# AN ASSESSMENT OF A BIOLOGICAL CONTROL AGENT, *Lixadmontia franki* (DIPTERA: TACHINIDAE), TO CONTROL *Metamasius callizona* (COLEOPTERA: CURCULIONIDAE), AN INVASIVE HERBIVORE DESTROYING FLORIDA'S NATIVE BROMELIADS

By

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To Mike Burton (1978 – 2008)

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*Metamasius callizona* is an invasive bromeliad-eating weevil that is destroying Florida's native bromeliads. *Lixadmontia franki* is a tachinid that parasitizes bromeliad-eating weevils and is a potential biological control agent for controlling *M. callizona*. Fly releases and monitoring were made from June 2007 to May 2009 at 4 release sites. Two F2 generation flies were recovered after the first release; no other recoveries were made. At one release site, the decline of the bromeliad population was monitored. The population declined by 97.4% in 2 years because of the weevil infestation.

Laboratory research was conducted to examine *L. franki*'s ability to assess host density; describe the larval stages of *L. franki*; and assess whether *L. franki* is ovoviviparous or viviparous. *Lixadmontia franki* showed the ability to assess host density. The fly has 3 larval stages that are easily distinguished. Data support that *L. franki* is ovoviviparous and not viviparous.

# CHAPTER 1
# INTRODUCTION

*Metamasius callizona* (Chevrolat), a bromeliad-eating weevil (Coleoptera: Curculionidae) from Guatemala and Mexico, was discovered in Florida (USA) in 1989 (Frank and Thomas 1994; Frank and Cave 2005). *Metamasius callizona* has been attacking and destroying many of Florida's native bromeliad species and, in the process, has spread to nearly fill its new potential range (Frank 1996).

*Metamasius callizona* is a specialist herbivore of bromeliads (Frank and Cave 2005). The larvae hatch from eggs inserted in leaf bases by gravid females (Frank and Thomas 1984; Frank and Cave 2005). The larvae mine first the leaf then the stem and leaf bases. The damage is extensive and includes the meristematic and intercalary tissue, which kills the plant. Adult weevils eat bromeliad leaves and can damage a plant but usually not fatally. The adults are winged and disperse to find new host plants.

Sixteen species of bromeliads are native to Florida and 12 of these are susceptible to attack by the weevil (Frank and Cave 2005). Ten of these species are listed as endangered or threatened and 1 species is precinctive to Florida. These bromeliads are important to Florida's ecosystems and are crucial to the survival of other species (Frank 1983; Frank and Fish 2008). *Tillandsia utriculata* L. (Bromeliales: Bromeliaceae) is a large, widespread bromeliad that is particularly susceptible to attack by *M. callizona*.

A biological control program was started in 1991 (Frank and Cave 2005). Beginning in 1992, searches were made for a classical biological control agent to import and release in Florida to regulate *M. callizona*. After many searches in the field and collecting and observing several bromeliad-eating *Metamasius* larvae, no parasitoids or

other specialist agents that might be regulating the weevil population in its homeland have been discovered.

A potential biological control agent, *Lixadmontia franki* Wood and Cave (Diptera: Tachinidae), was discovered in 1993 in Honduras on a related species of bromeliad-eating weevil, *M. quadrilineatus* Champion (Cave 1997; Wood and Cave 2006). *Lixadmontia franki* was shown to parasitize *M. callizona* at least as readily as it attacks *M. quadrilineatus* (Frank and Cave 2005). In 2007, permission was received to release the fly in Florida and releases were started at 4 sites, Lake Rogers (Hillsborough County), the Enchanted Forest Sanctuary (Brevard County), Loxahatchee National Wildlife Refuge (Palm Beach County), and Big Cypress National Preserve (Collier County). Fly releases and monitoring were made from June 2007 to May 2009.

*Lixadmontia franki* is multilarviparous and embryos develop into pharate larvae internally in a brood chamber (Suazo *et al*. 2008). Adult flies mate within 48 hours after emerging and females are ready to deposit neonate larvae by 8 days after mating (Suazo *et al*. 2008). Generation time for the flies is 5 to 6 weeks.

Tachinids use host plant – host volatiles for locating and assessing available hosts (Roth *et al*. 1982; Kainoh *et al*. 1999; Stireman 2002; Stireman *et al*. 2006). Gravid *L. franki* flies apparently locate hosts from a mix of odors arising from the host weevil larva and the plant material that has been chewed by the larva (Suazo *et al*. 2006). The fly does not make contact with a potential host but rather deposits neonate maggots on an infested bromeliad (Cave 2008). Because a bromeliad with more weevil larvae than a like bromeliad with fewer weevil larvae would have a greater accumulation of chewed host plant tissue and host frass, *L. franki* could use the amount of host plant – host

volatiles to adjust the number of maggots she deposits on a weevil-infested plant. It was unknown if *L. franki* is able to do this.

The maggots search for and parasitize weevil larvae inside the host plant (Cave 2008). The maggot is endoparasitic and a koinobiont. Once the maggot has consumed its host weevil internally, it emerges from the dead host and pupates. It was not known how many instars *L. franki* has nor were the instars described.

It was also unknown whether *L. franki* is ovoviviparous (pharate larvae in eggs hatch either while being deposited or shortly afterwards) or viviparous (pharate larvae hatch internally, before being deposited).

This dissertation assesses the likely usefulness of *L. franki* as a biological control agent; monitors the progression of a weevil infestation (degree of damage and weevil seasonality) on a *T. utriculata* population; examines the ability of gravid female flies to assess host density; describes the larval stages of *L. franki*; and assesses whether *L. franki* is ovoviviparous or viviparous.

# CHAPTER 2
# SEASONALITY, ABUNDANCE, AND BIOLOGICAL CONTROL OF AN INVASIVE HERBIVORE, *Metamasius callizona*, ON ITS HOST PLANT, *Tillandsia utriculata*, IN THE ENCHANTED FOREST SANCTUARY

## Introduction

*Metamasius callizona* (Chevrolat), a bromeliad-eating weevil (Coleoptera: Curculionidae) from Guatemala and Mexico, was discovered in Florida (USA) in 1989 (Frank and Thomas 1994; Frank and Cave 2005). *Metamasius callizona* has been attacking and destroying many of Florida's native bromeliad species and, in the process, has spread to nearly fill its new potential range (Frank 1996). *Tillandsia utriculata* L. (Bromeliales: Bromeliaceae) is a large, widespread bromeliad that is being devastated by *M. callizona*. A previous study showed *M. callizona* to be seasonal on *T. utriculata* and to be able to cause rapid death of a population, including the reproductive class (Cooper 2006; Cooper 2008). The *T. utriculata* population for that study was small (41 plants) and provided a data set that partially described the degree of destruction and pattern of seasonality. An effort was made to locate a larger *T. utriculata* population to monitor over a 2-year period to further understand the relationship between *M. callizona* and *T. utriculata*. In August 2006, *M. callizona* was discovered at the Enchanted Forest Sanctuary (Brevard County) attacking a large *T. utriculata* population (estimated at a few thousand bromeliads with longest leaf length ≥ 30 cm). From January to March 2007, the forest was mapped and prepared for monitoring and from March 2007 to June 2009 the bromeliad and weevil populations were monitored.

In May 2007, permission was received to release a biological control agent, *Lixadmontia franki* Wood and Cave (Diptera: Tachinidae), to attempt control of *M.*

*callizona*. *Lixadmontia franki* was discovered in Honduras on a closely related species of bromeliad-eating weevil, *M. quadrilineatus* Champion. The bromeliad population at the Enchanted Forest was declining rapidly and the decision was made to release the fly in the park and include this as part of the research. Five releases were made from August 2007 to August 2008 and one more in spring 2009. All releases were followed with monitoring using sentinel plants. This chapter documents this research and discusses the ecological and practical significance of the results.

## Materials and Methods

### Study Site

The Enchanted Forest Sanctuary is located on 190 hectares in Brevard County, Florida (Brevard County Board of County Commissioners 2008). Habitat includes oak scrub, mesic and hydric hammock, wet prairie, and pine flatwood. *Tillandsia utriculata* grew abundantly in the mesic and hydric hammocks along with *T. fasciculata* Swartz, which was rare in the Enchanted Forest. Both species are large bromeliads that range from central to south Florida and are being attacked by *M. callizona* (Frank and Cave 2005). The weevil arrived in the forest sometime between August 2003 and August 2006 (Frank 1996). In August 2003, the forest was checked for the presence of *M. callizona* and no weevils or weevil-damaged bromeliads were found. In August 2006, park management noticed suspected weevil damage on the bromeliads and the park was checked again. This time, *M. callizona* was found. By the time mapping began in January 2007, the ground was littered with bromeliads killed by *M. callizona*.

### The Species

*Metamasius callizona* is a specialist herbivore of bromeliads (Frank and Cave 2005). The larvae hatch from eggs inserted in leaf bases by gravid females (Frank and

Thomas 1984; Frank and Cave 2005). The larvae mine first the leaf then the stem and leaf bases. The damage is extensive and includes the meristematic and intercalary tissue, which kills the plant. Adult weevils eat bromeliad leaves and can damage a plant but usually not fatally. The adults are winged and disperse to find new host plants. In Guatemala and Mexico, where *M. callizona* is native, *M. callizona* is rarely found and has not been seen causing great damage to bromeliad populations. However, in Florida, *M. callizona* has been devastating bromeliad populations.

*Tillandsia utriculata* has suffered the greatest losses due to *M. callizona* (Frank and Cave 2005). Like all of Florida's bromeliads, *T. utriculata* is epiphytic. *Tillandsia utriculata* prefers shady understory and grows as a tank bromeliad, collecting water in the bases of its leaves (Benzing 1980; Frank 1983). The tank water supports an ecosystem that provides nutrients for the plant. Such nutrition is sparse compared to most terrestrial plants and *T. utriculata* grows slowly, requiring 10 to 20 years to reach maturity. Unlike Florida's other 15 species of bromeliads, which are polycarpic, *Tillandsia utriculata* is monocarpic and senesces while producing and releasing seed.

A small *T. utriculata* population, along with a larger *T. fasciculata* population, was studied in Myakka River State Park (Sarasota County) from June 2001 to June 2005 (Cooper 2006). The weevil was first discovered in Myakka in September 2000 (Frank 1996). On *T. fasciculata*, *M. callizona* was aseasonal, *i.e.,* found throughout the year in low numbers. On *T. utriculata*, *M. callizona* peaked seasonally in the months from March to July, with the highest peak occurring in June. The *T. utriculata* population was sparsely distributed throughout the park over an area of about 25 $km^2$. After 2 years, no further weevils were found on *T. utriculata*, though fallen, dead bromeliads killed by the

weevil continued to be encountered. Seed output was sparse and, at 3 years, ceased. Most of the *T. utriculata* died from falling to the ground or into water and subsequently rotting. Slightly more plants died from senescence than were killed by *M. callizona*. The combined median time to failure was 24 months. The *T. fasciculata* population declined too slowly to calculate a median time to failure in a 4-year study. At 43 months, the *T. fasciculata* population had declined by 62%. Mortality caused by *M. callizona* was much greater for *T. fasciculata* than mortality from natural causes; however, the rate of infestation on *T. fasciculata* was much slower than it was on *T. utriculata*. *Tillandsia fasciculata* continued to release seed while infested.

*Tillandsia utriculata*'s monocarpic habit and ability to support a seasonally abundant *M. callizona* population make *T. utriculata* more susceptible than *T. fasciculata* to *M. callizona*. *Tillandsia utriculata* experiences rapid destruction and elimination of seed output when infested by the weevil. *Tillandsia fasciculata*'s polycarpic habit allows a population to continue releasing seed during an infestation and its aseasonal, non-abundant *M. callizona* population has a slow growth rate and therefore a slow kill rate.

Because the *T. utriculata* population in Myakka was so small, an effort was made to find a larger population that could better demonstrate the seasonality and potential destruction of *M. callizona* on this species. Because *T. utriculata* populations can be so rapidly destroyed by *M. callizona*, a site was searched for that was near the beginning of a weevil infestation. The Enchanted Forest fulfilled these needs. On the first observation trip in November 2006, dead, fallen bromeliads already covered the ground, but a rough count of the medium-size and large living bromeliads in the canopy easily

and quickly reached 3,000. A more accurate count was not attempted. The park seemed to be an ideal site for monitoring *M. callizona* on *T. utriculata*.

In its native land, *M. callizona* does not cause the type of damage to wild bromeliad populations that it causes to most of Florida's bromeliads (12 of 16 species; Frank & Cave 2005). It is unknown what is regulating the weevil population in its homeland. It is suspected that it is controlled by a parasitoid though this has not been confirmed. A parasitoid of a related bromeliad-eating weevil, *M. quadrilineatus*, was discovered in Honduras in 1993 (Cave 1997; Wood and Cave 2006). The parasitoid, *L. franki*, was shown to parasitize *M. callizona* at least as readily as it will parasitize its native host. Only 1 non-target organism may be affected by *L. franki*, a bromeliad-eating weevil that is native to Florida, *M. mosieri* Barber. *Metamasius mosieri* is a small, rare species that does not cause excessive damage to its host plants (Cave *et al*. 2006). No parasitoids of *M. mosieri* have been found in Florida.

Gravid *L. franki* females apparently locate hosts from a mix of odors arising from the host weevil larva and the plant material that has been chewed by the weevil (Suazo *et al.* 2006). The fly does not make contact with a potential host but rather deposits neonate maggots on an infested bromeliad. The maggots search for and attack weevil larvae inside the host plant. The maggot is endoparasitic and a koinobiont. Once the maggot has consumed its host weevil internally, it emerges from the dead host and pupates. Adult flies mate 2 to 4 days after emerging and females are ready to deposit neonate larvae about 8 days after mating (Suazo *et al.* 2008). Generation time for *L. franki* is 5 to 6 weeks.

**Weevil and Bromeliad Monitoring**

The Enchanted Forest has a network of public trails. Figure 2-1 shows these trails and the areas that were mapped for bromeliad and weevil monitoring. The canopy over the trails was scanned for the presence of *T. utriculata* and/or *T. fasciculata* and those parts of the trails that passed under canopy that supported one or both of these species were mapped using a Global Positioning System (Trimble GPS Pathfinder Pro XRS). The area monitored was the length of the trail including 7.5 meters to either side of the trail. Area was calculated using the latitude and longitude recordings to measure the length of the trails in meters then multiplying by 15 meters. Table 2-1 lists the mapped areas as well as the calculated area and the starting and ending bromeliad population for each area.

Many dead bromeliads fallen from the canopy (most killed by *M. callizona*) littered the trail when mapping began at the park. The fallen, dead bromeliads were cleared from the trail before monitoring began in order to increase the visibility of freshly fallen plants and to reduce confusion of when a bromeliad had fallen out of the canopy. Several field trips were required to remove the dead plant mass.

Areas were monitored monthly for dead *T. utriculata* and *T. fasciculata* that had fallen from the canopy. Fallen bromeliads were identified to species and categorized according to size. Cause of death was determined. *Metamasius callizona* found in the fallout were collected and the life stage and condition were recorded. All weevil specimens were returned to the Entomology and Nematology Department at the University of Florida in Gainesville and added to the *M. callizona* colony in the laboratory. All larvae and pupae were kept separate until adults emerged, to confirm species identification.

On every 3rd field trip, the mapped trails were slowly walked and the number of living *T. utriculata* and *T. fasciculata* with longest leaf length ≥ 30 cm that could be seen from the trail was counted. Most of the plants were *T. utriculata*; when a *T. fasciculata* was spotted, this was noted. For each count the trails were walked in the same pattern between 9 am and 1 pm.

The mean was calculated for the number of bromeliads in the canopy, fallen dead bromeliads, and weevils counted per area and plotted over time, plus and minus 1 standard error. The plot of the bromeliad count was used to determine the rate and pattern of the infestation. Plots for the fallout and weevil specimens were used to determine weevil seasonality.

