectiveness of *A. nigriventris*, and turned breeding programs toward finding alfalfa cultivars that do not hinder the performance of its primary parasitoid. In another study, Mulatu et al. (2006) found that the presence of trichomes in tomato leaves had no effect on the establishment of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), but had a direct adverse effect on the parasitoid *Diadegma pulchripes* (Hymenoptera: Ichneumonidae). In contrast, Styrsky et al. (2006) found that the presence of trichomes in soybean plants did not inhibit fire ants (*S. invicta*) from foraging on plants, and that predation of herbivores was greater in pubescent plants when compared with glabrous plants.

The effects of host plants have proven to be especially important for relatively small parasitoids. Rabb and Bradley (1968) studied the egg parasitism of *Manduca sexta* (Lepidoptera: Sphingidae) by two parasitoids, *Telenomus sphingis* Ashmead (Hymenoptera: Scelionidae) and *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae), on tobacco and other solanaceous hosts. The tiny parasitoid *T. minutum* became stuck in the gummy exudates of the trichomes of the tobacco leaves and failed to parasitize eggs. However, it readily attacked eggs on other substrates. In contrast, *T. sphingis*, being larger than *T. minutum*, was able to parasitize eggs laid in tobacco and in other substrates (Rabb and Bradley 1968). There is increasing evidence that plant characters have a strong impact on the parasitism efficacy of trichogrammatid wasps. This family is characterized by grouping parasitoids that differ distinctly from most other parasitic wasps due to their minute size (Pinto and Stouthamer 1994). As a consequence, they are more affected by plant surface structures and have a low capacity for active flights (Romeis et al. 2005). In 1977, Burbulis et al. reported that *Trichogramma nubilale* Ertle and Davis is more successful at finding hosts that are located in the middle or lower portions of corn plants than hosts located in the top portion of these plants. In 2006, Olson and Andow observed the walking patterns of *T. nubilale* on leaves of several plants and artificial surfaces. They concluded that the type and size of trichomes on the different plants affected the walking speed, turning rates and the direction of the searching path of *T. nubilale*. Similarly, the searching behavior of *T. exiguum* Pinto and Platner was found to be affected by leaf trichome density and morphology of maize and pigweed plants (Keller 1987). Further studies using regression analyses found that trichome density on tomato plants accounted for the greatest proportion of the variance in *T. exiguum* parasitism of *Heliothis zea* (Boddie) and *H. virescens* (F.) eggs (Kauffman and Kennedy 1989). The searching success of *T. minutum* on paper and foliage

models also proved to be affected by structural complexity, plant leaf chemicals, and female age (Lukianchuk and Smith 1997). Romeis et al. (1998 and 1999) reported that parasitism efficiency of *Trichogramma* spp. on pigeon pea (*Cajanus Cajan* L.) depends mainly on the location of host (*Helicoverpa ormiguera* Hübner) eggs within the plant. Furthermore, they found that *H. ormiguera* preferred to oviposit on calyxes and pods where the efficiency of parasitism by *Trichogramma* spp. is the lowest due to physical and chemical plant characters that inhibit the searching behavior of these parasitoids. All these studies have served to underline the importance of habitat selection on the success of inoculative and augmentative biological control programs. Even though most of the evidence on the effects of plant surface structures on trichogrammatid species is restricted to the genus *Trichogramma* spp. (Romeis et al. 2005), one of the recently introduced parasitoids against *D. abbreviatus* (*H. sperata*) belongs to this family and could present similar interactions with the plants of its host. Given the wide range of host plants of *D. abbreviatus*, knowledge of the potential antagonism between the host plants and the parasitoids that were recently introduced against it is particularly important.

Another major factor that could affect the fitness of the introduced parasitoids is the application of pesticides in citrus and ornamentals. Rational use of pesticides in IPM relies in part on knowledge of the pesticide effects on beneficial insects and the development of strategies to minimize disruptive effects on the natural enemies. Such strategies include the use of selective compounds and altered rates and timings of pesticide applications. Ulmer et al. (2006b) reported differential susceptibility of *A. vaquitarum* to pesticides applied in citrus. Carbamate and organophosphate pesticides were the most toxic to *A. vaquitarum* adults, followed by neonicotinoid, pyrethroid and kaolin clay pesticides. Copper and phosphonate fungicides, petroleum oil, abamectin, diflubenzuron and bifenazate were slightly to non-toxic to *A.*

*vaquitarum*. Another study tested the effects of diflubenzuron on *C. etiennei* and *Q. haitiensis*, and concluded that this pesticide interferes with the development of *C. etiennei* but not on *Q. haitiensis* (Amalin et al. 2004). However, no information is available on the effects of pesticides on *H. sperata* and *F. dominica*, the two new egg parasitoids that are being released in citrus and ornamental groves where pesticides are applied. Information on the effect of pesticides on these two parasitoids is essential for integrating biological control into the pest management programs used in citrus and ornamental groves.

### **Objectives of Master of Science Thesis Research**

The study presented here emphasizes two important factors that will influence the fitness of *Haeckeliana sperata* as classical biocontrol agent of *D. abbreviatus*. Because *Diaprepes* is a polyphagous species, the different leaf morphology of the host plant species might influence parasitism by *H. sperata*. Furthermore, incompatibility with pesticide applications could hinder the regulatory effects of this natural enemy on *D. abbreviatus* populations. Optimum control by the parasitoid relies, in part, on knowledge of the influence of host plant traits and the effects that pesticides will have on their potential control. Such knowledge will permit the development of strategies to maximize control in suitable host plants and to minimize the disruptive effects of pesticides on natural enemies. The results of this study will provide information to design strategies to maximize the mortality effects of the parasitoids on *Diaprepes* populations. The specific objectives of this research are to:

**1. Determine the effect of host plants on successful parasitism of *Haeckeliana sperata* on *Diaprepes abbreviatus* eggs.** The effect of six major host plants on successful parasitism of *H. sperata* was assessed. The ability of *H. sperata* to parasitize *Diaprepes* eggs laid on leaves of

these host plants was studied. Furthermore, the effect of leaf thickness and leaf pubescence of the host plants on successful oviposition was determined.

**2. Determine the effect of leaf trichomes on the searching behavior of *Haeckeliana sperata* and its parasitism on *Diaprepes* root weevil.** Many failures in field releases and the effectiveness of suppression of various crop pests by trichogrammatid species have been ascribed to the physical characteristics of the host plant. The degree of control of *Diaprepes* populations by *H. sperata* may depend on the interaction of the parasitoids with the micro-environment created by the plant on the leaf surface. This study assessed the potential antagonism between the presence of trichomes on the leaves surface of the host plants of *D. abbreviatus*, and the levels of parasitism by *H. sperata*.

**3. Determine the relative susceptibility of *Haeckeliana sperata* to pesticides applied in citrus and ornamentals.** Pesticides are a critical component of insect pest management in citrus and ornamental production. This study assessed the relative susceptibility of *H. sperata* to leaf surface residues of seven pesticides used in citrus crops and ornamental plant production. This study provides information that will permit the development of strategies to minimize the disruptive effects of pesticides on *H. sperata*.

CHAPTER 2  
EFFECT OF HOST PLANTS ON SUCCESSFUL PARASITISM OF *Haeckeliana sperata*  
(HYMENOPTERA: TRICHOGRAMMATIDAE) ON *Diaprepes abbreviatus* (COLEOPTERA:  
CURCULIONIDAE) EGGS

**Introduction**

*Diaprepes abbreviatus* is a highly polyphagous weevil that was unintentionally introduced to Florida in the 1960s (Woodruff 1985). Since then, *D. abbreviatus* has spread throughout most of Florida where it is a pest of many native plants, fruit crops and ornamental commodities. The key pest status of *D. abbreviatus* in Florida is due to several factors including its polyphagy and lack of natural enemies. Many of the 260 plants from 60 families that have been reported as hosts of *D. abbreviatus* (Simpson et al. 1996, Mannion et al. 2003) grow in Florida where no native specialist natural enemies are present.

Classical biological control of *Diaprepes abbreviatus* is viewed as one of the components of integrated pest management programs with potential to effectively reduce weevil numbers (Peña et al. 2001, 2004). A promising biocontrol agent against *D. abbreviatus* is *Haeckeliana sperata* (Hymenoptera: Trichogrammatidae), a gregarious, egg-endoparasitoid that was collected from the island of Dominica in 2003 (Peña et al. 2006). *H. sperata* was found as a primary parasitoid parasitizing more than 50% of egg masses found in citrus groves and citrus nurseries in Dominica (Peña et al. 2006). Besides being a primary parasitoid of *D. abbreviatus* in its homeland, *H. sperata* was collected in areas of high altitude (509 meters above sea level) which suggests that it could have more cold hardiness than other imported parasitoids. If established, *H. sperata* could become a significant mortality factor against *D. abbreviatus*.

Failures in field releases and effectiveness of the suppression of various crop pests by trichogrammatid species have often been ascribed to intrinsic factors of the parasitoid strain (Olson and Andow 2002). However, the importance of extrinsic factors (i.e., the suitability of

the environment) has been increasingly recognized as determinant to the success of biological control programs (Olson and Andow 2003, 2006, Romeis et al. 1998, 1999). Given the wide host range of *Diaprepes*, a good understanding of the interactions between *H. sperata* and the food plant of its hosts could be key to the optimization of biological control programs.

Plants employ a battery of physical and chemical defenses against insect herbivores that are not necessarily compatible with parasitism of herbivore pests by natural enemies (Southwood 1986, Styrsky et al. 2006). One of the defense mechanisms that plants use against herbivores is the production of trichomes on the leaf surface (Jeffree 1986, Romeis et al. 1998, 1999, 2005). Trichomes are hair-like appendages that extend from the epidermis of above-ground plant tissues and play many functions (i.e., physiological, anchoring, water regulation, etc.) in plants. In some plants, trichomes serve as defense to herbivores; however, their presence may also affect their parasitoids. There is increasing evidence that the presence of trichomes has a negative effect on many trichogrammatid species and other minute hymenoptera (Jeffree 1986, Lukianchuk and Smith 1997, Romeis et al. 1998, 1999, 2005, Styrsky et al. 2006, Sütterlin and Van Lenteren 1997, 1998, 2000).

Plants with trichomes often impede the movement of parasitoids, thus weakening the parasitoid response (Price et al. 1980, Sütterlin and Van Lenteren 1997, Mulatu et al. 2006). Because of the small size of *H. sperata*, trichomes and trichome exudates can inhibit the parasitoid's movements. As with many other trichogrammatids (Schmidt 1994, Romeis et al. 2005), *H. sperata* searches for weevil eggs by walking on the leaf surface (D.C. personal observations). Thus, physical and chemical leaf surface characters can alter parasitoid searching and host encounter rates. Leaf surface characters can also alter the parasitoid's walking pattern by changing the distribution of turning angles, reducing their searching speed and ultimately

affecting their host finding ability (Olson and Andow 2006). Another factor that can affect the ability of *H. sperata* to parasitize *D. abbreviatus* eggs is the leaf thickness of the host plant. *H. sperata* inserts its ovipositor through the leaf until reaching the weevil egg mass on the other side of the leaf (Peña et al. 2004). This aspect of *H. sperata*'s behavior suggests that the relation between the length of the ovipositor and leaf thickness could be critical to the successful parasitism of *D. abbreviatus* in certain host plants.

In this study we hypothesize that the reproductive success of *H. sperata* is affected by the host plant of *D. abbreviatus*. The objectives of this investigation were to: (1) determine the successful parasitism of *H. sperata* on *D. abbreviatus* eggs on different host plants, (2) determine the effect of leaf trichomes on the searching behavior of *H. sperata* and (3) determine the relationship between searching speed, trichome density and leaf thickness to parasitism of *H. sperata* on *Diaprepes* eggs.