**Fly Release and Post-Release Monitoring**

A colony of *L. franki* was maintained at the Hayslip Biological Control Research and Containment Laboratory at the Indian River Research and Education Center in Ft. Pierce, based on a method designed by Suazo *et al.* (2006). *Metamasius callizona* larvae reared in pineapple crowns in the laboratory were used as hosts for the fly maggots. Newly emerged adult flies were collected from this colony 5-11 days before a release and held in a 60 cm × 60 cm × 64 cm cage. The flies mated in the cage and at the time of the release females should have been ready to deposit neonate maggots. Flies were transported to the release site in their cage and released at about 9 am. The release site was not located on a public trail but on the edge of the canal north of the mapped trails (Figure 2-1). Approximately 50 females and 50 males were released each time. Table 2-2 shows the date for each release and the number of female and male flies released. The releases were designed to have one release for each season of the year over the span of a year. The release in spring 2009 was a follow-up release.

Five weeks after a release, eight 0.6 m × 0.6 m × 0.1 m cedar boxes with mesh bottoms, each containing 6 pineapple tops infested with 2nd and 3rd instar *M. callizona*, were suspended from the canopy around the release site. Instar was determined by head capsule size and time since hatching (Salas and Frank 2001). The pineapple tops were inoculated with weevil larvae 1 week before the traps were placed in the field. Two to 3 weevil larvae were placed in each pineapple top; the amount varied depending on the availability of larvae from the weevil colony. The traps remained in the field for 2 weeks, then were retrieved and returned to the laboratory where the weevil larvae were monitored for parasitism. If a maggot of *L. franki* emerged from a weevil larva, then parasitism was affirmative; if no maggot emerged, then parasitism was negative.

For the monitoring trip following the release in June 2009, sentinel weevil larvae were taken from the colony at Ft. Pierce and returned there to be observed for parasitism. Sentinel pineapple tops were exposed to gravid *M. callizona* that deposited eggs in the leaf bases of the pineapple tops around four weeks before monitoring. Weevil larvae hatched from the eggs and grew on the tops. The pineapple tops were placed in the field when the weevil larvae were estimated to be 3rd instars.

The timing of the traps in the field should have coincided with the time that females from an F1 generation would be reproductively active. The expectation was that, if there was an F1 female in the field, she would parasitize the sentinel weevil larvae. *Lixadmontia franki* from these sentinel larvae would be F2 flies and would confirm survival of the fly in the Enchanted Forest.

## Results

### Weevil and Bromeliad Monitoring

### Bromeliad counts

This study began with 2,176 bromeliads with longest leaf length ≥ 30 cm (March 2007). The majority of the plants were *T. utriculata*. Four large, living *T. fasciculata* were counted in the canopy in March and June 2007. In September 2007, no *T. fasciculata* bromeliads were seen in the canopy. One large *T. fasciculata* was found as fallout in June 2007, killed by *M. callizona*. Coquina Trail, Biodiversity Loop II, and Mesic I had the largest areas and the highest number of bromeliads at the beginning and end of the study (Table 2-1). By the end of the study, Biodiversity Loop III and Magnolia Loop were reduced to 0 bromeliads and Biodiversity Loop I, Coquina Trail, Tomoka Trail, and Mesic II were reduced to bromeliad numbers less than 5.

Eighty-seven percent of the population was destroyed in the first 6 months of the study (Figure 2-2A). After this, the rate of decline slowed dramatically. From September 2007 to June 2008, the numbers were similar. In September 2008, the number dropped significantly compared to the previous September, reducing the population by 95.5%. Throughout the remainder of the study, the numbers were similar. At the end of the study, the bromeliad population was reduced by 97.4% at a count of 53 live plants.

Very few plants were seen releasing seed in the canopy. At the end of the study, seedlings and small plants were growing at the west ends of Biological Loop I and Coquina Trail, at the junction where Biological Loop I and II and Mesic I meet, and at the center of Biological Loop II and the end of Mesic I. The decline of the bromeliad population was constant except in December 2008 when the population had risen by 12

individuals; these were smaller plants that had grown into the countable range (longest leaf length ≥ 30 cm).

**Fallout and weevil counts**

One thousand and one fallen, dead bromeliads were collected; 98.8% of them were killed by *M. callizona*. Three (0.3%) were killed by falling branches and subsequent rot. Nine (0.9%) died naturally after releasing seed. Most of the fallout was fresh, except for the fallout that senesced after releasing seed; the latter was several months old and the plants were brown and dried out. Fallout and weevil counts were much higher in the first 6 months of the study; afterwards, activity greatly declined and fewer fallout and weevils were collected from September 2007 onward (Figure 2-2B-C). June 2007 had the highest peak for both fallout and weevils. The numbers declined in July 2007, August 2007, and September 2007. Weevil activity remained constant from September 2007 to December 2007 then ceased in January and February 2008. Activity resumed in March 2008, dipped in April and May, peaked again in June and was followed by a descent similar to that which followed the larger descent in 2007. The winter of 2008 – 2009 and spring of 2009 had no weevil activity. In May, 4 fallout and 3 weevils were collected and in June, 8 fallout and 3 weevils were collected.

**Fly Releases and Post-Release Monitoring**

From August 2007 to May 2009, 660 flies were released (Table 2-2). Estimated temperatures at the time of the releases ranged from a high of 30° C in the summer to a low of 15° C in the winter. The release site was very shady. The flies were usually reluctant to leave and required 10 to 15 minutes for the cage to empty. This exit time was aided by gently removing flies from the cage by hand and releasing them. Often after a release, a fly could be found on surrounding vegetation or on the brim of a hat.

The forest was always humid but more so in the summer months. The canal always held water and the level did not vary substantially.

Following the fly releases made from August 2007 to June 2008, 617 sentinel weevils were placed in the field and recovered at an average rate of 95% (Table 2-3). No flies were recovered.

## Discussion

### Survival

*Metamasius callizona* reduced the Enchanted Forest's *T. utriculata* population by 87% in the first 6 months of the study and, after 2 years, by 97.4%. This rate of decline was more rapid than expected. In Myakka River State Park, after 6 months the *T. utriculata* population had declined by 22% and at 4 years 10% of its population remained. *Metamasius callizona* killed almost all of the fallout in the Enchanted Forest (98.8%). In Myakka, 78% of the fallout was killed by *M. callizona*.

The greater rapidity and severity of the infestation at the Enchanted Forest compared to Myakka is probably due to a combination of factors. In Myakka, the *T. utriculata* population was sparsely distributed as small patches over an area of 25 $km^2$, making it less apparent to the weevil population. Some of the patches went undetected and were able to release seed. Other patches were completely eliminated by the weevil, including the reproductive class. In the Enchanted Forest, the *T. utriculata* population was large and mostly contiguous. Such a concentration of host plants allowed the weevil population to build rapidly and increase its epidemic potential.

In Myakka, the affects of *T. utriculata* on *M. callizona*'s seasonality and abundance were diluted by the more abundant weevil host, *T. fasciculata*, which maintained an aseasonal, endemic weevil population. In the Enchanted Forest, the weevil's host

population was primarily *T. utriculata*, a host plant that supports a seasonally abundant weevil population.

In Myakka, the infestation was observed over a period of 4 years. At the beginning of the study, losses by the weevil happened quickly, but as time progressed, the rate of the infestation declined. This same pattern is seen in the Enchanted Forest, though at a much exaggerated rate. It is unknown how the infestation progressed in the Enchanted Forest before March 2007. The first confirmed *M. callizona* sighting at the Enchanted Forest was in August 2006 (Frank 1996), which may have been the 1$^{st}$ or 2$^{nd}$ season since the weevil's arrival. When monitoring began at the Enchanted Forest in March 2007, the infestation would have been in its 2$^{nd}$ or 3$^{rd}$ season. At this time, the bromeliad population was experiencing cataclysmic losses, but after September 2007, weevils and fallen bromeliads killed by the weevil were found less frequently. Monitoring at Myakka began nearer the end of the cataclysmic stage, just as the infestation was slowing down.

An herbivore can inflict variable damage on a range of host plants (Underwood and Rausher 2000; Briese *et al.* 2002; Rudgers and Whitney 2006), and such is observed with *T. utriculata* and *T. fasciculata*. *Tillandsia utriculata* has a monocarpic habit and seasonally abundant *M. callizona* population that rapidly destroys the host plant population while *T. fasciculata* has a polycarpic habit and an aseasonal, non-abundant weevil population that is less aggressive. However, the outcome of a weevil infestation can vary for a species found in variable conditions. For *T. utriculata,* important conditions included patchiness and the ratio and type of other weevil host

bromeliads sharing the habitat. The conditions change as the infestation progresses and, as the host bromeliad population becomes sparse, the infestation slows down.

**Seasonality**

*Tillandsia utriculata* in the Enchanted Forest, as well as in Myakka, had highest peaks in weevil activity (number of fallen dead bromeliads, number of weevils, and number of weevils per host plant found per area) in June and showed overall greater activity in the months of March through August than in September through February. Weevil activity was severely reduced or ceased in the winter months. The decline in the bromeliad population at the Enchanted Forest followed the peaks in weevil activity. With each season, weevil activity and the rate of bromeliad loss declined. *Metamasius callizona* is seasonal on *T. utriculata* but seasonality becomes less pronounced as the bromeliad population becomes sparser.

Herbivore seasonality and abundance are often associated with host plant type and an herbivore can exhibit variable seasonality and abundance on a range of host plants (Wolda 1978; Hunter and Price 1992; Briese *et al.* 2002; Rudgers and Whitney 2006). The variable responses of the herbivore to its host plants can feed back and variably affect the survivability of the host plant. In this case, *M. callizona* grew seasonally abundant on *T. utriculata* and attained epidemic levels, whereas on *T. fasciculata*, the weevil was aseasonal and non-abundant.

**Biological Control**

No *L. franki* were recovered from the sentinel weevil larvae. The obvious answer is that the released flies did not make it through the F1 generation and therefore there were no flies to parasitize the sentinel weevil larvae for an F2 generation. However, it is also possible that there were F1 flies in the field but they failed to find the sentinel

weevil larvae because there were too few sentinel pineapple tops or the sentinel pineapple tops were not competitive with the attractiveness of the much larger population of wild, weevil-infested bromeliads. Another possibility is that we did capture a maggot from the F2 generation but failed to recognize this because the parasitized weevil was eaten by another weevil or because the maggot failed to reach maturity and emerge from the host larva.

Evidence based on the progression of the weevil infestation in the Enchanted Forest did not indicate establishment of *L. franki*. The decline of the bromeliad population and the pattern of weevil activity did not show anomalies that could be explained by parasitism of the weevil. If the releases did establish feral populations of *L. franki*, the effect on the weevil population was inconsequential.

It is difficult to predict whether a biological control agent will be successful or if an immigrant organism will become invasive (Barlow and Goldson 1993; Grevstad 1999; Shea and Possingham 2000). A critical part of undertaking a biological control project is to understand the ecology of the organisms involved and to use the ecological lessons learned to develop release and monitoring strategies. These strategies may vary when considering different species that are targeted by the invasive organism, but they may also need to be varied for a species under different conditions and stages of the infestation. For a *T. utriculata* population to be considered as a release site, the number of available host plants, the patchiness of the population, and type and proportion of other weevil host bromeliads that share the habitat should be considered. Infestations should be caught as early as possible because of the rapidity with which an infestation can grow. Releases of *L. franki* should be made frequently from March to September.

Rather than releasing in the same location, it might be prudent to release smaller numbers throughout the release site in areas where high weevil activity is found, on the same day as the release date.

Monitoring should be done more frequently. Gravid *L. franki* flies are attracted to the volatiles created by the weevil host larva and the chewed pineapple material (Suazo *et al.* 2006). If the chemicals that attract *L. franki* could be isolated and/or concentrated, smaller traps could be made. More traps could be placed in the field more frequently and, with the increased attractiveness, the chance of catching a maggot from the F2 generation would be increased. The trap would be improved with a design that separates weevil larvae and avoids weevil killing weevil. Sentinel weevil larvae should be dissected and searched for maggots and/or respiratory funnels.

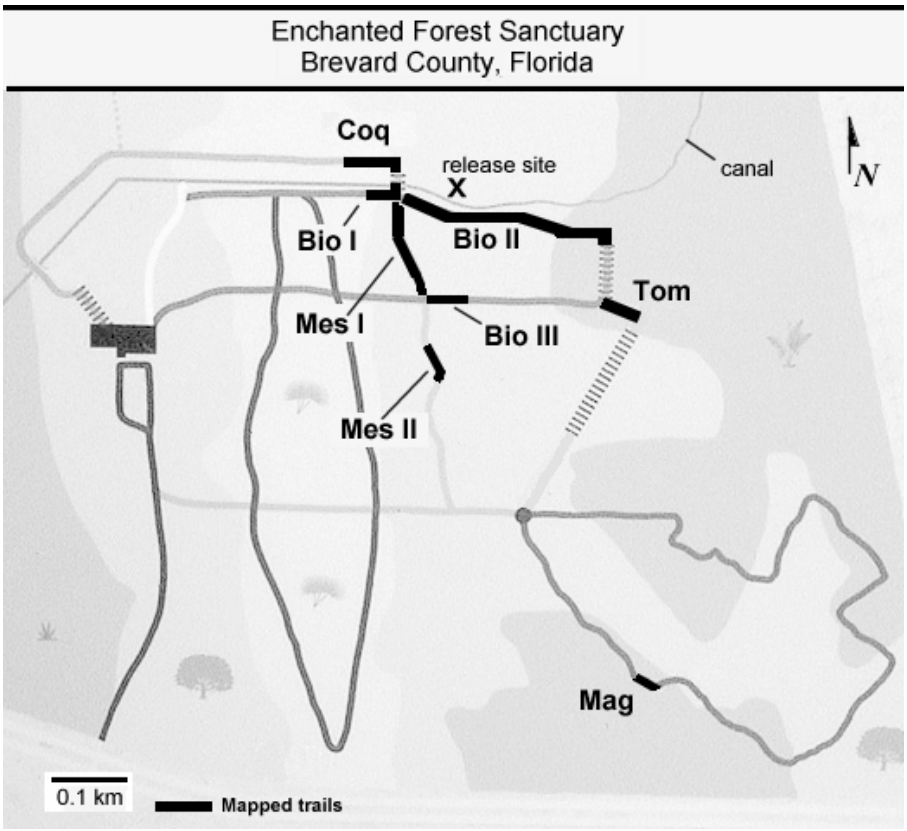



Figure 2-1. Mapped trails in the Enchanted Forest. The dark lines represent the area mapped and monitored in the Enchanted Forest Sanctuary (data super-imposed on modified map designed by John Norton for the Brevard County Environmentally Endangered Lands Program). Bio I = Biodiversity Loop I; Coq = Coquina Trail; Bio II = Biodiversity Loop II; Tom = Tomoka Trail; Mes I = Mesic I; Bio III = Biodiversity Loop III; Mes II = Mesic II; Mag = Magnolia Loop.