### **Material and Methods**

**Stock Colonies:** *H. sperata* used in each of the experiments described below were collected in Dominica in 2003 and reared for several generations at the Tropical Research and Education Center (TREC) insectary (L: D: 12:12 h,  $26.5 \pm 1^\circ\text{C}$ , and 75 % RH). Parasitoids were reared on *D. abbreviatus* eggs from adult weevils that were collected from a pesticide free commercial nursery in Homestead Florida. The weevils were placed randomly in groups of 300 in Plexiglass cages (30x30x30 cm) and fed on green buttonwood, *Conocarpus erectus*, two weeks prior the beginning of the experiments. Several collections of weevils were made during the course of all the experiments. All the parasitoids used in the experiments were mated, fed, naïve with respect to hosts, and were 1-day old at the time of testing.

**Plant Material:** Six host plants with varying trichome density and leaf thickness were chosen to study the successful parasitism on *D. abbreviatus* eggs. Among the fruit crops selected

for this study are lime, *Citrus aurantifolia* (Rutaceae), a much-branched, spiny shrub with elliptic ovate glabrous leaves (Bayley and Bayley, 1976), and Loquat, *Eriobotrya japonica* (Rosaceae), a small tree with alternate, short-petioled leaves with a high density of long, unicellular, nonglandular trichomes (Bayley and Bayley, 1976). Among the ornamental host plants the following were selected: pigmy palm, *Phoenix roebelinii* O'Brien (Arecaceae), a sometimes clustered slender palm with pinnate narrow pinnae with a sparse unicellular, non-glandular trichomes; button mangrove, *Conocarpus erectus* L., a native erect shrub or tree, with elliptic to ovate glabrous leaves; and silver button wood, *C. erectus* var. *sericeus* Fors ex. DC with silky-hairy leaves (Bayley and Bayley, 1976). The sixth host plant selected for this study is elephant grass, *Pennisetum prupureum* Schumach, (Poaceae) an invasive grass with often branched stems and elongate blades with a high density of long, unicellular trichomes.

#### Experiment 1. Determining the Successful Parasitism of *H. sperata* on *D. abbreviatus* Eggs on Different Host Plants.

No-Choice tests: Leaves free of any damage were removed from each host plant, leaving a 10 cm petiole, stem or other plant structure to hold the cutting firmly upright when formed into bouquets with exposed leaves (1500 cm<sup>2</sup> of leaf surface approximately). The bouquets were formed by inserting the stems into wet florist foam covered with aluminum foil, leaving the leaves exposed. The bouquets were placed separately into Plexiglass oviposition cages (30 x 30 x 30 cm) along with 250 adult weevils (50:50 ♂:♀) for 24 hours. After this period, the bouquets were removed from the weevil oviposition cages and the leaf area and number of egg masses were standardized (250 cm<sup>2</sup> of leaf surface and 5 egg masses per bouquet). The control treatment consisted of opened egg masses laid on wax paper that were obtained by hanging doubled strips of wax paper on the sides of a weevil cage during the oviposition period. The

bouquets were then placed individually in smaller cages (17x10x10 cm) bearing a small drop of honey as a food source for the parasitoids. Twenty 1-d-old adults of *H. sperata* (1: 1 ♀:♂) were introduced into each cage and parasitism was allowed for 24 hours. After the parasitism period, the bouquets were removed from the cages and checked under a microscope to make sure that no parasitoids were still in the bouquets. Seven days later, parasitized eggs were counted and percent parasitism was determined by dividing the number of parasitized eggs by the total number of eggs on each egg mass. At this stage parasitized eggs are easily recognizable by the presence of 4 to 6 compartments within a single *Diaprepes* egg, each holding one *H. sperata* individual (Ulmer et al. 2006c). Percent parasitism and natural egg mortality were recorded on each host plant and the control treatment. This experiment was replicated seven times, each time with new parasitoids.

Statistical analysis: ANOVAs (SAS institute, Inc. 1999) were used to detect the effects of host plants on the level of parasitism by *H. sperata* in the no-choice tests. Egg mass size (number of *Diaprepes* eggs per egg mass) was included as a covariate to control variation associated with host abundance. Means were compared using Tukey's honest significance test ( $p < 0.05$ ).

#### Experiment 2. Effect of Leaf Trichomes on the Searching Behavior of *H. sperata*.

This experiment was designed to determine the effect of leaf trichomes in two host plants on the walking speed of *H. sperata*. Only the two biotypes of buttonwood, *Conocarpus erectus* L. (Combretaceae), were used in this experiment. The searching speed of *H. sperata* females was measured on leaves kept intact or leaves where the trichomes were removed by two methods. A set of leaves of each buttonwood morph was washed with a 0.1% Triton X-100 solution, a non-phytotoxic detergent that helps removing trichomes and leaf-surface linked chemicals. Trichomes were removed from another set of leaves of the silver morph by shaving

them with a razor blade under a microscope. We included waxed paper as a non-leaf surface without the potential confounding effects from chemicals and epicuticular waxes. The mean searching speed on each plant was measured for ten mated, 1 day old and honey-fed *H. sperata* females. Leaf disks (6.3 cm<sup>2</sup>) of each surface (abaxial and adaxial) were excised and tightly placed in the bottom of a Petri dish of the same size. Female wasps were individualized in similar Petri dishes 30 min prior to each observation. Females were placed on the middle of each surface, the Petri dish was left uncovered which allowed ventilation and the females to leave the surface freely. The walking patterns were monitored using a video camera (Video Flex 7000 series ken-*a*-vision), equipped with a macro lens mounted 5 cm above the plane of the surface. Walking patterns were traced onto acetate sheets attached to a TV screen. The path and location were recorded every 10 s until the wasp left the surface or after 2 min had passed. The acetate sheets were scanned to convert the walking traces into digital images. The traces were processed using the image processing and analyzing software Scion Image ®. This procedure allowed us to calculate the total search time (s), the time spent motionless (s), the total distance traveled (mm), the walking speed (mm/s) and the searching speed of each female on each surface (mm/s). Searching speed is the average velocity a *H. sperata* during a certain observation, considering the total amount of time spent walking or immobile. Walking time refers to its average velocity only while walking. Searching speed is usually the variable of interest because it is a quantitative measure of how far a parasitoid searches in a given time (Van Hezewijk et al. 2000).

Statistical analysis: This experiment was replicated thirteen times, each time with new parasitoids and leaves. ANOVAs (SAS institute, Inc. 1999) were used to detect the effect of trichomes on the total search time, the time spent motionless, the total distance traveled, the walking speed and the searching speed of *H. sperata* females on buttonwood leaves kept intact or

leaves where the trichomes were removed. Means were compared using Tukey's honest significance test ( $p < 0.05$ ).

### Experiment 3. The Relationship of Searching Speed, Trichome Density and Leaf Thickness to Parasitism.

This experiment was designed to measure the degree to which variation in searching speed, trichome density and leaf thickness explained the variation in parasitism. The six host plants used in Experiment 1 were included in this experiment. A population of *H. sperata* was divided in two such that the proportion of hosts parasitized and the searching speed on the six plants could be measured simultaneously. All experiments including parasitoids were made between 1-5 pm. Leaves from each plant were collected and divided in three groups. The first group was used to test the proportion of hosts parasitized on each plant biotype, the second group was used to determine the searching speed on each leaf surface and the third to measure the trichome density and leaf thickness of each plant biotype.

The proportion of parasitized hosts was determined in an experiment similar to the no-choice tests. Bouquets of each host plant bearing 5 egg masses were introduced in 2-L jars (17x10x10 cm) provisioned with a drop of honey as food source for the parasitoids. Ten 1-d-old females of *H. sperata* were introduced into each cage. After the parasitism period, the bouquets were removed from the cages and checked under a microscope to make sure that no parasitoids were on the bouquets. Seven days later, parasitized eggs were counted and percent parasitism was determined by dividing the number of parasitized eggs by the total number of eggs on each egg mass. Percent parasitism and natural egg mortality were recorded on each host plant. Searching speed of *H. sperata* on the different host plant leaves was measured in the same way as in the previous experiment. This experiment was replicated ten times, each time with new parasitoids and plants.

Trichome density and leaf thickness were measured on ten expanded leaves on new shoots of the same plant material used in the previous experiments. Two squares (1 cm<sup>2</sup> each) from the middle part of the leaves were excised; one square was used to determine the leaf thickness and the other one for the trichome density for each leaf surface (abaxial and adaxial). Leaf thickness was determined by placing the cutting standing on a piece of clay and measuring its thickness under a microscope. Trichome density was established by counting the total number of trichomes on each surface (abaxial and adaxial) and making an average of the two values. Because of the high density of trichomes on silver buttonwood leaves, the 1 cm<sup>2</sup> squares were divided in four equal 0.25 cm<sup>2</sup> sections to determine the trichome density only in one of the four small squares.

Statistical analysis: Multiple regression (PROG REG) analysis was used to establish the relationship of searching speed, trichome density and leaf thickness to parasitism (SAS institute, Inc. 1999).

## **Results**

### Experiment 1. Determining the Successful Parasitism of *H. sperata* on *D. abbreviatus* Eggs on Different Host Plants.

The no-choice test revealed that *H. sperata* is able to parasitize *D. abbreviatus* eggs laid on the six host plants. However, *H. sperata* failed to parasitize egg masses on wax paper (Figure 2.1).

Interestingly the three plants with leaves having the higher densities of trichomes had a significantly lower parasitism when compared with the three plants that had either glabrous leaves (lime) or a low density of trichomes (pygmy palm and green buttonwood). Furthermore,

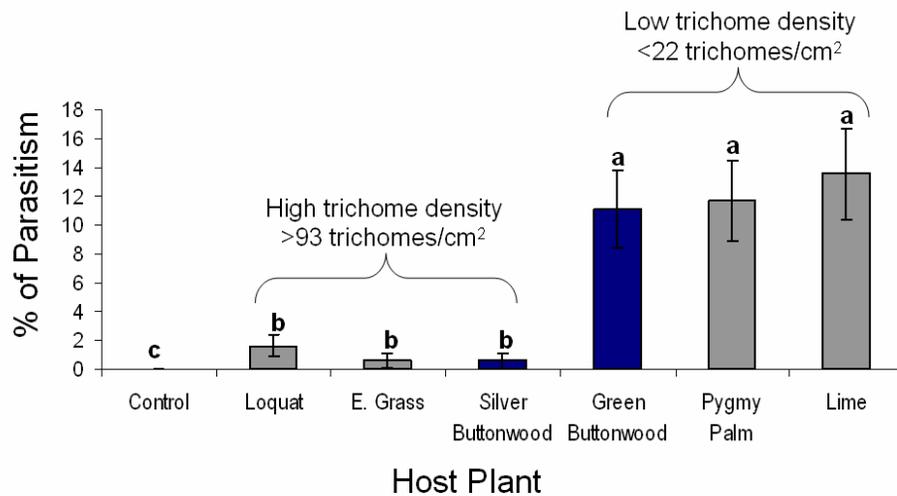


Figure 2-1. Effect of host plant on successful parasitism of *D. abbreviatus* eggs by *H. sperata*. No-Choice tests on six host plants and control treatment (wax paper). Different letters represents significant differences, Tukey ( $P < 0.05$ ). Error bars represent the standard error.

percent parasitism was more than ten times greater in the glabrous green morph of *C. erectus* when compared with the silver biotype that has a high density of trichomes on the leaf surface (Figure 2.1).