Table 2-1. Monitored area names, area, and the starting and ending bromeliad populations of *Tillandsia utriculata* and *Tillandsia fasciculata* with longest leaf length $\geq$ 30 cm in the Enchanted Forest Sanctuary.

| Area name | Area ($m^2$) | Starting bromeliad population (March 2007) | Ending bromeliad population (June 2009) |
|---|---|---|---|
| Biodiversity Loop I | 0.076 | 93 | 2 |
| Coquina Trail | 0.110 | 357 | 3 |
| Biodiversity Loop II | 0.402 | 1,090 | 28 |
| Tomoka Trail | 0.090 | 68 | 1 |
| Mesic I | 0.204 | 494 | 18 |
| Biodiversity Loop III | 0.096 | 26 | 0 |
| Mesic II | 0.087 | 32 | 1 |
| Magnolia Loop | 0.057 | 16 | 0 |
| Total: | 1.122 | 2,176 | 53 |

Table 2-2. Dates of releases of *L. franki* and number of female and male flies released.

| Date of release | Number of females | Number of males |
|---|---|---|
| 3 August 2007 | 69 | 63 |
| 26 October 2007 | 58 | 59 |
| 18 January 2008 | 56 | 57 |
| 28 April 2008 | 53 | 48 |
| 22 June 2008 | 52 | 48 |
| 27 May 2009 | 55 | 42 |
| Total: | 343 | 317 |

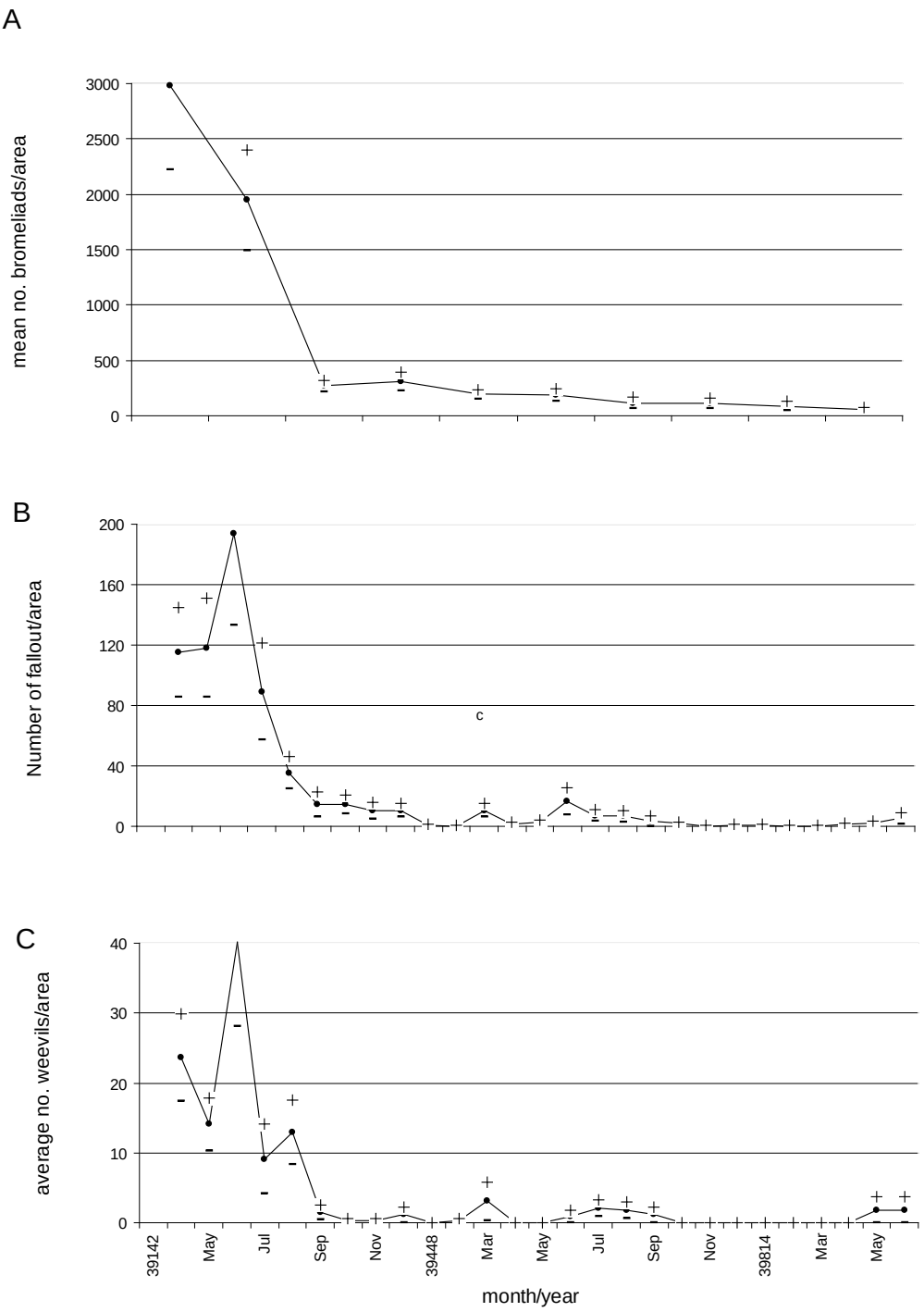



Figure 2-2. The mean number (plus and minus 1 standard error) of A) living bromeliads with longest leaf length ≥ 30 cm in the canopy counted every 3 months; B) fallout (dead bromeliads fallen from the canopy) found in the mapped areas counted every month; and C) weevil larvae, pupae and adults found in the fallout.

Table 2-3. Dates weevil-infested pineapple tops were placed in the field (following releases made from August 2007 to May 2008) and number of sentinel weevil larvae placed in the field and retrieved.

| Dates traps were placed in field | Number of sentinel weevil larvae in sentinel plants | Number of weevils retrieved from the field |
|---|---|---|
| 9 September to 23 September 2007 | 144 | 138 |
| 2 December to 16 December 2007 | 120 | 118 |
| 26 February to 13 March 2008 | 136 | 124 |
| 31 May to 13 June 2008 | 96 | 92 |
| 25 July to 13 August 2008 | 121 | 112 |
| Total: | 617 | 584 |

# CHAPTER 3
# RELEASE AND MONITORING OF A POTENTIAL BIOLOGICAL CONTROL AGENT, *Lixadmontia franki*, TO CONTROL AN INVASIVE BROMELIAD-EATING WEEVIL, *Metamasius callizona*, IN FLORIDA

## Introduction

*Metamasius callizona* (Chevrolat) (Coleoptera: Curculionidae), a bromeliad-eating weevil native to Mexico and Guatemala, was discovered established on native bromeliads in Florida in 1989 (Frank and Thomas 1994; Frank and Cave 2005). The weevil has become invasive and has spread to nearly fill its new range (Frank 1996). Sixteen species of bromeliads are native to Florida and 12 of these are susceptible to attack by the weevil (Frank and Cave 2005). Ten of these species are listed as endangered or threatened and 1 species is precinctive to Florida. These bromeliads are important to Florida's ecosystems and are crucial to the survival of other species (Frank 1983; Frank and Fish 2008). A biological control program was started in 1991 (Frank and Cave 2005). Beginning in 1992, searches were made for a classical biological control agent to import and release in Florida to regulate *M. callizona*. After several searches in the field and collecting and observing many *M. callizona* larvae, no parasitoids or other specialist agents that might be regulating the weevil population in its homeland have been discovered.

A potential biological control agent, *Lixadmontia franki* Wood and Cave (Diptera: Tachinidae), was discovered in 1993 in Honduras on a related species of bromeliad-eating weevil, *M. quadrilineatus* Champion (Cave 1997; Wood and Cave 2006). *Lixadmontia franki* was shown to parasitize *M. callizona* at least as readily as it attacks *M. quadrilineatus* (Frank and Cave 2005). In 2007, permission was received to release the fly and releases were started at 4 sites. Five releases for each site were made

throughout 2007- 2008 and a 6th round of releases was made in spring 2009. Monitoring followed each release. The first release resulted in a fly recovery which demonstrated that *L. franki* is capable of surviving and reproducing in Florida (Cave 2008). Since then, no other recoveries have been made. This paper evaluates the methods for releasing and post-release monitoring used in this study.

## Materials and Methods

### Insects and Plants

*Metamasius callizona* is native to Mexico and Guatemala and is a specialist of bromeliads (Frank and Cave 2005). Gravid weevils lay eggs at the leaf bases of a bromeliad and, after hatching, the larvae mine first the leaf and then the stem and leaf bases of the host plant (Frank and Thomas 1994; Frank and Cave 2005). *Metamasius callizona* larvae destroy the host plant's meristematic and intercalary tissue, which kills the plant. Adult weevils eat bromeliad leaves and can damage a plant but usually not fatally. The adults are winged and disperse to find new host plants and patches. In its native range, *M. callizona* is rarely found and has not been seen causing great damage to bromeliad populations. However, in Florida, where the weevil was discovered established in 1989, *M. callizona* has been devastating bromeliad populations.

Florida has 16 species of bromeliads and 12 of them are susceptible to attack by *M. callizona* (Frank and Cave 2005). Four of the 12 affected species were included in this study; they were *Tillandsia utriculata* L. (Bromeliales: Bromeliaceae), *T. fasciculata* Swartz, *T. balbisiana* Schultes and Schultes, and *T. simulata* Small. *Tillandsia utriculata* and *T. fasciculata* are both large bodied, long-lived bromeliads that range from central to south Florida. Both species have been heavily attacked by *M. callizona*, but *T. utriculata* has suffered the greatest losses. *Tillandsia balbisiana* is a small to medium

sized species that is often found growing in the same habitat as *T. fasciculata*; it does not appear to be as readily attacked as *T. fasciculata*. *Tillandsia simulata* is of particular interest because it is precinctive to Florida. *Metamasius callizona* has been observed infesting *T. simulata* in the field (Frank 1996), but it is unknown how damaging the weevil will be to this species.

It is suspected that *M. callizona* is controlled by a parasitoid in its homeland, though this has not been confirmed (Frank and Cave 2005). *Lixadmontia franki*, a parasitoid of a related bromeliad-eating weevil, *M. quadrilineatus*, was discovered in Honduras in 1993 (Cave 1997; Wood and Cave 2006). *Lixadmontia franki* was shown to parasitize *M. callizona* at least as readily as it will parasitize its native host (Frank and Cave 2005). Florida has 1 native bromeliad-eating weevil, *M. mosieri* Barber, a small, rare species that does not cause excessive damage to its host plants (Cave *et al*. 2006). Preliminary studies have shown that *L. franki* will parasitize *M. mosieri* but does not appear to do so as readily as it parasitizes *M. callizona* (Frank and Cave 2005). No parasitoids of *M. mosieri* have been found in Florida.

Gravid *L. franki* flies locate hosts from a mix of odors arising from the host weevil larva and the plant material that has been chewed by the larva (Suazo *et al*. 2006). The fly does not make contact with a potential host but rather deposits neonate maggots on an infested bromeliad. The maggots search for and attack weevil larvae inside the host plant. The maggot is endoparasitic and a koinobiont. Once the maggot has consumed its host weevil internally, it emerges from the dead host and pupates. Adult flies mate 2 to 4 days after emerging and females are ready to deposit neonate larvae by 8 days after mating (Suazo *et al*. 2008). Generation time for the flies is 5 to 6 weeks.

**The Release Sites**

Four release sites were chosen based on the size of the bromeliad population and the stage of the weevil infestation. The goal was to have an infested bromeliad population that would persist for at least 2 years before being destroyed by the weevil. Figure 3-1 is a map that shows the general locations and gives the latitudes and longitudes for the 4 release sites (Lake Rogers Park, Loxahatchee National Wildlife Refuge, Enchanted Forest Sanctuary, and Big Cypress National Preserve).

Lake Rogers Park is near the west coast of Florida in Hillsborough County. The release site was in a shady, humid swamp forest within the park. Bromeliad species included *T. fasciculata*, *T. balbisiana*, and *T. simulata*. The bromeliads were sparsely and singly distributed throughout the forest and grew from about shoulder height to high in the canopy. There was 1 very large *T. fasciculata* bromeliad, which was infested with the weevil at the time of the first observation of the site, the first release, and the first monitoring episode. The other bromeliads were small to medium sizes and several were infested by the weevil. The weevil was first seen at Lake Rogers in June 2007 (Frank 1996).

Loxahatchee National Wildlife Refuge is located on the northeast edge of the Everglades, in Palm Beach County. The release site was in a cypress swamp forest. Bromeliads included *T. fasciculata* and *T. balbisiana*. *Tillandsia fasciculata* was the predominant species. Both species grew on the trunks and upper branches of the trees. *Metamasius callizona* was first found in Loxahatchee in February 2001 (Frank 1996). In February 2001, the bromeliad population was dense and evenly spread throughout the forest. When releases were made, by rough estimate, the population had reduced in density by 75 to 80% but was still evenly spread.

The Enchanted Forest Sanctuary is near the east coast of Florida in Brevard County. The release site was in an oak hammock on the edge of a canal. Bromeliads included *T. utriculata* and rarely *T. fasciculata*. *Metamasius callizona* was first seen at the Enchanted Forest in August 2006 (Frank 1996). *Tillandsia utriculata* was the predominant species and grew abundantly in large patches on the trunks and branches of the trees high in the canopy. The bromeliad and weevil population was monitored monthly at the Enchanted Forest during the time of the releases (Chapter 2). The weevil infestation moved rapidly. From March 2007 to September 2007, 87% of the bromeliad population had been destroyed. By June 2009, less than 4% of the bromeliad population remained. As the bromeliad population declined, weevil activity declined.

Big Cypress National Preserve is located in Collier County in the Everglades. The release site was in a small cypress dome. Bromeliads included *T. fasciculata* and *T. balbisiana*. *Tillandsia fasciculata* was the predominant species and grew abundantly. The trees were short and grew in water that fluctuated from no water to about a meter high. Both bromeliad species grew low on the tree trunks, stopping at just above water line, and up into the canopy. The weevil was first seen in Big Cypress in February 2005 (Frank 1996).

**Releases**

Flies used for releases were reared in the Hayslip Biological Control Research and Containment Laboratory at the Indian River Research and Education Center in Ft. Pierce, Florida. The rearing method was based on a method designed by Suazo *et al*. (2006). *Metamasius callizona* larvae were used as hosts for the fly maggots. Newly emerged adult flies were collected from this colony 5 to 11 days before a release and

held in a 60 cm × 60 cm × 64 cm cage. The flies mated in the cage. Because 8 days are required for embryos to mature (Suazo *et al*. 2008), most of the females should have been ready to deposit maggots at the time of the release or within a few days afterwards.

*Lixadmontia franki* was released at 4 sites on 6 occasions. The first 5 rounds of releases were made from June 2007 to September 2008 and the 6$^{th}$ round in spring 2009. The releases made from June 2007 to September 2008 were scheduled approximately 3 months apart for each release site to ensure that releases were made for each season at each site. Table 3-1 shows the dates that flies were released for each of the release sites.

Analysis of variance was used to test the null hypotheses that similar numbers of flies were released at each site and for each season. In the event a null hypothesis was rejected, Tukey's method of multiple comparisons was used to determine which means were different.

Releases were made about 9:00 in the morning. Weather conditions were recorded. Releases were made from the same spot for each release site for each release. Latitude and longitude readings were taken for each point of release.

**Monitoring**

About 5 weeks after a release, traps were put out in the field and retrieved at about 7 weeks. The traps were 0.6 × 0.6 × 0.1 m cedar boxes with mesh bottoms that held 6 pineapple tops infested with sentinel weevil larvae per box. Two to 3 weevil larvae were inoculated in each pineapple top about a week before being placed in the field to allow the weevil larvae time to chew on the plant and create the necessary volatiles to attract *L. franki*. The weevil larvae were early 3$^{rd}$ instars when the traps

were initially placed in the field. Weevil larvae used in the traps came from a colony being maintained at the Entomology and Nematology Department at the University of Florida in Gainesville, Florida for monitoring following the releases made from June 2007 to September 2008. Table 3-4 shows the dates that traps were placed in the field then retrieved following the first 5 rounds of releases. Analysis of variance was used to test the null hypotheses that similar numbers of sentinel weevil larvae were put out in the field and recovered for each release site and for each season.

For the traps put out in the spring of 2009, weevil larvae were taken from a colony being maintained at Ft. Pierce. Sentinel pineapple tops were exposed to gravid *M. callizona* that deposited eggs in the leaf bases of the pineapple tops around four weeks before monitoring. Weevil larvae hatched from the eggs and grew on the tops. The pineapple tops were placed in the field when the weevil larvae were estimated to be 3$^{rd}$ instars. Table 3-5 shows the dates that traps were placed in the field then retrieved following the 6$^{th}$ round of releases. The recovery rate was not calculated for this set.

Traps were placed in the field about 5 weeks after a release to coincide with the time the F1 fly generation (if it existed) would be reproductively active. The goal was to attract F1 females to the trap that would deposit neonate maggots (F2 generation) which would then locate and parasitize sentinel weevil larvae inside the pineapple tops. The traps were suspended from rope and hung in the canopy around the point of release. The ropes were treated with Tangle Trap Insect Trap Coating® to prevent ants from getting into the trap. When possible, the traps were hung near wild bromeliads (infested with the weevil or not). Traps were suspended from the same location for each monitoring episode.

Traps were retrieved about 2 weeks after being placed in the field and returned to the laboratory. The weevil larvae were left in the pineapple tops in cages until the plants decomposed enough to easily retrieve the weevil larvae (usually about a week). After the weevil larvae were removed from the pineapple tops, they were reared separately in 5 mm × 20 mm Petri dishes on pineapple leaves. Parasitism was determined by the emergence of a maggot from a sentinel weevil larva.