#### Experiment 2. Effect of Leaf Trichomes on the Searching Behavior of *H. sperata*.

The overall results of this experiment show that the presence of trichomes has a significant negative effect in the searching efficiency of *H. sperata*. There was no effect of the leaf surface orientation (abaxial and adaxial) on any of the variables measured ( $p < 0.01$ ). The net displacement of the females was three fold greater on any of the surfaces that had no trichomes than on the pubescent surface (Table 2.1). When the trichomes were removed from the pubescent silver morph, by shaving them or treating the leaf with Triton X-100, the net displacement of *H. sperata* increased to levels similar to the glabrous green morph (Table 2.1).

Table 2-1. Effect of leaf trichomes on the searching behavior of *H. sperata*. N=13. Means followed by different letters within columns are significantly different at P<0.01

Leaf	Net displacement (mm) Mean ± SEM	Total Time on surface (s) Mean ± SEM	Walking Speed (mm/s) Mean ± SEM	Searching Speed (mm/s) Mean ± SEM
Wax Paper	79.02 ± 7.78 a	59.16 ± 5.51 c	7.76 ± 3.87 a	1.92 ± 0.34 a
Green Buttonwood	59.02 ± 4.28 b	72.30 ± 4.74 bc	5.68 ± 0.62 b	1.02 ± 0.15 b
Silver Buttonwood	17.92 ± 1.24 c	94.23 ± 6.25 a	1.87 ± 0.17 c	0.24 ± 0.04 c
Silver Buttonwood Shaved	54.96 ± 3.79 b	61.15 ± 6.10 c	5.09 ± 0.47 b	1.51 ± 0.39 ab
Green Buttonwood + Triton X-100	56.02 ± 3.80 b	76.92 ± 5.13 ab	5.51 ± 0.54 b	0.93 ± 0.17 bc
Silver Buttonwood +Triton X-100	50.77 ± 3.18 b	82.69 ± 6.16 ab	5.24 ± 0.51 b	0.81 ± 0.13 bc

In contrast *H. sperata* females spent more time on the pubescent surface and less on the smooth leaf surfaces. When the trichomes were shaved from the pubescent morph the total time spent on the surface decreased (Table 2.1). The walking speed was significantly higher on all the surfaces with no trichomes when compared with the pubescent surfaces. When the trichomes were removed by any method, the walking speed increased to levels similar to the smooth surfaces. A similar pattern was observed in the searching speed analysis; however, the searching speed on surfaces treated with Triton was significantly lower, which could suggest a confounding effect of this detergent that made the parasitoids stay longer on the surface.

### Experiment 3. The Relationship of Searching Speed, Trichome Density and Leaf Thickness to Parasitism.

The results of this experiment show that searching speed was a good predictor of the level of parasitism, whereas neither trichome density nor leaf thickness explained the variation on

parasitism (Table 2.2). It is likely that the type of trichomes and the exudates associated with them are more important than the number per unit of area.

Table 2-2. Relationship of searching speed, trichome density and leaf thickness to parasitism. Multiple regression analysis of the effect of searching speed, trichome density and leaf thickness on mean percent of parasitism of *H. sperata* on *D. abbreviatus* eggs laid on six host plants.

Model	Coef.	SE <sup>a</sup>	P <sup>b</sup>	R <sup>2c</sup>	P <sup>d</sup> (overall)
Parasitism					
= Searching Speed	2.82	0.23	<0.001	0.89	<0.01
+ Trichome Density	1.2 x 10 <sup>-6</sup>	3.0 x 10 <sup>-6</sup>	0.68		
+ Leaf Thickness	12.59	7.16	0.09		

<sup>a</sup> SE, standard error of the coefficient

<sup>b</sup> P values associated with each parameter, significance of the variable in the predicted response

<sup>c</sup> R<sup>2</sup>, proportion of the variance explained by the model

<sup>d</sup> P(overall) is for the test of significance of the model using GLM.

## Discussion

Even though *H. sperata* can parasitize eggs laid on different host plants, the parasitoid was affected by variations in the leaf surface structure of the host plants. We tested six host plants with various degrees of pubescence and found that the reproductive success of *H. sperata* is much higher in plants with smooth surfaces like green buttonwood or lime than on those with pubescence (silver buttonwood and loquat). Considering that *H. sperata* was originally collected from citrus groves and nurseries acting as a primary parasitoid, we could expect better chances of control and establishment in citrus plants which are one of the major hosts of *D. abbreviatus* in central and south Florida. Green buttonwood is used for rearing *H. sperata*, our results reaffirm that this plant is the best known option for rearing this parasitoid. Besides being available throughout the year in south Florida, we always found a high parasitism in this plant when compared with the other host plants that were tested. In contrast, parasitism was lowest on the

silver morph of buttonwood. These results are similar to those obtained by Amalin et al. (2005) working with another trichogrammatid parasitoid of *D. abbreviatus*, *Ceratogramma etiennei*. In a no-choice experiment with four of the same host plants that we used in our experiments, *C. etiennei* showed a high parasitism of *D. abbreviatus* eggs on lime, pygmy palm and green buttonwood, and a low parasitism on the pubescent green buttonwood. Interestingly, Mannion et al. (2003) found that *D. abbreviatus*, when given a choice, prefers to oviposit on silver buttonwood, where it has better larval survivorship. These results suggest that silver buttonwood, which is a common ornamental plant in south and central Florida, is an important host for *D. abbreviatus*, because a low parasitism by *H. sperata* could be expected. The results obtained in this host plant and the other pubescent plants (i.e. elephant grass and loquat) suggest that plants that have a high density of trichomes in their leaves are not good candidates for releasing *H. sperata*.

Our results show that the presence of trichomes in *C. erectus* has a negative effect on the searching efficiency of *H. sperata*. In theory, faster walking females have a higher chance of finding hosts as they can search a larger surface area per unit of time (Olson and Andow 2003). In fact, searching speed has been adopted by the IOBC working group on “quality control of mass reared organisms” as an important criterion for pre-introductory selection of a suitable strain of *Trichogramma spp.* parasitoids (Limburg and Pak 1991). Searching speed has been used to select strains and/or releasing sites on several trichogrammatid species (i.e. *Trichogramma brassicae*, *T. nubilale*, *T. exiguum* and *T. chilonis*) (Keller 1987). However, Van Hezewijk et al. (2000) reported that the level of parasitism in *T. minutim* was positively affected by the host acceptance, but was not related to the parasite's searching speed. Thus, searching speed cannot always be used to predict parasitoid quality or to select plants or sites to release.

Our results, however, suggest that searching speed is a parameter tightly linked to parasitism and that it is feasible to use this parameter to predict the suitability of host plants for releasing *H. sperata*.

*Diaprepes* has many host plants in Florida that represent an array of leaf structures and types of trichomes that could hinder the reproductive success of *H. sperata*. The degree of control of *Diaprepes* populations by *H. sperata* may depend, among other factors, on the interaction of the parasitoids with the micro-environment created by the plants on the leaves surfaces. As seen in other trichogrammatids (Flanders 1937), it is possible that *H. sperata* will become more prevalent in certain habitats or on specific plants. Also, parasitism levels of *D. abbreviatus* can vary widely, depending on the plants on which the eggs are found. In addition, parasitism by *H. sperata* may also vary with the plant structure or region of the plant on which the host eggs are located. Our results suggest that differences in the establishment and levels of control by *H. sperata* in different habitats with varying plant composition can be expected.

We conclude that the reproductive success of *H. sperata* is dependent on the host plant of *D. abbreviatus*. Plants with simplified leaf surfaces favor the searching efficiency of *H. sperata* and may allow higher levels of parasitism of *D. abbreviatus*. The presence of some types of leaf trichomes could have a negative effect in the searching efficiency and reproductive success of *H. sperata*. Finally, we propose using searching speed as one of the criteria to select suitable host plant for releasing *H. sperata*.

CHAPTER 3  
RELATIVE SUSCEPTIBILITY OF *HAECKELIANIA SPERATA* TO PESTICIDES USED IN  
CITRUS

**Introduction**

In order to maximize parasitism of *H. sperata* on the *Diaprepes* root weevil, biological control practices must be integrated with other pest management tactics. Pesticides are a critical component of insect pest management in citrus and ornamental production. Thus, awareness of their effects on *H. sperata* would be critical information to design tactics that could maximize the probability of establishment and control with this parasitoid. Rational use of pesticides in IPM relies in part on knowledge of the pesticide effects on beneficial insects. Two studies have addressed the toxicity of pesticides used in citrus to parasitoids of *D. abbreviatus*. Ulmer et al. (2006b) evaluated the toxicity of pesticides used in citrus to *Aprostocetus vaquitarum*. Their findings suggested that carbamate and organophosphate pesticides were the most toxic to *A. vaquitarum* adults, followed by neonicotinoid, pyrethroid and kaolin clay pesticides. Copper and phosphonate fungicides, petroleum oil, abamectin, and insect growth regulators were slightly to non-toxic to *A. vaquitarum*. In another study, Amalin et al. (2004) tested the effect of an insect growth regulator, diflubenzuron, on *Ceratogramma etiennei* and *Quadrastichus haitiensis*. Their study concluded that this pesticide interferes with the development of *C. etiennei* but not on the development of *Q. haitiensis*. Other examples of studies conducted on citrus to address the impact of pesticides on parasitoids in Florida, include evaluation of the toxicity of pesticides to *Ageniaspis citricola* (Hymenoptera: Encyrtidae) (Villanueva-Jimenez and Hoy 1998), a parasitoid of the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). These authors used an index of IPM compatibility, classifying the combination of each of these insecticides (azadirachtin, diflubenzuron and fenoxycarb) with oil and oil alone as IPM

compatible insecticides. Application of Neem oil and drenched imidacloprid were classified as semi-compatible insecticides. Abamectin + oil, ethion and imidacloprid applied as a spray were classified as IPM- incompatible insecticides.

In general, effects of pesticides on natural enemies could be direct or indirect. Direct effects are those caused by physical direct contact of the natural enemy with the toxin, and could be manifested as a short-term mortality or relatively long-term sublethal effects (Johnson and Tabashnik 1999). Indirect effects are caused when the natural enemy acquires the toxin through its host prey (Williams et al. 2003). *Haeckeliana* adults search for weevil egg masses while walking on the surface of the leaves of their host's plant, where they also mate and rest. In contrast, the immature stages of *Haeckeliana* develop inside the weevil eggs, which in turn are enclosed between two sealed leaves. These aspects of the biology and behavior of *H. sperata* suggest that the adults are more likely to get in direct contact with the pesticides whereas the immature stages are more likely to be affected indirectly by pesticides present in *Diaprepes* eggs. Adults will contact pesticides either directly during an application or by encountering pesticide residues while walking on the surface of the leaves. The fact that pesticide residues remain on the leaves for some time after the application suggests that the likelihood of encountering pesticide residues on the leaf surface should be much greater than the likelihood of getting affected by a direct spray. For this reason, a good starting point to determine the relative toxicity of pesticides to *H. sperata*, is to conduct bioassays to assess the effect of foliar residues of insecticides on acute mortality of *H. sperata* adults.

Evaluation of pesticide effects on minute Hymenoptera like *H. sperata* is complicated by their size and extreme fragility. Several bioassays have been used for this purpose by assessing pesticide residues on leaves or artificial surfaces enclosed in cages (Villanueva and Hoy 1998,

Hassan and Aldelgader 2001). However, recent studies have demonstrated that the toxicity of some pesticides may vary depending on which surface they are applied (Ternes et al. 2001). Considering that *H. sperata* walks on the foliage to search for hosts and mates, it is more appropriate to evaluate the effects of pesticides residues on leaf tissues which should approximate natural conditions in the field. Williams and Price (2004), developed a bioassay for assessment of contact residues of insecticides on *Trichogramma pretiosum* and *Anaphes iole* Girault (Hymenoptera: Mymaridae). Their methodology is especially appealing because it permits the leaf disks to stay fresh for a longer period of time, provides food (honey) for the parasitoids, allows ventilation, requires little manipulation of the parasitoids, and it is relatively easy to set up. After making preliminary tests of some of the proposed methodologies, and recognizing that no single bioassay method will provide definite and complete information to assess the effects of pesticides on insects, the methodology proposed by Williams and Price (2004) was chosen to assess the effect of pesticides on *H. sperata*.