## Results

### Fly Releases

In total, 2,279 flies were released, 1,198 females and 1,081 males (Table 3-1). The average number of flies released per release was 50 females (range 22 to 84) and 45 males (range 19 to 80). Statistically, there was no difference between the number of flies released at the 4 release sites and, for each site, similar numbers of females and males were released (Table 3-2). Greatest variance in the number of released flies was at Lake Rogers and the least variance was at the Enchanted Forest. Statistically similar numbers of flies were released in the summer, fall, and winter and in the summer, winter, and spring (Table 3-3). The number of flies released in the spring was slightly lower than those released in the fall.

Overall, conditions at the times flies were released ranged from cool or cold and dry in the winter to hot and humid in the summer. Driest conditions were in Big Cypress in May and June. Coldest conditions were in the Enchanted Forest when occasional freezes happened in the winter months. Lake Rogers remained the most constant with generally cool to warm, moist conditions. Loxahatchee was usually humid in the understory but was dry and exposed in the canopy, especially in the winter months.

Infested bromeliads and weevils were found around the release sites in the Enchanted Forest and at Lake Rogers at the beginning of the study but, by the end of the study, the bromeliad populations in both areas were severely diminished and weevil activity was reduced. Infested bromeliads and weevils were found throughout the study in Big Cypress and in Loxahatchee. In Big Cypress, the infested bromeliads and weevils were found consistently throughout the study in and around the release site. In Loxahatchee, infested bromeliads and weevils were found infrequently and usually distant from the release site.

**Post-release Monitoring**

Two flies were recovered from a single sentinel weevil larva following the first release at Lake Rogers on 29 June 2007. The trap with the parasitized weevil larva was suspended near the large *T. fasciculata* bromeliad that was actively infested by weevils at the time of the release and monitoring. No further flies were recovered from this site or from the other sites.

All traps were recovered intact. For the releases made from June 2007 to May 2008, 2170 sentinel weevil larvae were placed in the field and 1989 were recovered. The larvae were recovered at an average rate of 92% (Table 3-4). Statistically, there was no difference in the number of sentinel weevil larvae put out in the field and retrieved for the 4 release sites (Table 3-6) or for the seasons (Table 3-7).

## Discussion

Two flies were recovered only once, after the first release at Lake Rogers (Cave 2008). No other flies were recovered. The absence of further recoveries may be because no other flies survived to parasitize the sentinel weevil larvae, or flies did

survive but either did not parasitize sentinel weevil larvae or did but the parasitism went unnoticed.

Climate and elevation are strong influences in the range and distribution of many organisms and the success or failure of a biological control agent has often been attributed to the likeness or dissimilarity of the climate and elevation of the agent's home range compared to the range to which the agent is to be introduced (Samways 1989; Goolsby *et al*. 2005). Success was more likely in ranges with climate and/or elevations similar to a biological control agent's home range and failure more likely in ranges with dissimilar conditions. *Lixadmontia franki* comes from cool, humid cloud forests at high elevations and since the discovery of *L. franki* there has been concern that the fly would be unable to adapt to the hotter, lower elevations of Florida (Frank and Cave 2005). However, because the fly was once recovered, we know the fly is capable of surviving and reproducing in Florida, at least under certain conditions, and therefore has the potential to withstand Florida's climate and elevation.

The absence or reduction of a parasitoid's nectar source can affect the survival or effectiveness of a parasitoid (Walker *et al*. 1996; Wäckers 2004). In the laboratory, honey or nectar mixed with water is used as the nectar source for rearing *L. franki*. It is unknown what *L. franki* uses as a nectar source in its home range. The fly was able to find nectar at Lake Rogers Park so a source is available in Florida but it is unknown what or how many sources are available and if there are spaces and/or times when nectar for the fly is absent or insufficient.

The chance of a biological control agent becoming established increases as the number of individuals released and the number of releases increase (Grevstad 1999).

However, the number of individuals available may be limited. This was the case with *L. franki*. Difficulty in rearing the fly limited the number of flies available for a release to a range of 40 to 164 flies (Table 3-1) with a 50:50 female to male ratio (Table 3-6). Seasonal availability of the fly was nearly consistent, with slightly fewer flies available in the spring months compared to the fall (Table 3-7).

Were enough flies released to overcome the odds of at least one survival to the F2 generation? Multilarviparous tachinids that indirectly deposit their eggs or larvae tend to have high fecundity (Meier *et al*. 1999; Stireman *et al*. 2006) Eight days after mating an *L. franki* female can have about 50 neonate maggots and 80 or more developing eggs in her brood chamber (Suazo *et al*. 2008). The survival rate of the neonate maggots, once deposited, is unknown, but successful parasitism happens when 3 to 5 maggots are artificially larviposited on pineapple mash with a 3$^{rd}$ instar weevil (Chapter 4). These points, coupled with the fact that the single recovery was from the first release when only 27 female flies were released, suggest that the numbers of flies released were sufficient to overcome stochastic affects.

The success or failure of a biological control agent can vary in different habitats (Grevstad 1999; Manrique 2009). In this study there was only a single recovery of *L. franki*, a minimal success. Did the fly otherwise fail to survive to the F2 generation, or did the monitoring method fail to detect it? Attractiveness of a trap to a targeted organism may vary depending on the relative location of the trap to the targeted organism as well as the relative attractiveness of the trap to competing attractions (Bloem *et al*. 2005; Stephen and Rao 2005; Chu *et al*. 2006; Hall *et al*. 2007). The monitoring method in this study could have failed because the traps were not

advantageously situated or because the traps were less attractive to the fly compared to wild, infested bromeliads.

Only 8 traps were used around each release site thus limiting the area that could be monitored. In the Enchanted Forest and Loxahatchee, the traps were hung distantly from the wild bromeliads because the bromeliads were located high in the canopy. F1 flies may have ignored traps because the traps were located far from where the F1 generation emerged and likely mated and began its search for hosts. The only recovery happened in a trap that was placed near a large *T. fasciculata* plant that was actively infested by the weevil at the time of release and monitoring, lending mild support to the idea that traps in closer proximity to emerging *L. franki* flies would be more likely to be parasitized.

However, at Big Cypress, wild bromeliads grew easily within reach and a modest weevil infestation was present throughout the study. The traps were hung among the wild bromeliads and the release area was contained by the dimensions of the cypress dome. Yet, no flies were recovered from this site. It could be that the Big Cypress habitat was not compatible with fly survival and/or reproduction. An alternative reason for failing to capture a fly is that there was a fly, but it was more attracted to infested, wild bromeliads than to the sentinel pineapple tops. Parasitoids that use plant and host cues to locate a host are often influenced by plant species and/or the condition of an infested plant (Stireman *et al*. 2006). There is still much to learn about *L. franki*'s preferences.

The traps were placed in the field to coincide with the time that the F1 *L. franki* generation was reproductively active. Due to the great distances between the release

sites, the crowded schedule of releases and monitoring trips, and the limited number of field researchers, some traps remained in the field longer than 2 weeks. The longest was in Big Cypress from December 2007 to January 2008; these traps had the lowest recovery rate of sentinel weevil larvae (26 days at 74%; Table 3-4). Increased time in the field increases the chance that evidence of a captured fly may become lost or destroyed.

Most of the traps were recovered from the field after 2 to 3 weeks intact and in good condition. Weevil larvae were recovered at 92% (Table 3-4). The recovery rate was similar for the release sites (Table 3-6) and the seasons (Table 3-7). Such a high, consistent recovery rate makes *M. callizona* a good sentinel organism, but the recovery rate could be improved if the traps were designed to separate larvae or to decrease the time larvae share habitat to avoid larva killing larva, a behavior that could potentially eliminate a parasitized larva.

Following are suggestions to improve the chances of *L. franki*'s establishment and our ability to monitor the fly:

1. Make 3 to 4 releases of about 50 female and 50 male flies for each season of a year in a single release area that covers at least a few square kilometers and that has large, dense bromeliad populations with several localized outbreaks of weevil infestations.

2. The 3 to 4 releases made in the release area should be made in locations where weevils and bromeliads being killed by the weevil are found at the time of the releases. This will minimize the amount of time the parasitoid must spend locating a host, and will keep the releases in pace with the weevil infestation.

3. Following each release, place traps around the release site. Because the release areas will shift as the study progresses, the highest density of traps should always be clustered around the most recent release sites, but as much monitoring should be performed spatially and temporally as resources and people allow.

4. Use more, smaller traps that are more attractive to gravid flies than infested, wild bromeliads. This will require further research in understanding the cues that

attract the fly and in trap design. Trap design should include separation of sentinel weevil larvae.

5. Keep the traps in the field for a week (or less) and use several sets of traps for 5 to 10 weeks following a release.

In spite of the pessimistic results of this study, *L. franki* should continue to be considered as a potential biological control agent and releases should continue to be made. *Lixadmontia franki* is the only candidate biological control agent available and the potential losses from this biological invasion are too great to ignore the only possibility we have at present for controlling the weevil. Biological control agents can take several years before establishment happens (Grevstad 1999) and *L. franki* may eventually become established. If not, there is the possibility that the fly may be used as augmentative control in suitable habitats or seasons. Searches continue to be made for alternative biological control agents to control the weevil (Frank and Cave 2005). Information gained from studying *L. franki* will be useful in understanding other parasitoids or regulatory agents that may be found.

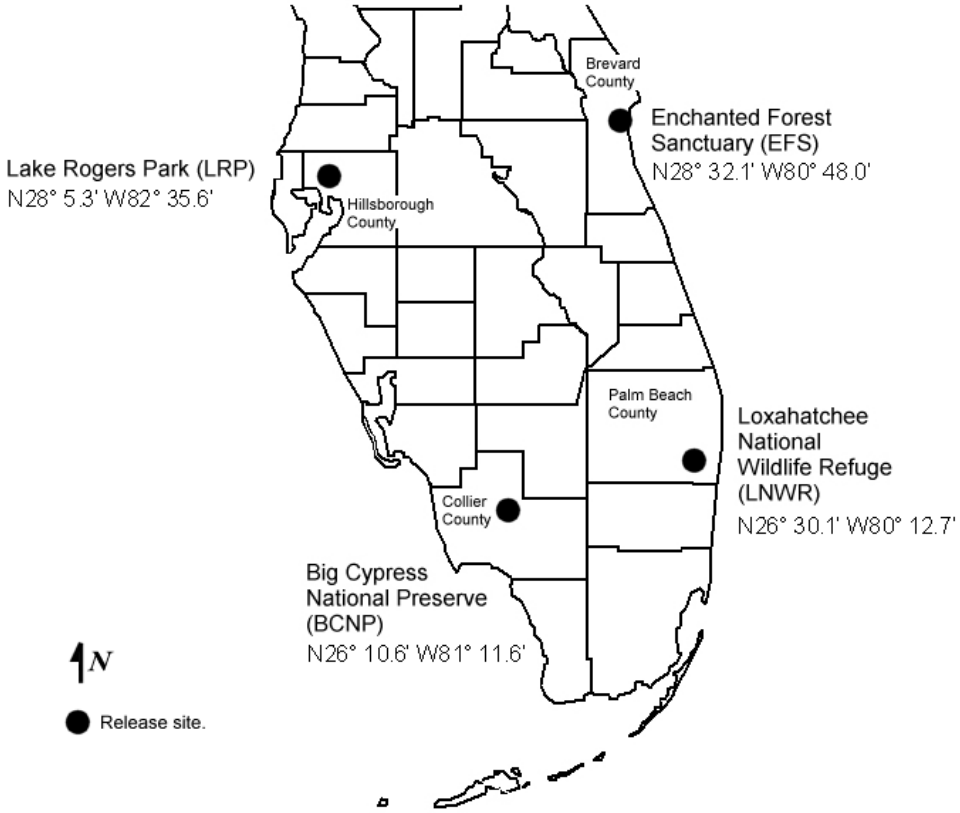


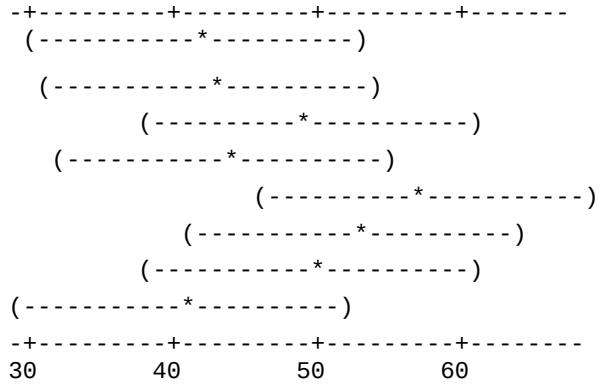

Figure 3-1. Map of *L. franki* release sites in Florida: Lake Rogers Park (Hillsborough County), Enchanted Forest Sanctuary (Brevard County), Loxahatchee National Wildlife Refuge (Palm Beach County), and Big Cypress National Preserve (Collier County).

Table 3-1. The date and number of female and male *L. franki* flies released per release number per release site.

| Release site | Release # | Date | # females released | # males released |
|---|---|---|---|---|
| Lake Rogers Park | 1 | 29 Jun 07 | 27 | 29 |
| | 2 | 21 Sep 07 | 84 | 80 |
| | 3 | 14 Dec 07 | 47 | 46 |
| | 4 | 3 Apr 07 | 33 | 36 |
| | 5 | 3 Jun 08 | 48 | 46 |
| | 6 | 24 Mar 09 | 22 | 19 |
| | Total: | | 261 | 256 |
| Loxahatchee National Wildlife Refuge | 1 | 20 Jul 07 | 59 | 32 |
| | 2 | 12 Oct 07 | 55 | 57 |
| | 3 | 11 Jan 08 | 68 | 62 |
| | 4 | 11 Apr 08 | 36 | 43 |
| | 5 | 13 Jul 08 | 37 | 30 |
| | 6 | 14 Apr 09 | 41 | 39 |
| | Total: | | 296 | 263 |
| Enchanted Forest Sanctuary | 1 | 3 Aug 07 | 69 | 63 |
| | 2 | 26 Oct 07 | 58 | 59 |
| | 3 | 18 Jan 08 | 56 | 57 |
| | 4 | 28 Apr 08 | 53 | 48 |
| | 5 | 22 Jun 08 | 52 | 48 |
| | 6 | 27 May 09 | 55 | 42 |
| | Total: | | 343 | 317 |
| Big Cypress National Preserve | 1 | 29 Aug 07 | 54 | 44 |
| | 2 | 21 Nov 07 | 61 | 48 |
| | 3 | 13 Feb 08 | 55 | 55 |
| | 4 | 12 May 08 | 41 | 32 |
| | 5 | 28 Jul 08 | 35 | 33 |
| | 6 | 5 May 09 | 52 | 33 |
| | Total: | | 298 | 245 |
| Grand total: | | | 1,198 | 1,081 |
| Average: | | | 50 | 45 |
| Range: | | | 22 to 84 | 19 to 80 |

Table 3-2. Comparison of the mean number of female and male *L. franki* flies released per release site using one-way analysis of variance.

| Release site | Fly gender | Mean number of flies released | Standard deviation | Individual 95% CIs for mean based on pooled standard deviation |
|---|---|---|---|---|
| LRP | ♀ | 43.50 | 22.44 | |
| | ♂ | 42.67 | 21.01 | |
| LNWR | ♀ | 49.33 | 13.22 | |
| | ♂ | 43.83 | 13.11 | |
| EFS | ♀ | 57.17 | 6.18 | |
| | ♂ | 52.83 | 8.04 | |
| BCNP | ♀ | 49.67 | 9.71 | |
| | ♂ | 40.83 | 9.62 | |




Pooled std. dev. = 14.06
n = 6 for all groups.
*P*-value = 0.455

Table 3-3. Comparison of the mean number of *L. franki* flies released per season using one-way analysis of variance. Similarities and differences between means are indicated by a, b, and c; based on results using Tukey's method of multiple comparisons with 95% simultaneous confidence intervals.

| Season | n | Mean number of flies released | Standard deviation | Individual 95% CIs for mean based on pooled standard deviation |
|---|---|---|---|---|
| | | | | ---+---------+---------+---------+------ |
| Summer | 8 | 47.63 | 13.86 | (-------*------) a b c |
| Fall | 4 | 64.50 | 13.23 | (----------*---------) a b |
| Winter | 4 | 56.50 | 8.66 | (----------*----------) a b c |
| Spring | 8 | 41.63 | 11.39 | (-------*------) a c |
| | | | | ---+---------+---------+---------+------ |
| | | | | 36 48 60 72 |
| Pooled std. dev. = 12.25 | | | | |
| *P*-value = 0.032 | | | | |

Table 3-4. The date and number of *M. callizona* sentinel weevil larvae put out in the field and recovered and percent weevil recovery per release number per release site following the releases made from June 2007 to May 2008.

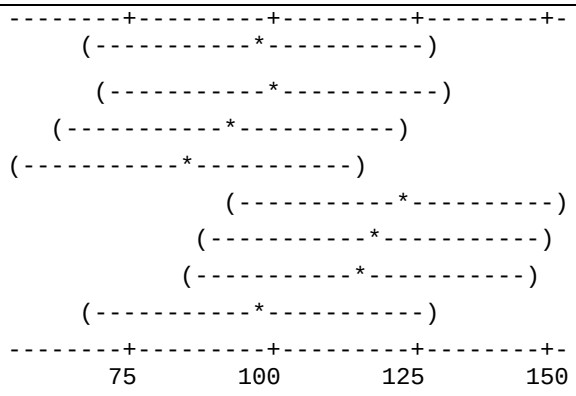
| Location | Release # | Date traps out | Date traps retrieved | # weevils out | # weevils recovered | % weevil recovery |
|---|---|---|---|---|---|---|
| Lake Rogers | 1 | 7 Aug 07 | 21 Aug 07 | 96 | 94 | 98% |
| Park | 2 | 1 Nov 07 | 15 Nov 07 | 60 | 58 | 87% |
| | 3 | 16 Jan 08 | 4 Feb 08 | 132 | 121 | 92% |
| | 4 | 7 May 08 | 22 May 08 | 138 | 128 | 93% |
| | 5 | 9 Jul 08 | 23 Jul 08 | 96 | 96 | 100% |
| | Total: | | | 522 | 497 | 95% |
| Loxahatchee | 1 | 30 Aug 07 | 13 Sep 07 | 72 | 68 | 94% |
| National | 2 | 20 Nov 07 | 5 Dec 07 | 51 | 42 | 82% |
| Wildlife | 3 | 12 Feb 08 | 27 Feb 08 | 48 | 34 | 71% |
| Refuge | 4 | 19 May 08 | 2 Jun 08 | 144 | 138 | 96% |
| | 5 | 21 Aug 08 | 4 Sep 08 | 144 | 141 | 98% |
| | Total: | | | 459 | 423 | 92% |
| Enchanted | 1 | 9 Sep 07 | 23 Sep 07 | 144 | 138 | 96% |
| Forest | 2 | 2 Dec 07 | 16 Dec 07 | 120 | 118 | 98% |
| Sanctuary | 3 | 26 Feb 08 | 13 Mar 08 | 136 | 124 | 91% |
| | 4 | 31 May 08 | 13 Jun 08 | 96 | 92 | 96% |
| | 5 | 25 Jul 08 | 13 Aug 08 | 121 | 112 | 93% |
| | Total: | | | 617 | 584 | 95% |
| Big Cypress | 1 | 2 Oct 07 | 23 Oct 07 | 96 | 85 | 88% |
| National | 2 | 19 Dec 07 | 14 Jan 08 | 96 | 71 | 74% |
| Preserve | 3 | 24 Mar 08 | 7 Apr 08 | 140 | 118 | 84% |
| | 4 | 16 Jun 08 | 30 Jun 08 | 144 | 131 | 91% |
| | 5 | 1 Sep 08 | 15 Sept 08 | 96 | 80 | 83% |
| | Total: | | | 572 | 485 | 85% |
| Grand total: | | | | 2,170 | 1,989 | 92% |

Table 3-5. The date and number of *M. callizona* sentinel weevil larvae put out in the field following the releases made in spring 2009.

| Location | Date traps out | Date traps retrieved |
|---|---|---|
| Lake Rogers Park | 5 May 2009 | 9 May 2009 |
| Loxahatchee National Wildlife Refuge | 26 May 2009 | 9 June 2009 |
| Enchanted Forest Sanctuary | 8 July 2009 | 22 July 2009 |
| Big Cypress National Preserve | 6 June 2009 | 30 June 2009 |

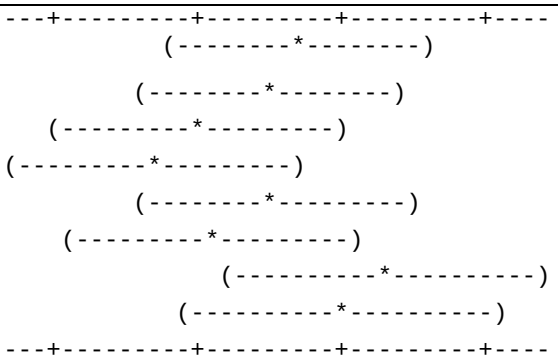
Table 3-6. Comparison of the mean number of *M. callizona* sentinel weevil larvae put out in the field and recovered per release site using one-way analysis of variance.

| Release site | Weevils put out in field and recovered | Mean number out and in | Standard deviation | Individual 95% CIs for mean based on pooled standard deviation |
|---|---|---|---|---|
| LRP | Out | 104.40 | 31.64 | |
| | Recovered | 99.40 | 27.56 | |
| LNWR | Out | 91.80 | 48.54 | |
| | Recovered | 84.60 | 51.68 | |
| EFS | Out | 123.40 | 18.38 | |
| | Recovered | 116.80 | 16.89 | |
| BCNP | Out | 114.40 | 25.23 | |
| | Recovered | 97.00 | 26.01 | |




Pooled std. dev. = 33.02
n = 5 for all groups.
*P*-value = 0.582

Table 3-7. Comparison of the mean number of *M. callizona* sentinel weevil larvae put out in the field and recovered per season using one-way analysis of variance.

| Season | n | Weevils put out in field and recovered | Mean # out and in | Standard deviation | Individual 95% CIs for mean based on pooled standard deviation |
|---|---|---|---|---|---|
| | | | | |  |
| Summer | 6 | Out | 112.17 | 29.12 | |
| | 6 | Recovered | 103.67 | 28.92 | |
| Fall | 5 | Out | 89.40 | 36.77 | |
| | 5 | Recovered | 80.60 | 36.45 | |
| Winter | 5 | Out | 106.40 | 36.18 | |
| | 5 | Recovered | 93.60 | 39.79 | |
| Spring | 4 | Out | 129.50 | 22.47 | |
| | 4 | Recovered | 119.00 | 19.77 | |
| Pooled std. dev. = 32.31 | | | | | |
| *P*-value = 0.369 | | | | | |

# CHAPTER 4
# INDIRECT ASSESSMENT OF HOST DENSITY BY *Lixadmontia franki*, A PARASITOID OF BROMELIAD-EATING WEEVILS

## Introduction

*Lixadmontia franki* Wood and Cave (Diptera: Tachinidae) is a parasitoid of bromeliad-eating weevils (Wood and Cave 2006; Cave 2008). The larval stage of the weevil is the host (Suazo *et al.* 2006). Gravid *L. franki* do not come in contact with a potential host because the weevil larvae grow and develop inside of the bromeliad, therefore the female uses a strategy that is used by other tachinids (Stireman *et al.* 2006). *Lixadmontia franki* is multi-ovolarviparous and deposits 1$^{st}$ instar maggots on weevil infested bromeliads and the maggots search for and parasitize weevil larvae inside of the host plant (Cave 2008; Suazo *et al.* 2008).

A weevil larva may support up to 9 *L. franki* maggots (Cave 2008). One to several weevil larvae may be found in a host bromeliad, depending on the size and species of the host bromeliad (Frank and Thomas 1994; Cooper 2006). Because parasitoids are limited to a single host for development, if a host is parasitized by too many parasitoids, superparasitism occurs and fitness is reduced by the loss of parasitoids that are unable to complete their development (Hardy *et al.* 1992; Vet *et al.* 1994; Reitz 1995; Royer *et al.* 1999). However, for species with short reproductive periods and that search for hosts in patchy environments, failing to parasitize available hosts would be a lost opportunity and, subsequently, fitness is reduced if a gravid parasitoid fails to parasitize before dying.

Because *L. franki* does not come in contact with the host, if the female is to make assessments about the host, she must use indirect cues. Suazo *et al.* (2006) showed that *L. franki* would not larviposit on bromeliads with a freshly inoculated weevil larva

(no accumulated chewed plant tissue or weevil frass), but would larviposit on bromeliads in which weevil larvae were given time to accumulate chewed plant material and frass. This suggests that, like other tachinids, *L. franki* uses chemical cues derived from accumulated chewed host plant tissue and/or host frass to detect larvipositional sites and to induce larviposition (Roth *et al.* 1982; Kainoh *et al.* 1999; Stireman 2002; Stireman *et al.* 2006).

Because a bromeliad with a greater number of weevil larvae would have a greater accumulation of chewed host plant tissue and host frass than a bromeliad with fewer weevil larvae, *L. franki* could use the amount of host plant – host volatiles that arise from this mix as a measure of host density and adjust the number of maggots she deposits on a weevil infested plant. This chapter examines whether *L. franki* can indirectly assess host density in this manner and respond by depositing more or fewer maggots with greater or lesser accumulated amounts of chewed host plant tissue and host frass.

## Methods and Materials

### Experimental Design

A randomized complete block design was used with 3 treatments and blocked for time.

### Experimental Unit

The experimental unit was a 20 mm × 60 mm Petri dish containing 2 host larvae (*Metamasius callizona* (Chevrolat)) in pineapple mash. To prevent weevil killing weevil, the Petri dishes had a sheet of plastic mesh with 1 mm holes spanning the center that separated the weevil larvae. The holes in the mesh were large enough to allow 1$^{st}$ instar maggots free movement while inhibiting the movement of the weevil larvae.

Weevil larvae came from a colony maintained at the University of Florida in Gainesville, Florida. Fresh pineapple leaf bases were exposed to gravid *M. callizona* kept in 25 ml vials. After 4 days, the leaf bases were inspected for eggs. Those with eggs were placed in covered 20 mm × 60 mm Petri dishes. Weevil larvae hatched from the eggs and, as they grew, fresh pineapple leaves were added every 4$^{th}$ day. Old leaves were not removed, thus chewed pineapple tissue and weevil frass accumulated. The weevil larvae were placed in the experimental units when they were 3$^{rd}$ instars, the preferred instar for parasitism by *L. franki* (Suazo *et al.* 2006). Instar was determined by days since hatching and head capsule size (Salas and Frank 2001).

**Treatments**

Three treatments were used with variable amounts of weevil-chewed pineapple and host frass added to the pineapple mash in the Petri dish. The treatments were:

- 0×: no weevil-chewed pineapple/frass added.
- 1×: weevil-chewed pineapple/frass from 2 weevil larvae added per experimental unit (an amount equal to what would be expected by the number of weevil larvae present).
- 2×: weevil-chewed pineapple/frass from 4 weevil larvae added per experimental unit (an amount twice as much as would be expected by the number of weevil larvae present).

The pineapple mash was prepared by chopping then blending pineapple stems and leaf bases in a food processor, separating the mash into 3 bowls, then adding variable amounts of the chewed pineapple and frass that accumulated while rearing the weevil larvae from egg hatch to 3$^{rd}$ instar. The first bowl had only pineapple mash. To the 2$^{nd}$ bowl, weevil-chewed pineapple/frass equal to the number of weevil larvae that would be used in making the experimental units was added to the mix. To the 3$^{rd}$ bowl, weevil-chewed pineapple/frass equal to 2× the number of weevil larvae that would be

used was added. Contents of each bowl were separately blended in the food processor again. The mash was packed into the bases of 20 mm × 60 mm Petri dishes and the mesh barrier was inserted. Two holes were poked in the pineapple mash, 1 on either side of the mesh barrier. The weevil larvae were inserted into the holes. The weevil larvae were left in the Petri dishes for 3 days before being used in an experiment.

**Experiments**

Two experiments were performed: 1) exposure of experimental units to a gravid *L. franki* population; and 2) artificial larviposition on the experimental units.

*Exposure to gravid* L. franki *population*. Experimental units were placed in a cage with a gravid *L. franki* population and the flies were allowed to choose the unit on which to larviposit. Forty *L. franki* puparia were collected from a colony maintained at the Hayslip Biological Control Research and Containment Laboratory at the Indian River Research and Education Center in Ft. Pierce, Florida. Rearing is based on a method designed by Suazo *et al.* (2006) and *M. callizona* larvae reared in pineapple crowns in the laboratory are used as hosts for the fly maggots. The pupae were taken to the Entomology and Nematology Department in Gainesville, placed in a 0.6 m × 1.0 m × 1.8 m cage, and kept under moist paper towels. The cage was kept indoors with a controlled temperature set at 20° C. The cage was placed near an east-facing window to allow the flies to receive morning sunshine. The cage was draped with transparent plastic on all sides and the top. From 5:00 am to 3:00 pm, the plastic was opened on 3 sides (including the side facing the window). The cage was lightly sprayed with water about every 45 minutes to maintain humidity. From 3:00 pm to 5:00 am, the plastic was lowered and secured around the cage to hold in humidity.

The adult flies that emerged were sexed and counted daily. The population was observed every 45 minutes for mating behavior. Observations and manipulations were performed when the plastic was open.

*Lixadmontia franki* requires 8 days after mating for embryo maturation (Suazo *et al.* 2008). Male flies were removed from the cage 7 days after the 1st observed mating. Experimental units were exposed to the fly colony 8 days after the 1st observed mating and thereafter for the lifespan of the colony. Experimental units were placed in the cage in a series of experimental runs. Each run was 4 days (the time the experimental units remained in the cage) and had 3 replications for each treatment. The experimental units were placed in the same location within the cage for each experimental run but randomly placed within that location. Eleven experimental runs were performed. Because the experimental runs were performed over time, time was blocked in the analysis.

*Artificial larviposition.* First instar maggots were dissected from female flies and artificially larviposited on the experimental units. Flies came from the colony in Ft. Pierce. Female flies at 8 to 14 days after mating were placed in 25 ml vials filled with water and left undisturbed until the fly settled down. The female was grasped by the thorax with a pair of forceps, removed from the vial, and placed in a dissecting dish with tap water. The abdomen was removed and opened, exposing the oviduct. If there were viable maggots in the brood chamber, the maggots were released by tearing the brood chamber open with forceps and prodding the maggots to leave by nudging them with the bristles of a paintbrush. Mobile maggots were sucked up in a dropper, and then gently squirted into the holes in the pineapple mash into which the weevil larvae had

been inserted. Three maggots were deposited per hole (6 per experimental unit). Two experimental runs were made to complete enough replications. Three replications for each treatment were performed for each experimental run. Time was blocked to account for variability between the 2 runs.

For both experiments, weevil larvae were removed from the experimental units 2 days after they were removed from the cage and 2 days after artificial larviposition and reared on pineapple leaves, again, in a covered Petri dish. The larvae were monitored daily for maggot emergence. Dead weevil larvae were dissected and examined for maggots.

**Responses**

For both experiments, 2 responses were measured:

1. The number of living maggots per experimental unit.
2. The number of dead maggots per experimental unit.

**Analysis**

Analysis of variance was used to compare the treatment means for responses 1 and 2 for both experiments. If analysis resulted in rejecting the null hypothesis ($H_0$: $\mu_{treatment\ 1} = \mu_{treatment\ 2} = \mu_{treatment\ 3}$), then Tukey's method of comparison was used to determine which means were different.

For the experimental units that were exposed to the gravid flies, the responses were a measure of the number of maggots that were larviposited per experimental unit. The artificial larviposition experiment was conducted to test whether the maggots experienced differential survival in the treatments. Artificial larviposition was done to distinguish whether the outcome from the experimental units exposed to the gravid flies

was a result of differential larviposition by the female flies or differential survival of the maggots after being larviposited but before successfully parasitizing a host.

## Results

### Experimental Units Exposed to Gravid Flies

From the 40 puparia, 13 females and 22 males emerged. The 1st fly mating was observed 5 days after the 1st fly emerged. Observed matings continued up to the 10th day and were observed daily. The males were removed on the 12th day and the first experimental run began on the 13th day at which time 10 female flies were alive. Eleven experimental runs were performed. Parasitism occurred only from the 3rd to the 9th experimental runs, during which time the female population dropped from 8 to 3 flies. Analysis was based on data from the 3rd to 9th experimental runs (7 runs total). The last fly died on the 37th day.