Some studies have demonstrated that deposition of pesticide residues on the leaf surface under field conditions is variable. Ebert et al. (1999a) reported that the uniformity of the deposition of the active ingredients on the target area depends largely on the volume of water in the pesticide solution and the application equipment that is used. In general, changing the application volume not only affects coverage of the application but also changes the concentration of active ingredient deposited per unit of area (Ebert et al. 1999a). In addition, there is evidence showing that different application equipment applies different amounts of active ingredient to different portions of the plants (Ebert et al. 1999b). As a consequence pest insect and natural enemies are often exposed to a range of pesticide concentrations that include the label concentration but also sub-label rates. Some studies have underlined the importance of

studying the effect of different pesticide rates on natural enemies (Villanueva-Jimenez and Hoy 1998, Delpuech et al.1999). Some pesticides have shown significant variation in effects on natural enemies at different doses. Moreover, some pesticides are registered for controlling different pests in a single crop but with different rates depending on the target pest.

The objective of this study is to evaluate the relative susceptibility of adult *H. sperata* to some pesticides used in citrus and ornamental plant production.

### **Materials and Methods**

Stock Colonies: *H. sperata* used in each of the experiments described below were collected in Dominica in 2003 and reared in the Tropical Research and Education Center (TREC) insectary (12 hour photoperiod,  $26.5 \pm 1^\circ\text{C}$ , and 75 % RH) for several generations. Parasitoids were reared on *D. abbreviatus* eggs from adult weevils that were collected from a pesticide free commercial nursery in Homestead Florida. All the parasitoids used in the experiments were mated, fed, naïve with respect to hosts and less than 1-day old.

Bioassay: Williams and Price (2004) bioassay methodology developed for minute hymenoptera was used in this study. The purpose of the bioassays was to expose parasitoids to 24h old pesticide residues on leaf surfaces to determine their effects on the longevity of *H. sperata*.

Four concentrations of seven pesticides were included in the bioassays. Leaves from a pesticide-free lime grove were collected and leaf disks (2.3 cm diam.) were excised with a cork borer. The leaf disks were dipped for 20 min in the different concentrations of the pesticides and in water for the control, and left to dry for 24 hours. The concentrations were chosen based on label rates. The concentration of each pesticide tested consisted of a dilution series where the

starting and more concentrated solution was the recommended label rate for field applications, assuming a standard volume of water of 100 gallons/acre. The pesticides tested (including trade name, class, active ingredient, application rate, label concentration, and bioassay concentrations) are listed in Table 3.1

The bioassay chambers consisted of one piece of transparent PVC tube (2.54cm ID x 3.5 cm long) with organically covered ventilation holes, two vial scintillation caps (kimble glass inc. cat. no. 74521-22400) each containing 3ml of agar and a treated leaf disk (each pair treated with the same concentration and placed on top of the agar), a piece of dialysis membrane, and a feeding tube. The chambers were assembled by sliding one cap into each end of the tube so that the edge of leaf disk was aligned with the edge of the ventilation holes. The upper surface of the leaf disk formed the floor and the under surface of the leaf disk formed the ceiling of the chamber. A strip of dialysis membrane was used to seal the chambers. A piece of borosilicate glass capillary (5 cm long x 1.5 mm diam.) that was previously flamed was used to make a small hole in one of the ventilation holes. Through this hole 10 parasitoids (presumed mated and 1:1 ♀:♂) were introduced to each chamber using an aspirator constructed with a capillary of the same type used for making the holes. The hole was then covered with a feeding tube, one tip (1.5 cm long) from a Finntip 5-300 µl pipette tip was filled with honey-water solution (1:1) as a food source for the parasitoid. Once assembled, the chambers were placed in a room maintained at  $26.5 \pm 1^{\circ}\text{C}$ , 12:12 L:D, and 75 % RH.

Mortality was scored under a stereoscope every three hours after the parasitoids were exposed to the pesticide residues on the leaf discs. Mortality was defined by immobility and a complete lack of movement by mouthparts, wings and legs. Five replicates per insecticide concentration were evaluated.

Table 3-1. List of pesticides tested on *H. sperata*, including trade name, class, active ingredient, application rate, label concentration, and bioassay concentrations.

Trade Name	Class / Active Ingredient	Manufacturer	application rates	Label concentration <sup>a</sup>	Bioassay concentrations <sup>b</sup>
Sevin XLR <sup>®</sup>	Carbamate/ Carbaryl (44.1%)	Bayer CropScience	1.5 qt/acre	3.7 ml/L	(ml/L) 3.7, 1.8, 0.9, 0.4, 0
Lorsban 4E <sup>®</sup>	Organophosphate / Chlorpyrifos (44.9%)	Micro Flo Company	2-7 pt/acre	8.7- 2.5 ml/L	(ml/L) 5.0, 2.5, 1.2, 0.6, 0
Provado 1.6F <sup>®</sup>	Neonicotinoid/ Imidacloprid (22%)	Bayer CropScience	10-20 fl.oz/acre	1.5- 0.7 ml/L	(ml/L) 1.0, 0.5, 0.2, 0.1, 0
Danitol 2.4 EC <sup>®</sup>	Pyrethroid/ Fenprothrin (30.9%)	Valent USA Corporation	16-21.3 fl.oz/acre	1.6- 1.2 ml/L	(ml/L) 1.5, 0.7, 0.3, 0.1, 0
Alliete WDG <sup>®</sup>	Phosphonate/ Aluminium tris (80%)	Bayer CropScience	5.0 lb/acre	5.9 gr/L	(gr/L) 6.0, 3.0, 1.5, 0.7, 0
AgriMek 0.15 EC <sup>®</sup>	Glycoside/ Abamectin (2%)	Syngenta	10-20 fl.oz/acre	1.5- 0.7 ml/L	(ml/L) 1.0, 0.5, 0.2, 0.1, 0
Citrus Soluble Oil <sup>®</sup>	Petroleum oil (99.3%) FC 435	Platte Chemical Company	5 qt/acre	12.5 ml/L	(ml/L) 12.5, 6.2, 3.1, 1.5, 0

<sup>a</sup> Solutions were calculated using a standard volume of water of 100 gallons/acre.

<sup>b</sup> bioassay concentrations are a dilution series using formulated pesticides at label rates as the primary solution and a standard volume.

Data Analysis: Lethal time 50 (LT50) and lethal time 90 (LT90) were estimated for each insecticide concentration using the SAS-PROBIT procedure (SAS Institute, 1999). Significant differences between lethal times were indicated when the 95% fiducial limits of one concentration did not overlap with the fiducial limits of the other concentrations. Lethal concentrations LC50<sub>s</sub> and LC90<sub>s</sub> were calculated 12, 24 and 48, hours after parasitoids were exposed to the pesticides using the SAS-PROBIT procedure (SAS institute, Inc. 1999). Abbott's transformation (Abbott 1925) was used to correct for control mortality.

## Results

Carbamate, organophosphate and pyrethroid insecticides used in the bioassays were highly toxic to *H. sperata*. Contact with any of the pesticides from these groups at any of the

concentrations resulted in death of all *Haeckeliana* wasps within a few hours of being exposed (Figure 3.1). LT50<sub>s</sub> of *H. sperata* exposed to residues of these pesticides ranged from 2.4-8.1 h (Sevin®), 2.4 – 2.8 h (Lorsban®) and 1.1- 3.43 h (Danitol®) (Table 3.2). In some cases, the analyses did not produce lethal concentration values or fiducial limits. This was probably caused by the high mortality registered in the first evaluations; 42%, 82% and 61% of the total number of wasps were dead three hours after being exposed to Sevin®, Lorsban® and Danitol®, respectively. No differences in the LT50<sub>s</sub> of the four tested concentrations were observed for Lorsban® and Danitol®, suggesting that the lower concentrations (dilutions) are as toxic as the high concentrations (label rate concentrations). In contrast, lower concentrations of Sevin® had a significantly lower toxic effect on *H. sperata* than the higher concentrations (Figure 3.1). The lethal concentrations (LC 50<sub>s</sub>) were out of the range of tested concentrations. Considering that all the tested concentrations of these insecticides produced a rapid and high mortality on *H. sperata*, the LC<sub>s</sub> of these pesticides will necessarily be lower than the ones used in these bioassays. The minimum concentrations used in the bioassays were one eighth of the label recommended concentrations which suggest that these insecticides are incompatible with *H. sperata*.

Contact with Agrimek® and Provado® also caused a high mortality of *H. sperata* but it occurred later than on those exposed to Sevin®, Danitol® and Lorsban®. LT50<sub>s</sub> for wasps exposed to Agrimek® and Provado® ranged from 9.42 to 25.37 hours and 7.66 to 29.03 hours, respectively. In both cases an effect of the concentration on the time of death was observed. The two lower concentrations (0.125 and 0.25 ml/L) had a significantly longer LT50<sub>s</sub> than the two higher concentrations (0.5 and 1.0 ml/L) (Table 3.2).

Table 3-2. Lethal Time (50 and 90) of *H. sperata* exposed to of 4 concentrations of selected pesticides.

Pesticide	Concentration	LT 50		LT 90		$\chi^2$	Slope
		(h)	LT 50 FL (h)	(h)	LT 90 FL (h)		
Alliete WDG®	0 gr/L	184.6 a	154.0 - 234.7	978.6 a	660.8 - 1665	15.35	1.76
	0.75gr/L	39.7 b	38.7 - 40.7	62.1 b	60.2 - 64.2	19.1	6.6
	1.5gr/L	33.0 c	30.71 - 35.48	58.5 b	52.4 - 68.1	71.25	5.15
	3gr/L	30.9 cd	28.3- 33.7	58.8 b	51.5 - 71.1	88.63	4.59
	6gr/L	21.6 d	17.0 - 26.7	57.8 b	43.1 - 100.5	198.4	3
Provado 1.6F®	0 ml/L	184.6 a	154.1- 234.7	978.7 a	660.8 - 1665	15.35	1.76
	0.12 ml/L	29.0 b	25.2 - 32.8	104.1 b	86.0 - 134.8	147.4	2.31
	0.25 ml/L	26.5 b	22.8 - 30.1	99.0 b	81.6 - 128.8	135.9	2.24
	0.50 ml/L	13.8 c	11.8 - 15.8	65.0 c	56.2 - 77.7	54.67	1.9
	1.00 ml/L	7.6 d	6.4 - 8.8	52.8 c	46.67 - 61.18	26.76	1.52
AgriMek 0.15 EC®	0 gr/L	184.6a	154.1- 234.7	978.7 a	660.8 - 1665	15.35	1.76
	0.12 ml/L	25.3 b	22.1- 28.6	77.6 b	66.3 - 95.2	157.1	2.64
	0.25 ml/L	20.5 b	17.8 - 23.2	74.6 b	63.7 - 91.2	92.57	2.29
	0.50 ml/L	15.2 c	14.1- 16.4	55.0 c	51.1 - 59.9	17.58	2.29
	1.00 ml/L	9.4 d	8.5 - 10.2	30.7 d	28.2 -33.6	13.57	2.5
Citrus Soluble Oil®	0 ml/L	179.3 a	150.6- 226.1	908.1 a	621.5 - 1515	15.99	1.81
	1.56 ml/L	26.4 b	24.4 - 28.4	162.8 b	141.7 - 191.7	34.06	1.62
	3.12 ml/L	20.5 c	18.1- 22.9	136.2 b	114.2 - 169.5	47.36	1.55
	6.25 ml/L	13.4 d	10.5 - 16.3	62.2 c	51.5 - 79.4	146.8	1.92
	12.5 ml/L	12.1 d	10.1 - 14.1	50.5 c	44.1 - 59.2	85.27	2.07
Sevin XLR®	0 ml/L	179.3 a	150.6 - 226.1	908.1 a	621.5 - 1515	15.99	1.81
	0.45 ml/L	8.1 b	6.3 - 9.7	23.3 b	18.2 - 34.7	19.13	2.79
	0.94 ml/L	4.8 b	3.1 - 6.4	13.6 bc	10.4 - 21.2	29.48	2.88
	1.87 ml/L	3.8 bc	2.6 - 4.9	12.3 c	10.0 - 16.3	12.88	2.54
	3.75 ml/L	2.4 c	1.7 - 2.9	7.9 d	6.9 - 9.2	2.56	2.48
Lorsban 4E®	0 ml/L	179.3 a	150.6 - 226.1	908.1 a	621.5 - 1515	15.99	1.81
	0.63 ml/L	2.8 b		3.3 b			22.06
	1.25 ml/L	2.7 b		3.1 b			20.93
	2.50 ml/L	2.7 b		3.1 b			20.93
	5.00 ml/L	2.4 b		2.8 b			18.38
Danitol 2.4 EC®	0 ml/L	179.3 a	150.6 - 226.1	908.1 a	621.5 - 1515	15.99	1.81
	0.19 ml/L	3.4 b	2.9 - 3.9	8.3 b	7.4 - 9.6	4.26	3.34
	0.38 ml/L	2.7 b	2.3 - 3.1	5.2 c	4.6 - 6.0	2.33	4.63
	0.75 ml/L	2.6 b	2.14 - 2.96	5.1 c	4.5 - 5.9	1.97	4.37
	1.50 ml/L	1.1		3.7		2.91	2.51

\*LT50<sub>s</sub> or LT90<sub>s</sub> followed by an asterisk are not significantly different because of fiducial limits overlap.

Accordingly, LT90<sub>s</sub> for wasps exposed to the lower concentrations were significantly longer than those exposed to higher concentrations (Table 3.2). In both cases the LC50<sub>s</sub>

calculated 12 hours after exposure to the insecticides were less than the labelled field rates (Tables 3.1 and 3.3). Results of the bioassays suggest that these two pesticides are less toxic to *H. sperata* than the Sevin®, Lorsban® and Danitol®. An effect of the concentration on the mortality of *H. sperata* was observed, suggesting that a reduction in the insecticide concentration favors the longevity of *H. sperata* (Figure 3.1).

Table 3-3. Lethal Concentrations (50 and 90) at 12, 24 and 48 hours after *Haeckeliana sperata* adults were exposed to leaf disks treated with seven pesticides at four different concentrations.

Pesticide	Time after exposure to insecticide	LC 50 ml/L	LC 50 Fiducial limits ml/L	LC 90 ml/L	LC 90 Fiducial limits ml/L	$\chi^2$	Slope
Alliete WDG®	12	19.92	11.27 - 77.24	111.59	38.50 – 1543	1.3	1.7
	24	9.34	6.46 - 18.17	73.18	31.68 - 371.66	1.8	1.4
	48	0.26	0.07 - 0.47	3.01	2.19 - 5.24	4.3	1.2
Provado 1.6F®	12	0.64	0.48 - 1.02	10.62	4.20 - 74.84	0.5	1.0
	24	0.28	0.20 - 0.36	3.56	1.92 - 11.68	2.0	1.1
	48	0.17	0.12 - 0.21	1.21	0.86 - 2.12	3.0	1.5
AgriMek 0.15 EC®	12	0.81	0.56 - 1.68	22.27	6.34 – 487	2.6	0.8
	24	0.18	0.07 - 0.28	1.77	1.14 - 3.90	4.4	1.3
	48	0.12	0.07 - 0.15	0.82	0.61 - 1.35	1.3	1.5
Citrus Soluble Oil®	12	11.86	6.73 - 87.29	2091	179.65 – 22090	0.6	0.5
	24	4.78	3.17 - 7.56	231.56	66.90 – 10572	0.1	0.7
	48	0.8	0.30 - 1.29	9.62	6.70 - 19.04	4.5	1.1
Sevin XLR®	12	0.21		1.55		5.6	1.5
	24	0.36		0.42		0	18.3
	48	*		*			
Lorsban 4E®	12	*		*			0
	24	*		*			
	48	*		*			
Danitol 2.4 EC®	12	0.14		0.17			17.6
	24	*		*			0
	48	*		*			0

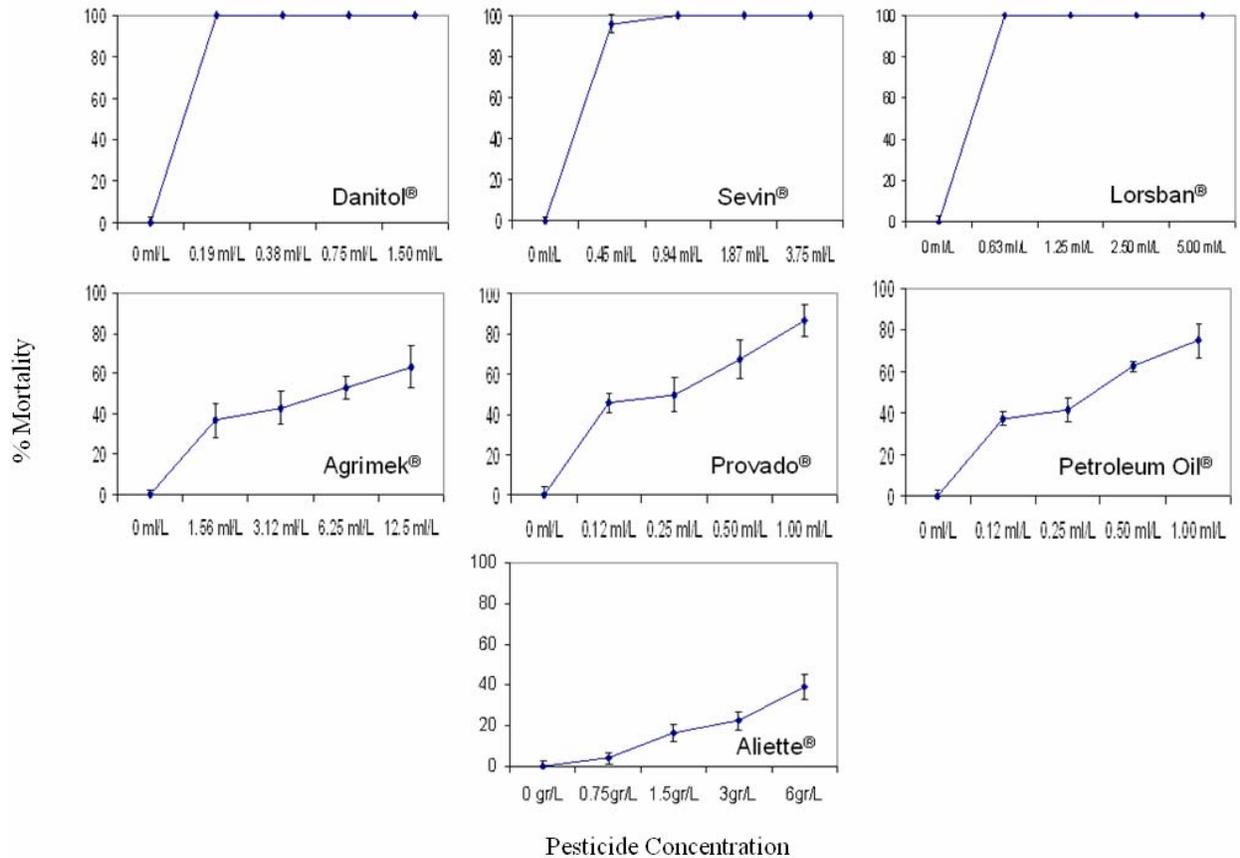


Figure 3-1. Percent mortality of *H. sperata* 24 hours after exposure to four different concentrations of pesticide residues on lime leaves. The concentrations for each pesticide consisted of a dilution series using the label rate for field applications as t

The petroleum oil alone showed results similar to those of Provado® and Agrimek® (Figure 3.1). LT50<sub>s</sub> for wasps exposed to petroleum oil ranged from 12.16 to 26.42 hours. The two lower concentrations (1.56 and 3.12 ml/L) had a significantly longer LT50<sub>s</sub> than the two higher concentrations (6.25 and 12.5 ml/L) (Table 3.2). LT90<sub>s</sub> of wasps exposed to leaf residues of petroleum oil at the two lower concentrations were larger than those of any of the other tested pesticides (Table 3.2). LC50<sub>s</sub> at 12, 24 and 48 hours after exposure to petroleum oil were equal to or lower than the label rates which suggest that contact with fresh residues of petroleum oil at label concentrations will cause the death of half of the *Haeckeliana* wasps in less than 12 hours.

The only fungicide evaluated in this study, Aliette® WDG, was the pesticide that showed the lowest effect on *H. sperata*. LT50<sub>s</sub> for wasps exposed to Aliette® ranged from 21.67 to 39.74 hours, being significantly longer at the lowest concentration that was tested (0.75 gr/L) (Table 3.2). However, LT90<sub>s</sub> of wasps exposed to Aliette® were very similar to the other pesticides such as Provado® and Agrimek®, but lower than that of petroleum oil. This was the only pesticide for which the LC 50 and LC 90s at 12 and 24 hours after exposure were significantly higher than the label concentrations (Tables 3.1 and 3.3). However, LC50 and LC90 48 hours after exposure to the pesticide were lower than the label concentration. These results suggest that contact with fresh residues of Aliette® at label concentrations will cause the death of half *Haeckeliana* wasps in less than 48 hours. An effect of the concentration of Aliette® on the mortality of *H. sperata* was observed, suggesting that a reduction in the fungicide concentration favors the longevity of *H. sperata* (Figure 3.1). These results suggest that Aliette® has a lower acute toxicity than the other tested pesticides (Figure 3.1). However, the long-term effects seem to be relatively similar to the other pesticides used in the bioassays.

### Discussion

In our bioassays, the organophosphate, carbamate and pyrethroid pesticides showed a rapid and strong toxic effect on *H. sperata*. Moreover, no reduction in mortality was caused by the dilutions of the pesticide solution, which were up to eight times lower than the recommended label rates. This suggests that these three insecticides have a high acute toxicity to *H. sperata*. These results are similar to those found on *A. vaquitarum*. Ulmer et al. (2006b) reported that organophosphate and carbamate insecticides tested at label rates were more toxic to *A. vaquitarum* adults than other insecticides. However, *H. sperata* was affected similarly by carbamate, organophosphate and pyrethroid pesticides, whereas pyrethroid insecticides were less toxic to *A. vaquitarum* than the organophosphate or carbamate pesticides. These three pesticides interfere

with the transmission of nervous impulses in the nervous system of the insect. Organophosphate and carbamate insecticides inhibit the acetylcholinesterase enzyme (Scharf 2003). This enzyme regulates the sodium channel by removing acetylcholine from its postsynaptic receptor, and ultimately, modulates the initiation of action potential at precise, exact intervals (Scharf 2003). Inhibition of the acetylcholinesterase enzyme by organophosphate and carbamate insecticides results in the prolonged binding of acetylcholine to its receptor, and ultimately, in the insect death from prolonged neuroexcitation. Pyrethroids alter the gating kinetics of the sodium channel causing a prolonged flow of Na<sup>+</sup> currents into neurons, and ultimately, neuronal dysfunction because of excessive neuroexcitation (Scharf 2003). Insecticides that target the nervous system are considered to be broad spectrum pesticides. It is not surprising that neurotoxic insecticides could have a negative effect on natural enemies, as reported in other studies (Villanueva-Jimenez and Hoy 1998, Wakgari and Giliomee 2001, 2003).