For the 7 experimental runs that were analyzed, 63 experimental units (126 larvae) were exposed to the adult female *L. franki* population. Fifty-three larvae were parasitized. From these weevils, 55 maggots emerged and 96 dead maggots were found inside hosts after dissection.

Experimental units with 1× and 2× weevil-chewed pineapple/frass added had similar numbers of living maggots per experimental unit and both had significantly higher numbers of living maggots than the treatment with no weevil-chewed pineapple/frass added (Table 4-1). The mean and maximum numbers of living maggots found per experimental unit were 0.86 and 6. The mean and maximum numbers of living maggots per larva were 0.44 and 5.

The experimental units with 0× and 1× weevil-chewed pineapple/frass added had similar numbers of dead maggots per experimental unit and both had significantly lower

numbers of dead maggots per unit than the treatment with 2× weevil-chewed pineapple/frass added (Table 4-2). The mean and maximum numbers of dead maggots found per experimental unit were 1.52 and 17. The mean and maximum numbers of dead maggots per larva were 0.76 and 9.

**Experimental Units Artificially Larviposited**

One hundred and eight maggots were artificially larviposited on 36 weevil larvae. Twenty-three of the larvae were parasitized, from which 24 maggots emerged and 11 dead maggots were found inside the dissected larvae.

The experimental units that were artificially larviposited had a similar number of living maggots per unit for the 3 treatments (Table 4-3) and a similar number of dead maggots per unit for the 3 treatments (Table 4-4). The mean and maximum numbers of living maggots found per experimental unit were 1.33 and 3. The mean and maximum numbers of living maggots per larva were 0.67 and 2. The mean and maximum numbers of dead maggots found per experimental unit were 0.55 and 4. The mean and maximum numbers of dead maggots per larva were 0.30 and 3.

## Discussion

The experimental units with weevil-chewed pineapple/frass added had on average a significantly greater number of living maggots per unit than the units that had no weevil-chewed pineapple/frass added (Table 4-1). This indicates that accumulated chewed pineapple tissue and/or frass are important cues for the fly's larviposition behavior.

The experimental units with 2× weevil-chewed pineapple/frass had a significantly greater number of dead maggots than the other units, which on average had similar numbers of dead maggots (Table 4-2). The experimental units with 2× weevil-chewed

pineapple/frass had twice the amount of accumulated chewed pineapple/frass than the amount that would have been present based on the number of weevil larvae in the pineapple mash. The female flies apparently responded by larvipositing too many maggots which resulted in not only a higher level of superparasitism but also higher maggot mortality; this is what would be expected if the fly was using volatiles from the accumulated chewed host plant and host frass as an indirect assessment of host density.

Because the experimental units that were artificially larviposited had on average similar numbers of living maggots per unit per treatment (Table 4-3) and similar numbers of dead maggots per unit per treatment (Table 4-4), the maggots evidently did not suffer differential mortality in the 3 treatments. Therefore, the outcomes from the experimental units that were exposed to the gravid fly population were a result of the females larvipositing differentially and not because the maggots had differential survival after being larviposited but before parasitism.

These experiments suffered from high variability, particularly for the experimental units of treatment 0× that were exposed to the gravid flies because so few units were parasitized (2 out of 21). However, the outcomes from these 2 experiments indicate that the fly has some ability to make an assessment of host density based on the amount of accumulated weevil-chewed host plant tissue and host frass that is present. Because understanding what attracts an *L. franki* female to a larvipositional site and then what induces her to larviposit is crucial for improving the design and management of traps and/or sentinel plants that are used for monitoring the fly population, as well as for designing laboratory experiments, it would be worthwhile to further pursue research

on how the chewed pineapple/frass affects fly behavior.  This would include isolating the chemicals that act as the attractant and inducer as well as further studies on fly choice and larvipositional behavior based on bromeliad species, presentation of food, and amount of weevil-chewed host plant tissue and/or host frass.

Table 4-1. For the experimental units exposed to the female flies, the following table compares the average number of living maggots per experimental unit for the 3 treatments (no weevil-chewed pineapple added; 1x weevil-chewed pineapple added; 2x weevil-chewed pineapple added) using analysis of variance and Tukey's HSD. *P* value for comparing treatments = 0.016. *P* value comparing time = 0.088; n = 21.

| Treatment: | 0× | 1× | 2× |
|---|---|---|---|
| Average # of living maggots +/- 2 standard errors per experimental unit: | 0.19 ± 0.06 | 1.14 ± 0.13 | 1.24 ± 0.13 |
| Tukey's method of comparison: | a | b | b |

Table 4-2. For the experimental units exposed to the female flies, the following table compares the average number of dead maggots per experimental unit for the 3 treatments (no weevil-chewed pineapple added; 1x weevil-chewed pineapple added; 2x weevil-chewed pineapple added) using analysis of variance and Tukey's HSD. *P* value for comparing treatments = 0.005. *P* value comparing time = 0.090; n = 21.

| Treatment: | 0× | 1× | 2× |
|---|---|---|---|
| Average # of dead maggots +/- 2 standard errors per experimental unit: | 0.09 ± 0.10 | 1.00 ± 0.47 | 3.48 ± 1.11 |
| Tukey's method of comparison: | a | a | b |

Table 4-3. For the experimental units that were artificially larviposited, the following table compares the average number of living maggots per experimental unit for the 3 treatments (no weevil-chewed pineapple added; 1x weevil-chewed pineapple added; 2x weevil-chewed pineapple added) using analysis of variance. *P* value for comparing treatments = 0.490. *P* value comparing time = 0.625; n = 12.

| Treatment: | 0× | 1× | 2× |
|---|---|---|---|
| Average # of living maggots +/- 2 standard errors per experimental unit: | 0.67 ± 0.22 | 0.83 ± 0.21 | 0.50 ± 0.15 |

Table 4-4. For the experimental units that were artificially larviposited, the following table compares the average number of dead maggots per experimental unit for the 3 treatments (no weevil-chewed pineapple added; 1x weevil-chewed pineapple added; 2x weevil-chewed pineapple added) using analysis of variance. *P* value for comparing treatments = 0.391. *P* value comparing time = 0.419; n = 12.

| Treatment: | 0× | 1× | 2× |
|---|---|---|---|
| Average # of dead maggots +/- 2 standard errors per experimental unit: | 0.25 ± 0.13 | 0.50 ± 0.26 | 0.17 ± 0.11 |

# CHAPTER 5
# DESCRIPTION OF THE LARVAL STAGES OF *Lixadmontia franki* (DIPTERA: TACHINIDAE)

## Introduction

*Lixadmontia franki* (Wood and Cave) is a parasitoid of bromeliad-eating weevils that was originally discovered on its host, *Metamasius quadrilineatus* Champion, in Honduras in 1993 (Cave 1997). Adult flies mate within 48 hours after emergence (Suazo *et al.* 2008). The females are multi-ovolarviparous and embryos develop internally to 1st instars in a modified vagina that functions as a brood chamber, a reproductive method used by other tachinids (Meier *et al*. 1999; Stireman *et al*. 2006). The embryos require at least 8 days to develop into 1st instars (Suazo *et al*. 2008). The female flies deposit 1st instar maggots on bromeliads that are infested with host weevils. The maggots search for and attack weevil larvae inside the host plant. The maggot is endoparasitic and a koinobiont. Once the maggot has consumed its host weevil internally, the final instar emerges from the dead host and pupates.

This paper describes the larval stages of *L. franki*. Features that are described include body size and shape, presence or absence of spinulae, absence or presence and size of posterior and anterior spiracles, size and shape of the mouth hooks and the cephalopharyngeal skeleton, and presence or absence of a respiratory funnel. These features have proven useful in describing other tachinid larval stages (Thompson 1960; Ichiki and Shima 2003; Michalková *et al*. 2009). Wood and Cave (2006) described the adult of *L. franki*.

## Methods and Materials

Adult females of *L. franki* were taken from a colony that is maintained at the Hayslip Biological Control Research and Containment Laboratory at the Indian River

Research and Education Center in Ft. Pierce, Florida, based on a method designed by Suazo *et al.* (2006). Larvae of *Metamasius callizona* (Chevrolat) reared in pineapple crowns in the laboratory were used as hosts for the fly maggots.

First instars of *L. franki* were dissected from adult females under a microscope in tap water 9 to 14 days post-mated; the females were stunned but still alive. The fly's abdomen was opened and the brood chamber removed. The writhing pharate first instars were released by ripping open the brood chamber with forceps and gently nudging the maggots out with the bristles of a paint brush. Mobile maggots were sucked up with a dropper and gently squirted onto the pineapple mash (stems and leaf bases that had been chopped and blended in a food processor) in 35-ml cups containing 3$^{rd}$ instar *M. callizona*. Five maggots were deposited per cup, with 1 host larva per cup. Thirty cups were artificially larviposited and held at 25 °C. At 1 through 10 days after artificial larviposition, 3 weevil larvae were dissected each day and searched for *L. franki* maggots developing inside of the host.

Measurements of body size (length and width), width of the respiratory funnel, and length of a mouth hook (from the apex to the base of the mouth hook) and cephalopharyngeal skeleton (from the base of the mouth hook to the apex of the dorsal wing) were taken for 1$^{st}$, 2$^{nd}$, and 3$^{rd}$ instars (n=3 for each instar) and the mean and standard error were calculated for each instar. An Auto-Montage® image was taken of a respiratory funnel in a 2$^{nd}$ instar. Drawings were made of 1$^{st}$, 2$^{nd}$, and 3$^{rd}$ instar bodies and mouth hook – cephalopharyngeal complex using a drawing tube attached to a compound microscope (Leica MZ16). A scanning electron microscope was used to

take images of the posterior spiracles and spinulae of 2nd and 3rd instars and the anterior spiracle of the 3rd instar.

## Results

### Instars and Tracheal Attachments

*Lixadmontia franki* has 3 instars. Early 1st instars and late 3rd instars (before emerging from the host) were not attached to host tracheae. Late 1st instars, 2nd instars, and early 3rd instars were attached to the lateral longitudinal trunk of the host trachea, usually near the anterior or the posterior host spiracle, though some were found centrally attached. Very few 1st instars were found unattached; when they were discovered, they were found living freely beneath the integument. When 1st instars molted, the exuviae remained attached to the respiratory funnel and the 2nd instar developed upon the existing respiratory funnel (Figure 5-1). The number of respiratory funnels counted equaled the number of maggots counted.

Respiratory funnels of 1st and 2nd instars were sufficiently dissimilar in width to be able to distinguish instars (0.18 mm *vs* 0.85 mm; Table 5-1). Second and 3rd instars had respiratory funnels with similar widths (0.85 mm *vs* 0.87 mm; Table 5-1). Most of the 3rd instars discovered were already disconnected from the funnel. Second instar exuviae were found either attached to the respiratory funnel or nearby. First and 2nd instar exuviae were distinguishable by the mouth hook – cephalopharyngeal complex (2nd instars mouth hooks and cephalopharyngeal skeleton were twice as long as 1st instars) and spinulae patterns (1st instars had distinct bands encircling the thorax and abdominal segments while 2nd instars had little spinulae).

**First Instar**

Body length, 1.31 ± 0.46 mm; width, 0.48 ± 0.33 mm (Table 5-1). Translucent. Head and anterior edges of thoracic and abdominal segments encircled by a wide band of spinulae (Figure 5-2A). Mouth hook 0.05 ± 0.02 mm; cephalopharyngeal skeleton 0.10 ± 0.02 (Table 5-1, Figure 5- 3A). Anterior spiracles absent. First instars were found 2-5 days after artificial larviposition.

**Second Instar**

Body length, 2.91 ± 0.63 mm; width, 0.97 ± 0.07 mm (Table 5-1). Semi-opaque, whitish color. Anterior edges of thoracic and abdominal segments encircled with 5–6 rings of spinulae ((Figures 5-2B and 5-3). Spinulae triangular and approximately 0.8 µm wide and 5 µm long (Figure 5-9). Mouth hook 0.11 ± 0.04 mm; cephalopharyngeal skeleton 0.26 ± 0.02 (Table 5-1, Figure 5-3B). Anterior spiracles absent (Figure 5-4). Posterior spiracles approximately 25 µm wide and 28 µm long (Figure 5-7). Second instars were found 2-8 days after artificial larviposition.

**Third Instar**

Body length, 7.59 ± 1.12 mm; width, 2.30 ± 0.53 mm (Table 5-1). Yellow-cream colored, stout body. Head and anterior edges of thoracic and abdominal segments encircled with 5–6 rings of spinulae (Figures 5-2C and 5-4). Spinulae triangular, approximately 15 µm wide and 28 µm long (Figure 5-10), or ovoid with an inverted cup on the base and approximately 20 µm high and 40 µm long (Figure 5-11). Ovoid spinulae located ventrally. Mouth hook, 0.24 ± 0.05 mm; cephalopharyngeal skeleton, 0.58 ± 0.05 (Table 5-1, Figure 5-3C). Anterior spiracles present on mesothorax (Figures 5-5 and 5-6). Posterior spiracles approximately 130 µm wide and 200 µm long

and ringed by spinulae (Figure 5-8). Third instars were found 7-10 days after artificial larviposition.

## Discussion

The instars of *L. franki* are easily distinguishable by the body size, shape and color, the amount and type of spinulae, and the size and shape of the mouth hook and the cephalopharyngeal skeleton. Respiratory funnels can be used to distinguish between 1st and 2nd instars but not between 2nd and 3rd instars. The number of respiratory funnels counted in a host can be used as a reliable count of the number of maggots that successfully parasitized a host and lived long enough to attach to the host tracheae and construct a respiratory funnel.

First instars are mobile because they must search for their hosts. Third instars are also mobile because, after emerging from the host, they move away to find a place to pupate. The dense spinulae on the 1st instar and the relatively larger, ventral, ovoid, and cup-shaped spinulae on the 3rd instar likely function to aid mobility. The 2nd instar, which is not mobile outside the host, has fewer and relatively smaller spinulae.

In this experiment, instars were found across a wide range of days (1st instar, 2-5 days after maggots were deposited on the pineapple mash; 2nd instars, 2-8 days; and 3rd instars 7-10 days). This may be due to different times required for the maggots to find and parasitize a host. Suazo *et al.* (2008) showed that development time of *L. franki* from penetration of the host to pupation ranged from 13 to 21 days in *M. quadrilineatus* at 21° C, suggesting there may be high variability in the growth rate of these maggots, which could also cause variability. More replications are necessary to determine an average time for instar development.

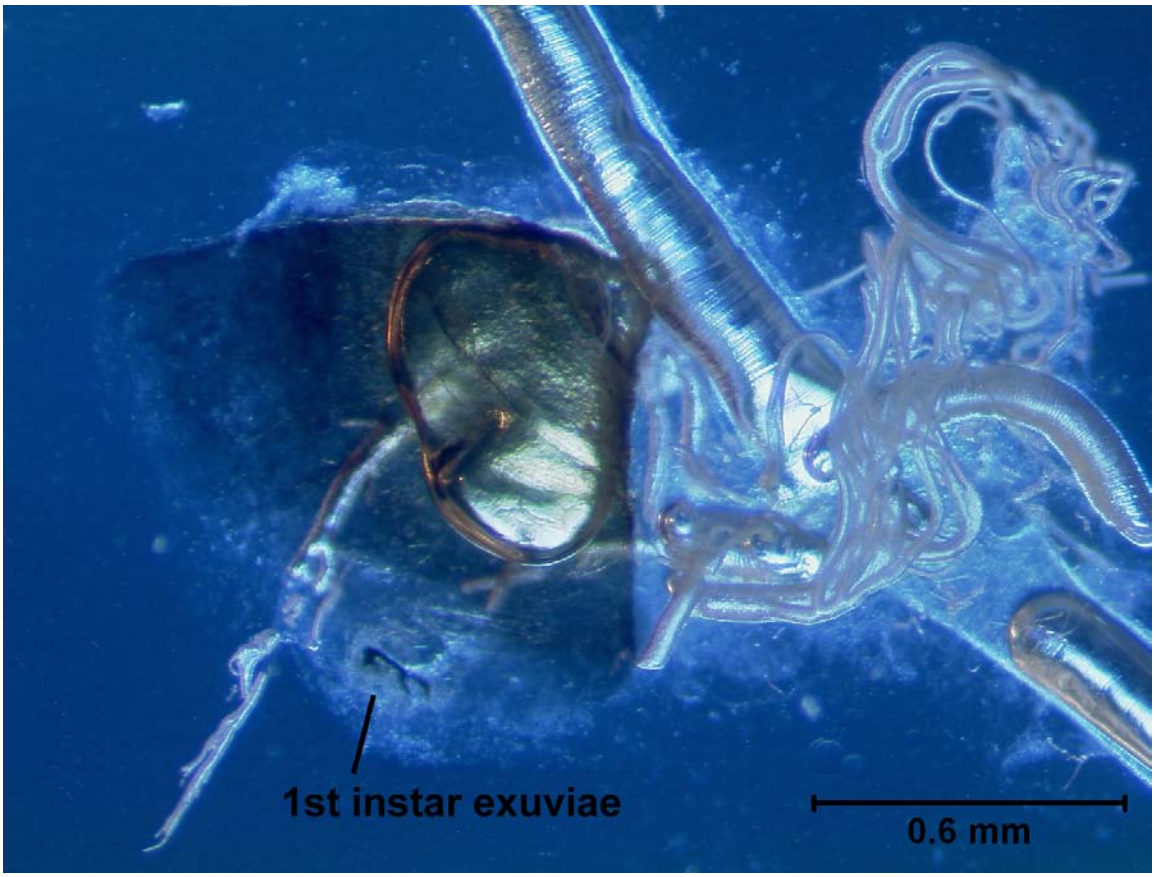



Figure 5-1. Respiratory funnel of a 2nd instar *L. franki* with 1st instar exuviae attached.