The carbamate, organophosphate and pyrethroid insecticides that were used in our experiments are registered to control several citrus pests with varying doses depending on the target pest. Sevin XLR® is recommended for the control of various citrus pests at doses ranging from 1.5 - 3 qt/acre ( $\approx$  7.5 ml/L - 3.37 ml/L solution assuming a volume of 100 gal/ acre) (Browning et al. 2007, Stansly et al. 2007). For root weevils, the dose is much higher (1-2 gal/ acre  $\approx$  10-20 ml/L) and application is recommended in mixture with petroleum oil (+ 1 gal/ acre  $\approx$  10 ml/L of Petroleum oil) (McCoy et al. 2007). Lorsban 4EC® is registered for the control of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, at a rate of 5 pt/ acre ( $\approx$ 6.25 ml/L) (Rogers and Stansly 2007). Danitol® is recommended for control of several pests including: *D. citri*, flower thrips (*Frankliniella* spp.) and orchid thrips (*Chaetanaphothrips* spp.) at a rate of 1 pt/acre ( $\approx$ 1.25 ml/L), and for citrus root weevils at a rate of 16-21 fl. oz./ acre ( $\approx$ 1.25-1.63ml/L) (Rogers

and Stansly 2007, Stansly et al. 2007). Results of this study suggest that applications with any of these pesticides at any the recommended doses will be extremely toxic to *H. sperata*. We conclude that these insecticides are non selective to *H. sperata*.

Provado® is a formulation of imidacloprid applied as a foliar spray that was also highly toxic to *H. sperata*. Imidacloprid is another neurotoxic molecule that has translaminar action and causes the insect death because of prolonged neuroexcitation. Foliar residues of imidacloprid were found to be highly toxic to several predatory insects (i.e., *Hippodamia convergens* and *Olla v-nigrum* (Coleoptera: Coccinellidae); *Chrysoperla rufilabris* (Neuroptera: Chrysopidae); *Deraeocoris nebulosus* (Hemiptera: Miridae); *Geocoris punctipes* (Hemiptera: Lygaeidae)), but less toxic to predatory mites (i.e., *Neoseiulus collegae*, *Phytoseiulus macropilis* and *Proprioseiopsis mexicanus* (Acari: Phytoseiidae) ((Mizell and Sconyers 1992). Williams and Price (2004) reported that residues of imidacloprid on leaves were highly toxic to *Anopheles iole*. Moreover, Villanueva-Jimenez and Hoy (1998) reported that foliar sprays of imidacloprid were highly toxic to the parasitoid *Ageniaspis citricola* and only slightly affected its host, the citrus leaf miner, *Phyllocnistis citrella*. However, drenched imidacloprid had a moderate effect on the parasitoid while controlling the pest. In this study Provado® was highly toxic to *H. sperata* but it allowed the parasitoids to live longer than the carbamate, organophosphate and pyrethroid insecticides. *Haeckeliana* wasps exposed to low concentrations of imidacloprid lived significantly longer than those exposed to higher concentrations. These results suggest that even though detrimental, Provado® had a lower acute toxicity on *H. sperata* than the previous pesticides. These results agree with those reported by Ulmer et al. (2006b) which stated that Admire® (the drench version of imidacloprid) is detrimental to *A. vaquitarum* but it does not act as fast as carbamate and organophosphates. Provado® is registered for control of the asian citrus

psyllid, *Diaphorina citri* and for control of several species of aphids at a rate of 10- 20 fl. oz/acre ( $\approx 0.78$  -1.56ml/L) (Browning et al. 2007, Rogers and Stansly 2007). At this rate, Provado® had a negative effect on *H. sperata*. However, within the synthetic insecticides used in the bioassays, Provado® was the one that allowed the wasps to live longer which could suggest that this insecticide is more IPM compatible than the other insecticides.

Agrimek® had similar effects on *H. sperata* as those caused by Provado®. Agrimek® is an avermectin that also targets the nervous system, but, in contrast to the former pesticides, it acts at glutamate-gated chloride channels (Scharf 2003). Avermectins agonize glutamate-gated chloride channels causing increased chloride current flow into neurons, which results in neuron inhibition and flaccid paralysis (Scharf 2003). Agrimek® was also highly toxic to *H. sperata* but it allowed the parasitoids to live longer than the carbamate, organophosphate and pyrethroid insecticides. *Haekeliana* wasps exposed to low concentrations of this pesticide lived significantly longer than ones exposed to the higher concentrations. Similar to what was observed with Provado®, this pesticide was detrimental to *H. sperata*, but showed a lower acute toxicity than the carbamate, organophosphate and pyrethroid insecticides. These results are different to those reported by Ulmer et al. (2006b) on which Agrimek® caused a slight increase in mortality of *A. vaquitarum* without affecting its longevity, but partially agree with those reported by Villanueva-Jimenez and Hoy (1998), that found that this pesticide was highly toxic to *A. citricola* and inappropriate for IPM programs.

Agrimek® is recommended in a mixture with petroleum oil for the control of *P. citrella*, rust mites (*Aculops pelekassi* and *Phyllocoptruta oleivora*) and broad mites (*Polyphagotarsonemus latus*). However, the recommended rate for controlling the citrus leaf miner (5 fl. oz/ acre of Agrimek®  $\approx 0.39$  ml/L + 1 gal/ acre of petroleum oil  $\approx 10.0$  ml/L) and the

mites (10 fl. oz/ acre of Agrimek®  $\approx$  0.78 ml/L + 3 gal/ acre of petroleum oil  $\approx$  30.0 ml/L) are different (Childers et al. 2007, Rogers and Stansly 2007). Based on the results obtained with the concentrations used in the bioassays, we could expect that applications of Agrimek® with petroleum oil targeting the citrus leaf miner should have less impact on *H. sperata* than those made targeting mites. Our results suggest that this product was toxic to *H. sperata*. However, Agrimek and Provado were less toxic to *H. sperata* than the carbamate, organophosphate and pyrethroid insecticides.

Petroleum oil caused an acute mortality similar to those of Provado® and Agrimek®, but was the “insecticide” that allowed the parasitoids to live longer. Observations made during the bioassays suggest that the effect of petroleum oil on *H. sperata* is mechanical. It appears that the oil sticks progressively to the wasps until it renders them immobile. However, this effect was only observed during the first evaluations. Our results contrast with those reported by Ulmer et al. (2006b) and Villanueva-Jimenez and Hoy (1998), the former reported that petroleum oil showed no contact toxicity to *A. vaquitarum* whereas the later considered it an IPM compatible product. One explanation to these contrasting results is the fact that *H. sperata* is much smaller in size than the other parasitoids, and could be more mechanically affected by the petroleum oil. Another explanation could be that our bioassays did not include treatments aging the leaf residues, which was found to have a large effect on the toxicity of the petroleum oils (Villanueva-Jimenez and Hoy 1998) probably because of the short residual effect that has been observed with this product. Moreover, our experiments did not include the effects of petroleum oil mixed with other insecticides that could be more favorable for *H. sperata*. Petroleum oil alone is recommended for control of various citrus arthropod pests at a rate of 5 gal/ acre ( $\approx$ 50.0 ml/L). It is also recommended for control of greasy spot, *Mycosphaella citri* at a rate of 5-10

gal/ acre ( $\approx 50.0 - 100.0$  ml/L) (Timmer and Chung 2007). These concentrations are much higher than those used in our bioassays and could have an acute toxic effect on *H. sperata*. We conclude that this product caused a high acute mortality similar to that caused by Provado® and Agrimek® but lower than that caused by carbamate, organophosphate and pyrethroid insecticides.

The fungicide Aliette WDG®, was the pesticide that showed the lower impact on *H. sperata*. Ulmer et al. (2006b) found somewhat coinciding results that showed that Aliette® was not toxic to *A. vaquitarum*. Our results suggest that Aliette® is toxic to *H. sperata* but to a lower extent than all the other tested pesticides. Moreover, the wasps that were exposed to lower concentrations of the product had a higher survival rate than those exposed to the label rate. Aliette® is a protectant, curative and systemic fungicide recommended for the control of *Phytophthora* spp. foot rot and brown rot of fruit at a rate of 5 lb/ acre ( $\approx 6$  gr/L) (Graham and Timmer 2007). At this rate Aliette® was toxic to *H. sperata*, however the parasitoids survived longer at the lower concentrations.

We conclude that all the pesticides that were included in our experiments had a negative effect on *Haeckeliana*'s survivorship. Based on this study, we could not say that these products are selective to *H. sperata*. In other words, we didn't find that any of the tested pesticides preserves the ability of *H. sperata* to control *D. abbreviatus*. However, within the registered insecticides, there are some that cause significantly less harm to this parasitoid. Results presented here and those reported by Ulmer et al. (2006b) suggest that the organophosphate, carbamate and pyrethroid pesticides are not good candidates to preserve the natural control of *D. abbreviatus* by any introduced egg parasitoids. Our results showed that Provado®, Agrimek®, Petroleum oil® and Aliette® allowed *H. sperata* to live longer than the previous pesticides, which suggests a certain degree of selectivity of these pesticides. Moreover, our results show that

*Haeckeliana* adults exposed to lower concentrations than the recommended rates of Provado®, Agrimek®, Petroleum oil®, and Aliette® have more chances of surviving than those exposed to the label concentrations. It is unclear if those parasitoids that can live for a certain period of time after being exposed to a pesticide remain reproductively active and continue parasitizing hosts. There is evidence that the behavior and physiology of beneficial arthropods could be affected by the exposure of sublethal doses of some pesticides (Desneux et al. 2007). For example, exposure of *T. brassicae* to sublethal doses of deltamethrin, a pyrethroid, modified its sex pheromonal communication which reduced mating and therefore the fitness of this biological control agent (Delpuech et al. 1999). This kind of pesticide effects could also be present in *H. sperata*.

We propose the use of products that have less toxic effects on the introduced parasitoid. This will increase the chances of *H. sperata* to control of *D. abbreviatus* and might reduce the application frequency of pesticides targeting the weevil. Within the tested pesticides, some had lower effects on *H. sperata* but none showed to be clearly selective. However, this study only evaluated a few pesticides amongst the many other products used in citrus and ornamental plant production. In order to have a better understanding of the impact of pesticide applications on *H. sperata*, further study of effects of other pesticides and the effects of aged residues on the leaves is recommended.

APPENDIX  
CHECKLIST OF ENTIMINAE SCHROEDER 1823 (COLEOPTERA CURCULIONIDAE)  
SPECIES AND KEY TO SOME SPECIES PRESENT IN FLORIDA

Entiminae is recognized as a monophyletic subfamily of the family Curculionidae and superfamily Curculionoidea (Marvaldi 1997). The subfamily Entiminae groups the “broad-nosed” weevils that possess a short and stout rostrum not used to prepare oviposition sites (Marvaldi 1997, Anderson 2002). Nearly all Entiminae have a mandible that bears a deciduous cusp used by newly emerged adults to escape from the pupal cell. The cusp is subsequently lost, leaving a definite scar at the point of attachment on the outer face of the mandible (Fig. 2.B) (Barratt and Kuschel 1996). However not all Entiminae possess this feature (i.e. *Sitona*, *Thecesternus*, and members of the tribe Alophini) and their inclusion in the subfamily is based in other characters. Entiminae also possess a short tooth or spine in the inner angle at the apex of the hind tibia (Anderson 2002). The antennal scape of some species also extends to or beyond the anterior margin of the eye, a feature otherwise only found in Dryophtorinae. Most are flightless, with elytra fused together along the suture and the hind wings are vestigial.