Table 5-1. Length and width of the body, length of the mouth hook and cephalopharyngeal skeleton, and width of the respiratory funnel in each instar of *L. franki*.

| Instar | Body length (mm) | Body width (mm) | Mouth hook length (mm) | Cephalo-pharyngeal skeleton length (mm) | Respiratory funnel width (mm) |
|---|---|---|---|---|---|
| 1 | 1.31 ± 0.46 | 0.48 ± 0.33 | 0.05 ± 0.02 | 0.10 ± 0.02 | 0.18 ± 0.02 |
| 2 | 2.91 ± 0.63 | 0.97 ± 0.07 | 0.11 ± 0.04 | 0.26 ± 0.02 | 0.85 ± 0.16 |
| 3 | 7.59 ± 1.12 | 2.30 ± 0.53 | 0.24 ± 0.05 | 0.58 ± 0.05 | 0.87 ± 0.18 |

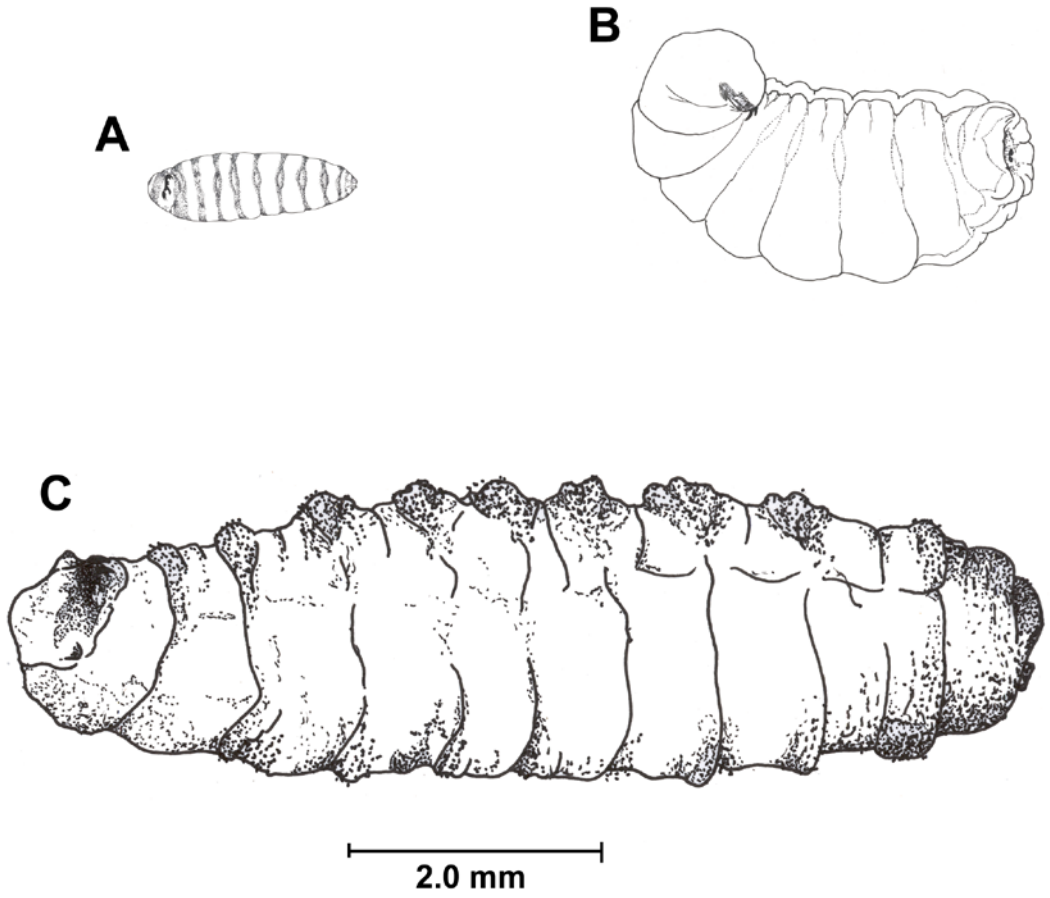



Figure 5-2. Left lateral view of larval L. franki. A) 1st instar; B) 2nd instar; and C) 3rd instar. Anterior end faces left and ventral side is up for all instars.

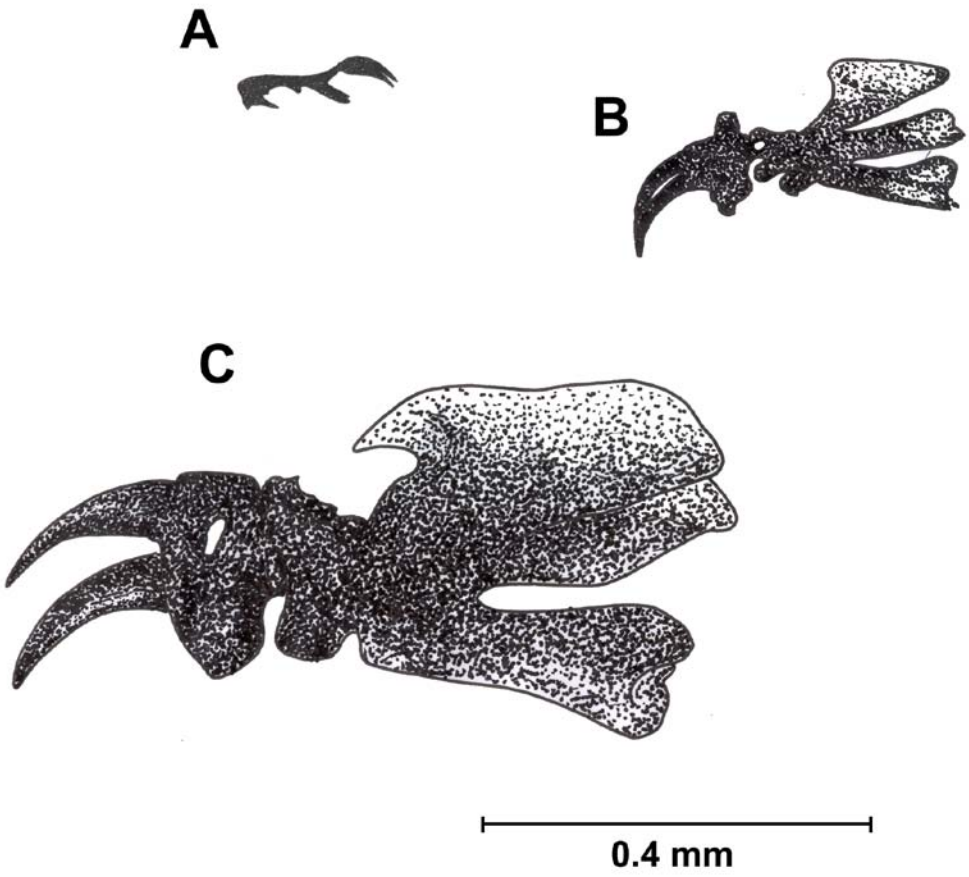



Figure 5-3. Left lateral view of mouth hook and cephalopharyngeal skeleton of larval *L. franki*. A) 1st instar; B) 2nd instar; and C) 3rd instar. Anterior end faces left and dorsal side is up.

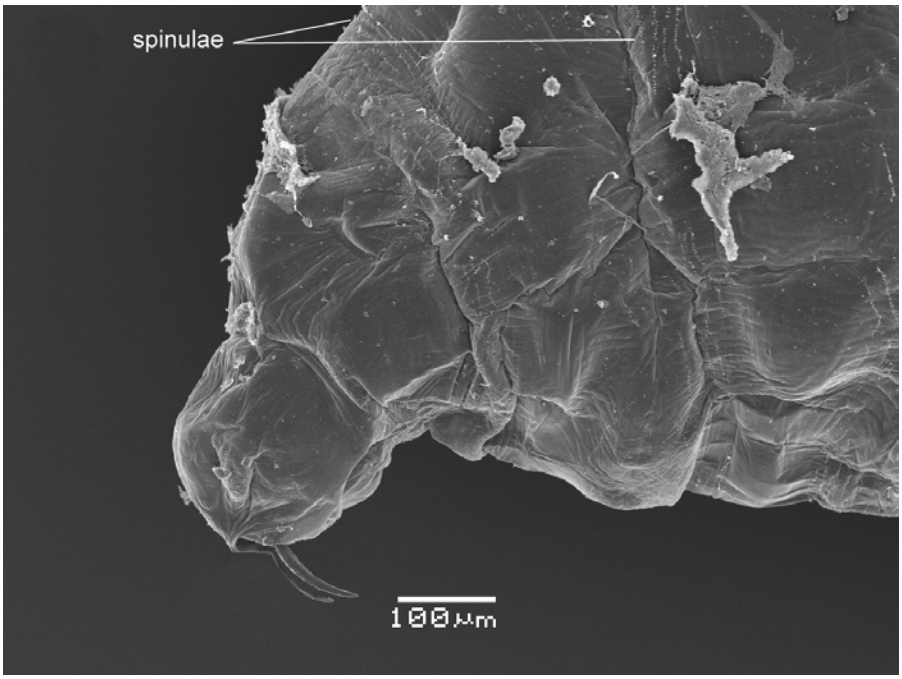



Figure 5- 4. Left lateral view of head and thorax of 2nd instar *L. franki*; no anterior spiracle is present; spinulae are present.

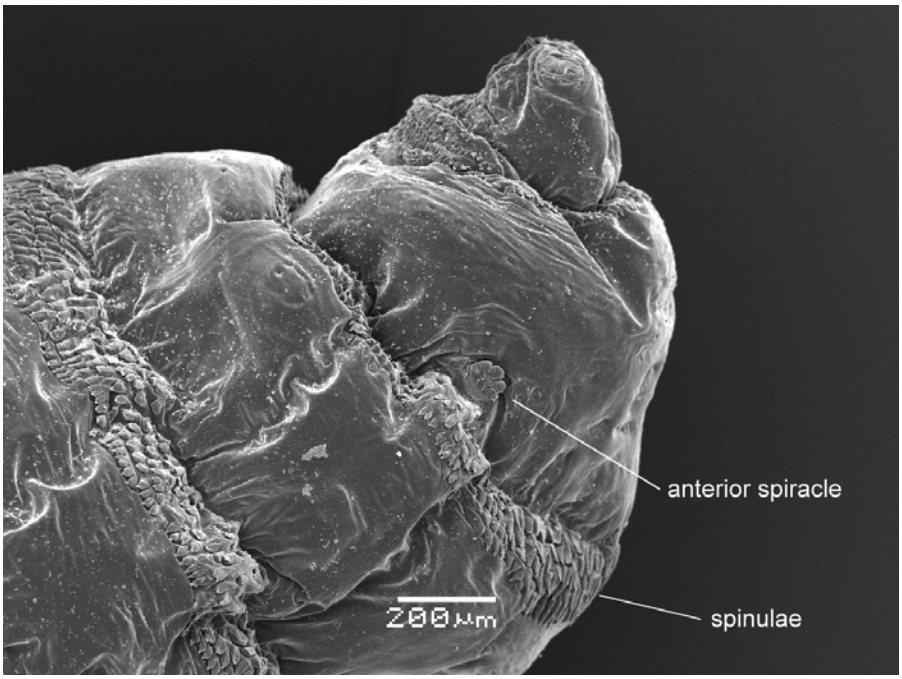



Figure 5-5: Left lateral view of head and thorax 3rd instar *L. franki*, showing anterior spiracle and spinulae.

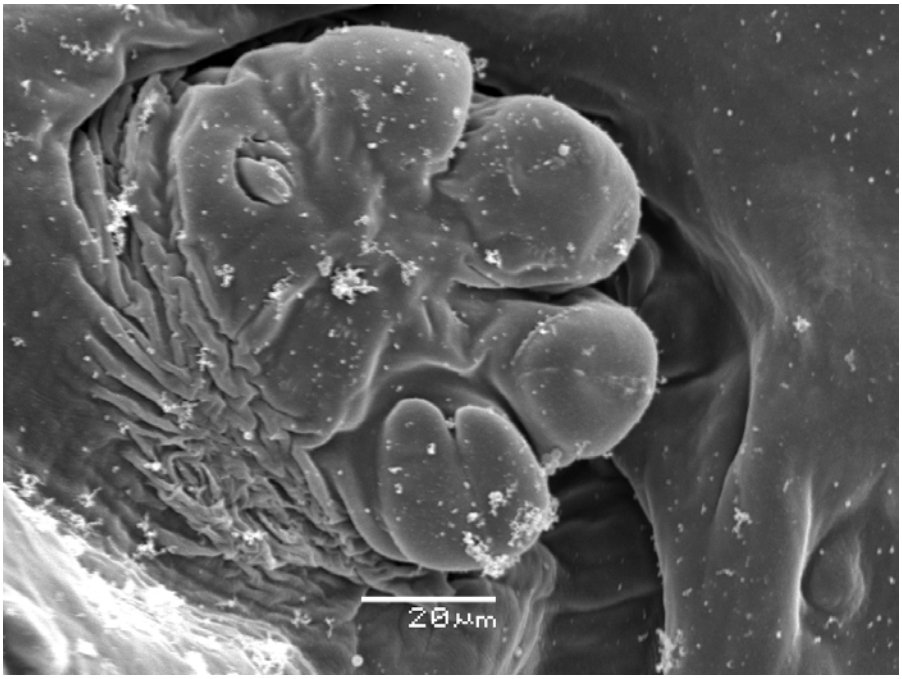



Figure 5-6. Anterior spiracle of 3rd instar *L. franki*.

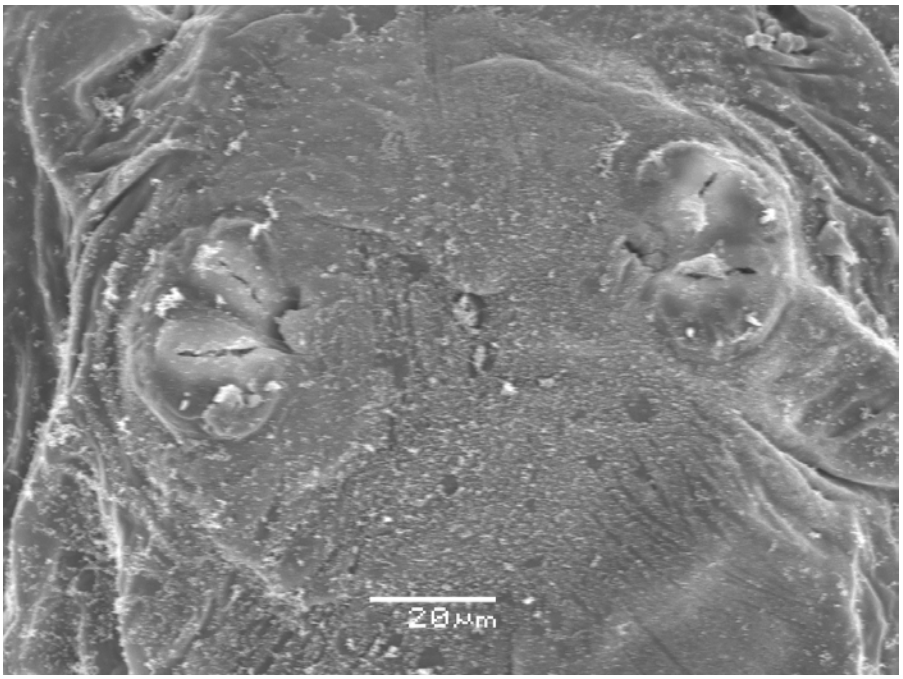



Figure 5-7. Posterior spiracles of 2nd instar *L. franki*.