This is a large and widely distributed group containing 124 genera in 23 tribes recognized in North America (Anderson 2002). Many species feed on a very broad host range both as adults and larvae. A number of species are considered pests on citrus, fruit and ornamental production.

The following checklist includes the names of 24 genera and 36 species of weevils that belong to the subfamily Entiminae and that are known to be present in Florida (Anderson 2002, Peck and Thomas 1998, O’Brien and Wibmer 1982). The known distribution of the species in Florida is given by a code that represents the counties where it has been reported (Table A.1), followed by a brief comment on the biology and economical importance of some species. The common name, when available is provided.

This checklist was assembled based on the classification used in the book American Beetles (Anderson 2002) and complemented with the checklists of Beetles of Florida (Peck and Thomas 1998) and Weevils of North America (O'Brien and Wibmer 1982). The distribution information was taken mainly from the checklist of the beetles of Florida (Peck and Thomas 1998) and complemented with other sources (Bloem et al. 2002).

### List of the Entiminae occurring in Florida

#### *Agraphus* Say 1831

*A. bellicus* (Say 1831)<sup>1,2,3</sup>; ESC, LEV, OKA, SLU, TAY, VOL; Adults are associated with sandy soils along the Atlantic coast and in central Florida.

#### *Paragraphus* Blatchley 1916

*P. setosus* Blatchley 1916<sup>1,2,4</sup>; LEE, OKE, OSC; Endemic.

#### *Cyrtepidomus* Marshal 1913

*C. castaneus* (Roelofs 1873)<sup>1,3</sup>; ALA, ESC, HIL, LEO; "Asiatic oak Weevil"; Adventive, native from Japan.

#### *Myllocerus* Schoenherr 1823

*M. undatus* Marshall 1916<sup>2,4</sup>; BRO, DAD, ORA, PAL, PIN, SAR; Adventive, native from Sri Lanka. Pest of many ornamental and native trees and shrubs.

#### *Neoptochus* Horn 1876

*N. adpersus* (Boheman 1834)<sup>1,2,4</sup>; ALA, CIT, COLL, COLU, DAD, FRA, JAC, HIG, LEO, LEV, MAN, MON, ORA, PUT, SAR, WAK; Found in pinelands.

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<sup>1</sup> O'Brien, C.W., and G.J. Wibmer. 1982. Annotated checklist of the weevils (*Curculionidae sensu lato*) of North America, Central America, and the West Indies (Coleoptera: Curculionidae). Mem. Am. Entomol. Inst. 34: 29-49.

<sup>2</sup> Peck, S. B., and M.C. Thomas. 1998. A Distributional Checklist of the Beetles of Florida. <http://www.fsca-dpi.org/Coleoptera/Mike/curculio.htm>

<sup>3</sup> Bloem, S., R.F. Mizell, and C.W. O'Brien. 2002. Old Traps for new weevils: New records for Curculionids (Coleoptera: Curculionidae), Brentids (Coleoptera: Brentidae) and Anthribids (Coleoptera: Anthribidae) from Jefferson Co., Florida. Fla. Entomol. 85: 632-644.

<sup>4</sup> Anderson, R.S. 2002. Curculionidae, pp. 722-815. In R.H. Arnett Jr., M. Thomas, P.E. Skelley, and J.H. Frank (eds.), American Beetles: Polyphaga: Scarabaeoidea through Curculionoidea, vol. 2. CRC Press. Boca Raton, FL.

***Eudiagogus*** Schoenherr 1840

*E. marye* Warner 1979<sup>2</sup>; BRE, BRO, DAD, DES, DUV, LEO, LIB, MAN, POL, SAR, SJO, WAK, WAS; Adults feed on foliage of species of *Sesamia* (Fabaceae); larvae feed on nitrogen-fixing root nodules in the soil.

*E. pulcher* Fahraeus 1840<sup>2</sup>; ALA, DAD, IND, OKE, SEM, SLU.

*E. rosenschoeldi* Fahraeus 1840<sup>1,2</sup>; ALA, CIT, CLA, DUV, HIG, HIL, LAK, NAS, OKA, TAY, WAK.

***Brachystylus*** Schoenherr 1845

*B. acutus* Say 1824<sup>2</sup>; JEF.

***Diaprepes*** Schoenherr 1823

*D. abbreviatus* (Linnaeus 1758)<sup>1,4</sup>; DAD, BRO, PAL, COLL, LEE, HIG, IND, SLU, POL, MAN, HIL, ORA, SEM, LAK, VOL, GLA, HEN, MARI, SUM, DES, PAS, OSC, MAR; “Diaprepes root weevil”; Adventive, native from the lesser Antilles. Highly polyphagous species, considered pest of citrus and various ornamental plants. Adults feed and oviposit on the foliage of the plant; the neonate larvae fall and burrow into the soil to feed on the roots of the plants (Woodruff 1985, Mannion et al. 2003).

***Epiacaerus*** Schoenherr 1834

*E. formidosus* Boheman 1842<sup>1,4</sup>; ALA, DAD, DES, HAM, HAR, HER, HIG, JAC, LAK, LEO, LEV, MAN, MARI, ORA, PAS, PIN, POL, SAR, TAY, VOL; Adults feed on foliage.

***Lachnopus*** Schoenherr 1840

*L. floridanus* Horn 1876<sup>2,4</sup>; DAD, MON; Native and polyphagous species.

*L. argus* (Rieche 1840)<sup>2,4</sup>; MON; Adventive. Doubtfully established in Florida.

*L. hispidus* (Gyllenhal 1834)<sup>2,4</sup>; DAD Adventive. Doubtfully established in Florida.

***Artipus*** Sahlberg 1823

*A. floridanus* Horn 1876<sup>1,4</sup>; DAD, BRE, BRO, GLA, HEN, IND, LAK, MART, MON, ORA, PAL, SLU, VOL; Minor pest of Citrus plants.

***Artrichonotus*** Buchanan 1939

*A. taeniatulus* (Berg 1881)<sup>1,2,4</sup>; OKA; Adults feed on foliage of various plants, but most frequently Fabaceae.

***Naupactus*** Dejean 1821

*N. godmanni* (Crotch 1867)<sup>4</sup>; ALA, BAK, DAD, DES, ESC, GAD, HAR, HER, HIG, HIL, HOL, IND, JAC, JEF, LAK, LEO, MAN, MARI, MART, MON, OKA, OKE, ORA, OSC, PAS, POL, SAR, SEM, SLU, SRO, SUW, UNI, VOL, WAL; Adventive, native from South America. Adults are considered pests and feed on foliage of various plants.

*N. minor* (Buchanan 1942)<sup>2,4</sup>; ESC, SRO.

*N. peregrinus* (Buchanan 1939)<sup>2,4</sup>; ALA, CAL, COLU, GAD, GUL, JAC, JEF, LEO, LIB, OKA, SRO, WAL, WAS.

*N. leucoloma* (Boheman 1840)<sup>2,4</sup>; ALA, ESC, HOL, JAC, LEO, OKA, SRO, WAL.

**Otiorhynchus** Germar 1822

*O. ovatus* (Linnaeus 1758)<sup>1,2,4</sup>; “Strawberry root weevil”. Adventive, possibly not established in Florida. The Adults and larvae feed on a variety of plants and are considered serious pests. Adults are flightless.

**Aphrastus** Say 1831

*A. griseus* Blatchley 1916<sup>1,2</sup>; LIB; Adults feed on foliage of various plants.

*A. taeniatus* Say 1831<sup>1,2</sup>; GAD, LEO.

**Sitona** Germar 1817

*S. californicus* (Fahraeus 1840)<sup>1,2,4</sup>; JEF; Adventive. Adults feed on the foliage of the plant and the larvae on the roots of the plants.

*S. lineellus* (Bonsdorff 1785)<sup>1,2</sup>; DAD; “Sweetclover weevil”; Immigrant, Pest.

*S. hispidulus* (Fabricius 1776)<sup>1,2,4</sup>; LEO; “Clover root curculio”; Adventive, native from Europe.

**Pachnaeus** Schoenherr 1826

*P. litus* (Germar 1824)<sup>1,2,4</sup>; Southern Florida; Native, Pest in citrus industry.

*P. opalus* (Oliver 1807)<sup>1,2,4</sup>; Northern Florida; Native, Pest in citrus industry.

**Pandeleiteius** Schoenherr 1834

*P. hilaris* (Herbst 1797)<sup>1,2</sup>; ALA, CAL, COLU, DAD, DES, DIX, FRA, HIG, HIL, HOL, LEO, LEV, MAN, MARI, ORA, PIN, PUT, SAR, SRO, WAK; Adults feed on Fagaceae and other plants.

*P. nodifer* Champion 1911; BRO, DAD; Adults are found in various ornamental plants.

**Scalaventer** Howden 1970

*S. subtropicus* (Fall 1907)<sup>1,2,4</sup>; DAD, MON, adults have been collected on *Bumelia celastrina* (Sapotaceae).

**Tanymecus** Germar 1817

*T. lacaena* Herbst 1797<sup>1,2,4</sup>; ALA, BAK, BAY, BRE, BRO, CIT, DES, HEN, HIG, HIL, IND, LEE, LEO, MAN, MON, NAS, ORA, OAS, PAL, PIN, POL, PUT, SAR, SEM, SLU, VOL; Adults have been collected on *Sesuvium. portulacastrum* (L.) (Aizoaceae) in southern Florida.

*T. confusus* Say 1831<sup>1,2</sup>; FLA, LEO.

**Cercopeus** Schoenherr 1842

*C. komarecki* O'Brien 1977<sup>1,2,4</sup>; LEO; Adults are flightless and found in leaf litter.

**Pseudocneorhinus** Roelofs 1873

*P. bifasciatus* Roelofs 1880<sup>2,4</sup>; WAS; “ Twobanded Japanese Weevil”; Adventive, native from Japan. The adults and larvae feed on various plants.

***Trachyphloeosoma* Wollaston 1869**

*T. advena* Zimmerman 1956<sup>1,4</sup>; LEO; Adventive, native from Japan.

Table A-1. Codes for Florida Counties.

Code	County	Code	County	Code	County
ALA	Alachua	HAM	Hamilton	OKE	Okeechobee
BAK	Baker	HAR	Hardee	ORA	Orange
BAY	Bay	HEN	Hendry	OSC	Osceola
BRA	Bradford	HER	Hernando	PAL	Palm Beach
BRE	Brevard	HIG	Highlands	PAS	Pasco
BRO	Broward	HIL	Hillsborough	PIN	Pinellas
CAL	Calhoun	HOL	Holmes	POL	Polk
CHA	Charlotte	IND	Indian River	PUT	Putnam
CIT	Citrus	JAC	Jackson	SAR	Sarasota
CLA	Clay	JEF	Jefferson	SEM	Seminole
COLL	Collier	LAF	Lafayette	SJO	St. Johns
COLU	Columbia	LAK	Lake	SLU	St. Lucie
DAD	Dade	LEE	Lee	SRO	Santa Rosa
DES	De Soto	LEO	Leon	SUM	Sumter
DIX	Dixie	LEV	Levy	SUW	Suwannee
DUV	Duval	LIB	Liberty	TAY	Taylor
ESC	Escambia	MAD	Madison	UNI	Union
FLA	Flagler	MAN	Manatee	VOL	Volusia
FRA	Franklin	MARI	Marion	WAK	Wakulla
GAD	Gadsden	MART	Martin	WAL	Walton
GIL	Gilchrist	MON	Monroe	WAS	Washington
GLA	Glades	NAS	Nassau		
GUL	Gulf	OKA	Okaloosa		

**Literature based Key to some Entiminae Species of Florida**

This literature based key to some Entiminae species found in Florida was assembled based on the classification used in the book American Beetles (Anderson 2002) and complemented with the checklists of Beetles of Florida (Peck and Thomas 1998) and Weevils of North America (O’Brien and Wibmer 1982).

1. Mandibles large, hemispherical externally, inner surface slightly cupped; surface of mandible densely squamate except narrow median edge glabrous; mandible without deciduous process; bucal cavity large, maxillary palpus fully or mostly exposed, scrobes lateral; rostrum similar to head in length and width; anterior edge of prothorax straight laterally, not lobbed beneath the eye.....*Sitona*
- Mandibles various; if large and densely squamate, then with postocular lobe (Fig. 1-A) and/or with scrobe dorsal and/or deciduous process (Fig. 3-B).....2
- 2(1). Side of prothorax with anterior margin produced into slight to very large rounded postocular lobe (Fig. 1-A); eye tear drop shaped.....3
- Side of prothorax with anterior margin straight; eyes various.....6
- 3(2). Mandible with four or more large setae; ventral edge of postocular lobe very abrupt; elytra with humeral angle slanted from stria 7 outwards; scutellum very wide; prothorax and elytra patterned with lines and other markings; body length 4.1-8.0 mm....*Ediagogus*
- Mandible with three large setae, femur with a tooth on inner edge distally.....4
- 4(3). Elytra with humerus rounded; Pterygium closed apically; eye small, flattened, with approximately 20 facets along longest axis; prothorax 1.4x longer dorsally than ventrally; body length 3.0-4.0 mm.....*Neoptochus adspersus*
- Elytra with humeral angle .....5
- 5(4). Femora with very large tooth bearing two smaller teeth on distal edge (Fig. 1-B); scape reaching anterior third of prothorax; base of elytron forming a large lobe between scutellum and interval 5; dorsal elytral setae minute, color dark brown-black with whitish scales with irregular pattern; body length 6.0-7.0 mm (Fig. 1-C).....*Mylocherus undatus*
- Femora with a single small tooth; dorsal elytral setae long; base of elytra straight; eye large, separated from anterior margin of prothorax by single row of scales; pronotum and disc of elytra with scales very sparse or absent; scales becoming more numerous laterally; body length 4.5-5.8mm.....*Cyrtepestomus castaneus*



Figure A-1. *Mylocherus undatus* A. Postocular lobe B. Femora tooth C. Adult

- 6(2). Anterior edge of prothorax laterally with postocular vibrissae in a cluster or tuft (Fig. 2-A) .....7
- Anterior edge of prothorax without postocular vibrissae or if postocular vibrissae present not in cluster or tuft.....11
- 7(6). Eye large, flattened; rostrum thick; front coxae contiguous or apparently so; body length 5.0-12.0 mm.....8
- Eye smaller; front coxae distinctly separated by continuous prosternal integument; body length less than 5.0 mm.....10
- 8(7). Hind tibia with straight comb of setae on outer edge, comb at least as long as width of tibia at apex; postocular vibrissae set on edge of prothorax; color dorsally dark, vaguely patterned at most; corbel open; body length 5.5-10.0 mm.....*Tanymecus*
- Hind tibia without straight comb of setae; postocular vibrissae set on a knob or rounded tooth on edge of prothorax; corbel various; color pastel gray, green; body length 6.4-12.0mm.....9
- 9(8). Elytra produced forward at basal center, appearing bisinuate (Fig. 2-B); humeral angle also projecting forward; color usually bright blue green or aqua; found only in southern half Florida (Fig. 2-C).....*Pachnaeus litus*
- Elytra not noticeably produced forward, the juncture between elytra and pronotum slightly irregular but not appearing sinuate; humeral angle rounded, not projecting. Color more variable; most often pale gray-green, but occasionally bright aqua. Found in northern half of Florida.....*Pachnaeus opalus*



Figure A-2. *Pachnaeus litus* A. Postocular vibrissae B. Base of Elytra C. Adult

- 10(7). Anterior margin of abdominal ventrites 3, 4 and 5 without modification; contour and vestiture more or less uniform; mandible without scales; postocular vibrissae various, well developed in most species; front legs distinctly to greatly larger than middle and hind legs.....*Pandeleiteius*

- Anterior margin of abdominal ventrites 3, 4 and 5 deeply, narrow sulcate across width of abdomen; posterior margin of sulcus carinate, right angled in female, more rounded in male; front legs slightly larger than middle and hind legs.....*Scalaventer subtropicus*
- 11(6). Scrobe dorsal or dorsolateral, indefinite caudad of antennal insertion; scape in repose not situated in scrobe, usually passing over eye.....12
- Scrobe lateral, scape in repose situated in scrobe.....20
- 12(11). Corbel closed; tarsal claws free; humeral angle well developed.....13
- Corbel opened or not distinctly closed; tarsal claws free or connate; humeral angle rounded.....14
- 13(12). Scape very thick, short, no longer than thickness of rostrum; apex of rostrum with 20-30 long setae; mandible with many long setae directed to mandibular scar; body length 5.5-7.5 mm.....*Brachystylus acutus*
- Scape longer, extended to or beyond eye; eye slightly encroaching on dorsum; anterior margin of prothorax with a row of 20 or more very fine long setae of graduated lengths directed toward the edge of the eye (Fig.3-A); humeri quadrate, prominent; base of elytra very slightly produced; body length 13.5-18.0 mm (Fig. 3-C).....*Diaprepes abbreviatus*

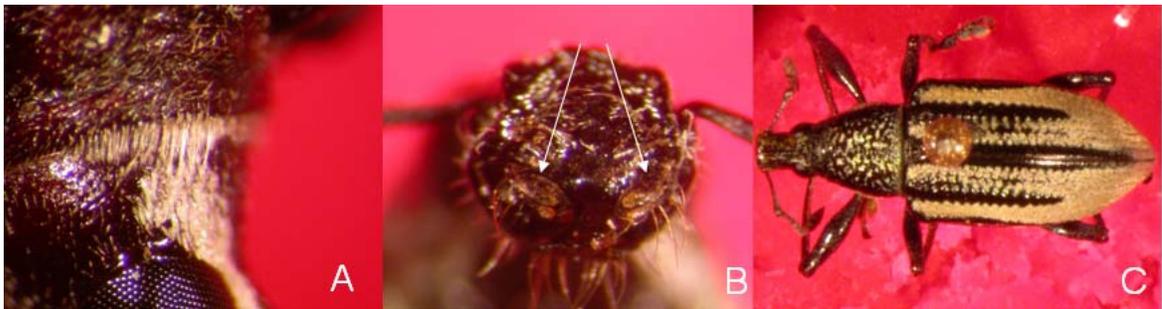


Figure A-3. *Diaprepes abbreviatus* A. Postocular setae B. Mandible scar C. Adult

- 14(12). Funicle with six articles; tarsal claws free.....15
- Funicle with seven articles; tarsal claws various.....16
- 15(14). Prothorax lacking median sulcus; surface punctuate; elytral intervals flat; corbel plate large, glabrous, oval; body length 5.8-6.8 mm.....*Agraphus bellicus*
- Prothorax with median longitudinal sulcus; elytral intervals 3, 5 and 7 more prominent; corbel plate intermediate; body length 6.2 mm.....*Paragraphus setosus*

- 16(14). Tarsal claws connate.....17  
     Tarsal claws free.....18
- 17(16). Eye large, almost touching prothorax; anterior margin of prothorax with postocular lobe; corbel narrowly closed; elytra very convex, sides greatly rounded .....*Pseudocneorhinus bifasciatus*  
     Eye smaller, prothorax without postocular lobe; corbel narrowly closed; rostrum in dorsal view more or less rectangular in outline; entire body and appendages densely scaled; scrobe completely dorsal.....*Aphrastus*
- 18(16). Antenna with scape with vestiture of fine setae and round flat scales; body size small 2.3-4.5 mm .....19  
     Antenna with scape with vestiture of fine setae only or with at most a few scattered elongate, recumbent scales intermixed; elytra with few sparse or no scales; body size 4.0-13.0 mm.....*Otiorhynchus ovatus*
- 19(18). Epistoma large, distinct, occupying approximately half the anterior margin of rostrum, triangular, limited by distinct carina; scrobe dorsal, very short and deep, not reaching eye; front and middle tibiae with single, strong, almost horizontal apical tooth; hind tibia with pair of short vertical apical spines.....*Cercopus komarecki*  
     Epistoma very small, indistinct; scrobe lateral, long, passing backward and below the lower angle of eye; all tibiae with apical spine or tooth; vestiture of long, suberect, fine hair-like setae; eye with less than 5 facets across greatest width; elytra rounded, subglobose.....*Trachyphloeosoma advena*
- 20(11). Eyes partly encroaching on head; epistoma poorly defined; humeri rounded (except *Lachnopus*, southern Florida only).....21  
     Eyes lateral; humeri rounded; rostrum with longitudinal sulcus or impressed line reaching from interantennal line to head, continuing or not with fine impressed line reaching beyond eyes(Fig. 4-A).....22
- 21(20). Mesepimeron triangular, anterior margin running straight to angle between elytron and peduncle of mesothorax, mesepisternum not touching side margin of elytron; metepisternal suture complete; humeri well-developed, quadrate; scales on body sparse, not imbricate, prothorax and elytra lacking erect scales or setae (Fig. 4-C).....*Lachnopus*



Figure A-4. *Artipus floridanus* (A &B) *Lachnopus floridanus* (C) A.Longitudinal sulcus B. Adult C. Adult

Mesepimeron short trapezoidal, anterior margin running to side margin of elytron, mesepisternum touching elytron on broad contact; metepisternal suture obliterated in basal half; rostrum (excluding mandibles) in dorsal view from anterior margin of eye to apex slightly longer than greatest width in apical region; antenna with short, narrow scales and sparse, long, fine setae; prosternum lacking two close adjacent tubercles behind front coxae.....*Epiacaerus formidolosus*

22(20). Epistoma conspicuous, very wide, occupying most of anterior edge of rostrum; prothorax and elytra with very irregularly shaped, randomly situated large foveae (Fig.4-B) .....*Artipus floridanus*

Epistoma inconspicuous, occupying half or less of anterior edge of rostrum; prothorax and elytra with only regular sculpture.....23

23(22). Scutellum glabrous, glossy; antennal funicle with article 2 approximately as long as article 1; corbel plate narrow .....*Artrichonotus taeniatulus*

Scutellum squamate; antennal funicle with article 2 approximately 1.5 to 2.0x longer than article 1; corbel plate absent or present.....*Naupactus*

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## BIOGRAPHICAL SKETCH

Daniel Carrillo was born in Pereira, Colombia. Daniel got his bachelor's degree in agronomic engineering in the Universidad Nacional de Colombia in the year 2004. During his time as an undergraduate student, Daniel got interested in the study of insects and their interactions with plants. After graduating, Daniel worked with farmers promoting the integration of biological control tactics to pest management programs in horticultural systems. In the year 2006, Daniel started a master's program in entomology at the University of Florida. For the past two years Daniel has worked in a biological control project in the Tropical Research and Education Center in Homestead, Florida.