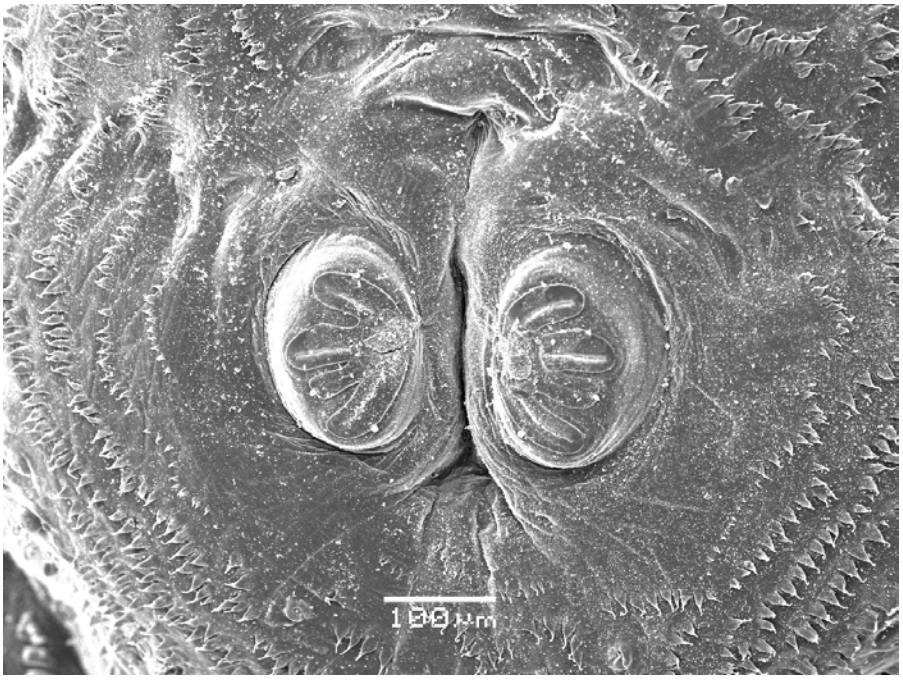



Figure 5-8. Posterior spiracles of 3rd instar *L. franki*.

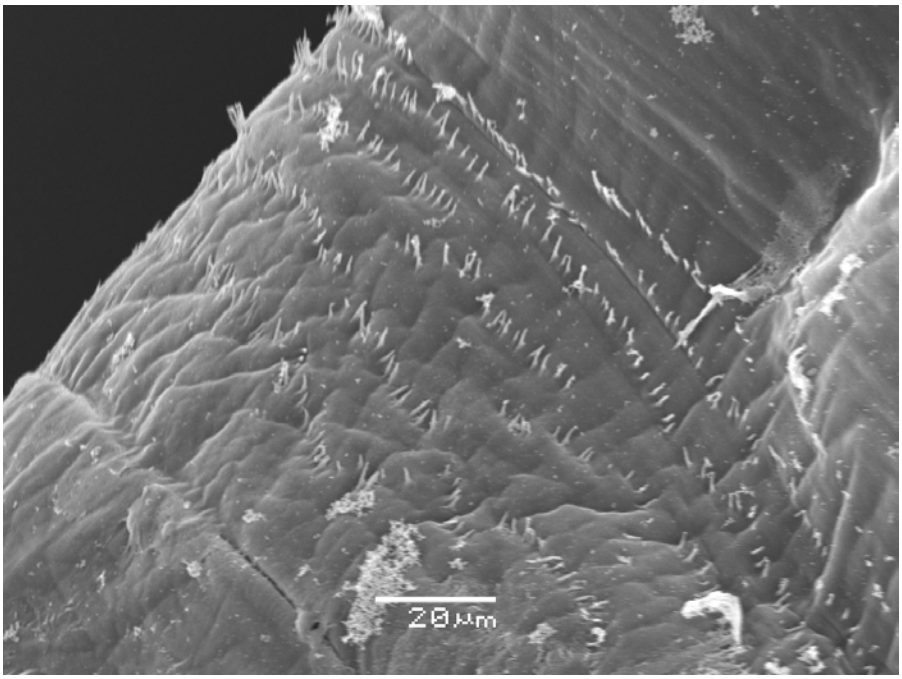



Figure 5-9. Spinulae of 2nd instar *L. franki*.

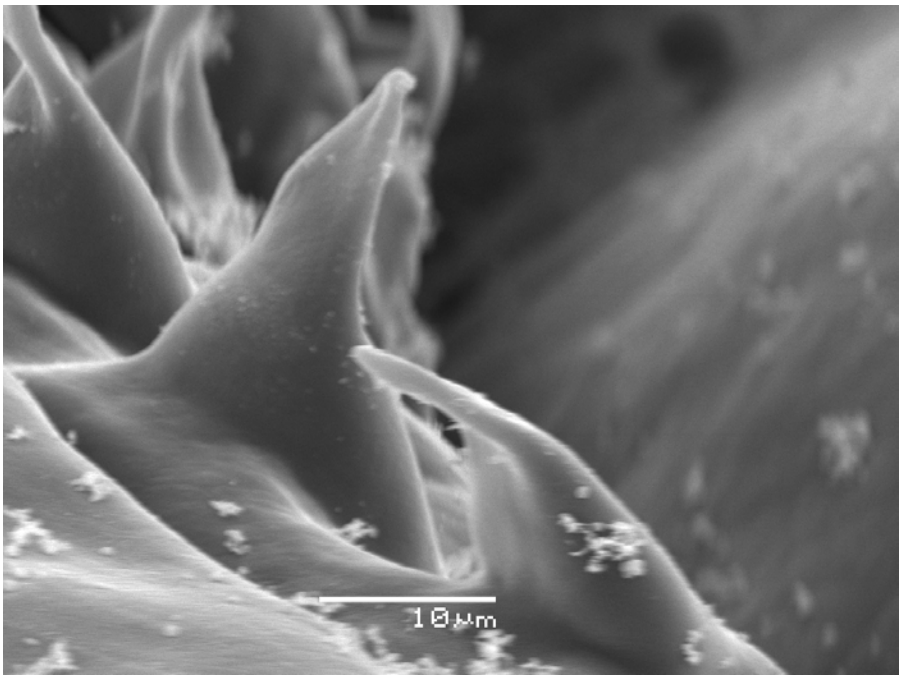



Figure 5-10. Triangular spinulae of 3rd instar *L. franki* circling anterior ring of mesothorax.

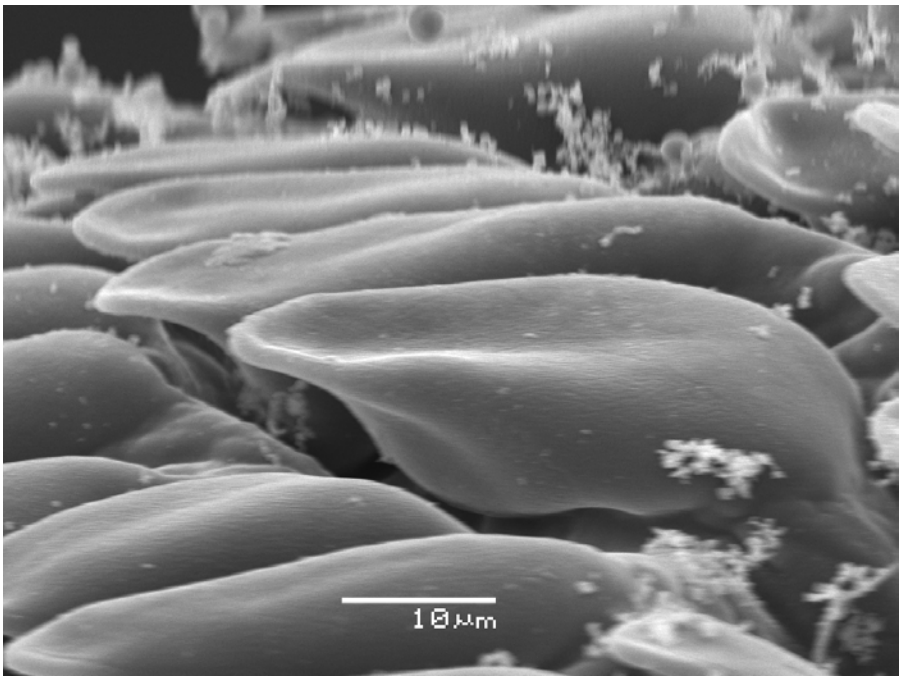



Figure 5-11. Ovoid spinulae of 3rd instar *L. franki* on ventral side of metathorax.

# CHAPTER 6
# *Lixadmontia franki*: OVOVIVIPARITY VERSUS VIVIPARITY

## Introduction

Ovoviviparity and viviparity are common in tachinids (Meier *et al*. 1999; Stireman *et al*. 2006). Because these are reproductive strategies that require changes in physiology and behavior and may affect associations with other organisms, they are traits that can be used in understanding the phylogeny as well as the ecology of Tachinidae. There is some confusion with terminology concerning ovoviviparity and viviparity, therefore I will define and use the following terms for this paper (taken from Meier *et al*. 1999; Gordh and Headrick 2001; Stireman *et al*. 2006):

1. Larviparity: eggs incubate internally and the female deposits pharate larvae in eggs that hatch after or during departure from the mother's abdomen, or she deposits neonate larvae that hatch from eggs before departure from the mother's abdomen. This term includes both ovoviviparity and viviparity.
2. Ovoviviparity: eggs incubate internally and the female deposits pharate larvae in eggs that hatch either while being deposited or shortly afterwards.
3. Viviparity: eggs incubate internally, pharate larvae hatch internally, and the female deposits neonate larvae already hatched from eggs within her abdomen.

*Lixadmontia franki* Wood and Cave is a tachinid species from Honduras and Guatemala that specializes on bromeliad-eating weevils (Cave 1997; Wood and Cave 2006). There is no doubt that this species is larviparous. The median oviduct of the female reproductive tract is modified to function as a brood chamber (Suazo *et al*. 2008). Dissected females at 8 days post-mated have easily recognizable pharate maggots lined up in the brood chamber ready to be deposited. These maggots can be extracted and used successfully in artificial larviposition (Suazo *et al.* 2008; chapters 4 and 5). Pharate maggots have been photographed exiting recently killed females (Suazo *et al*. 2008). Females deposit maggots on weevil-infested bromeliads and the

maggots search for and parasitize hosts (Cave 2008). All of these traits are solid evidence that this species is larviparous (as defined by Meier *et al*. 1999).

Evidence of ovoviviparity versus viviparity requires observation of the absence or presence of a chorion at the time of larviposition, cast egg shells in the reproductive tract of the female, pharate maggots living freely (hatched) inside the brood chamber, and neonate maggots emerging from the female's ovipositor (Meier *et al*. 1999). Tachinids may be ovoviviparous, viviparous, or exhibit both reproductive habits. It is unknown which habit is used by *L. franki*. This chapter examines whether *L. franki* is ovoviviparous, viviparous, or both.

## Methods and Materials

### Data Collection

Data were gathered from March 2009 to October 2009 from flies from 2 colonies, one at the Hayslip Biological Control Research and Containment Laboratory at the Indian River Research and Education Center in Ft. Pierce, Florida and the other at the Entomology and Nematology Department at the University of Florida in Gainesville, Florida.

The median oviducts of gravid females 8 to 15 days post-mated were examined for the presence of pharate maggots and/or egg castings left over from hatching. The presence of either would be solid evidence supporting viviparity. The absence of these, particularly after a large number of observations has been made, can be used to support ovoviviparity.

Changes that occurred in the reproductive tract of mated females over time since mating were monitored. This was of interest because some apparently ovoviviparous

species can become viviparous if no hosts are available for parasitism (Stireman *et al*. 2006).

**Observation of Female *L. franki* Flies from the Gainesville Colony**

The fly colony in Gainesville was supported from puparia collected from the Ft. Pierce colony. Puparia were collected on 5 occasions. The populations from each collection were discrete and topped out at 10 to 40 flies. The reproductive tract, eggs, and maggots were examined as the female flies died and were dissected or when the flies were dissected as part of an experiment. Because the fly populations were discreet and small, time since mating could be estimated for dissected flies. Depending on the experiment being conducted, the dissected flies may or may not have been given host weevil larvae to parasitize.

**From the Ft. Pierce Colony: Isolated Female *L. franki* Flies after Mating**

Adult flies emerging from puparia collected from the Ft. Pierce fly colony were collected over a period of days, sexed, and isolated in 60 cm × 60 cm × 64 cm cages. *Lixadmontia franki* mates within 48 hours after emergence (Suazo *et al*. 2008). Males were removed 2 days after the last calculated mating. The female flies remained in the cages. No host larvae were provided to the females for larviposition. When the flies were in a range of 8 to 15 days since mating, they were dissected and the reproductive tract and condition of the eggs and maggots were examined. Living maggots were used for artificially larvipositing host weevil larvae for other experiments.

Flies were collected and isolated on 3 occasions. On the 1$^{st}$ occasion, approximately 40 female and 40 male flies were collected and kept in 2 cages. The females were dissected when they were in a range of 8 to 12 days after mating.

On the 2nd occasion, approximately 40 female and 40 male flies were collected over a period of 7 days and kept in 2 cages. Females were dissected in the range of 8 to 11 days and from 12 to 14 days after mating.

On the 3rd occasion, approximately 28 female and 28 male flies were collected over 8 days and kept in 4 cages. The females were dissected in the ranges of 8 to 9 days, 10 to 11 days, 12 to 13 days, and 14 to 15 days after mating.

## Results

The average number of pharate maggots per female (including all dissected females) was 16 with a range of 5 to 75. From the Gainesville colony, 80 females were dissected and 67 had pharate maggots. From the Ft. Pierce colony, 64 females were dissected and 35 had pharate maggots. More than 2,000 pharate maggots were observed in the reproductive tracts of dissected females and all of them were encased in a chorion (Figure 6-1).

Those flies that were less than 12 days post-mated had eggs and maggots neatly aligned in the brood chamber in order of development (Figure 6-2). The posterior end of the brood chamber was uncluttered. Occasionally, 1 to 5 degraded eggs and/or dead maggots were found.

Those females that were 12 days or more post-mated had higher numbers of degraded eggs and dead maggots, usually chaotically packed (Figure 6-3). The posterior end of the brood chamber was packed with degraded eggs (Figure 6-4). The dead maggots were all encased in a chorion (Figure 6-5).

Females that larviposited, as well as females that were deprived of host weevil larvae, showed an increase in degraded eggs, dead maggots, and chaotic packing as time since mated increased.

## Discussion

Over 2,000 pharate maggots were examined in dissected gravid *L. franki* and all of them were encased in a chorion (Figure 6-1). The number of dead maggots and degraded eggs in a female's reproductive tract increased as the time since mating increased (Figures 6-2 and 6-3). Observations indicated that these were dead eggs and not cast egg shells because the interior of the eggs had substance and the shells appeared intact (Figure 6-4). It is unknown whether this increase in degraded eggs and dead maggots is the natural progression of embryo and maggot development for this species or is a result of inbreeding in the fly colony.

Pharate 1$^{st}$ instars in dissected brood chambers were observed to maneuver around living and dead maggots and developing and degraded eggs without attempting to break out of the chorion. Observations were shortened, however, because the maggots were being used for artificial larviposition and could not be overly stressed.

Because no hatched maggots or egg castings were found in the reproductive tract of *L. franki*, evidence supports the argument that the fly is ovoviviparous, not viviparous. This argument is further supported by the observed behavior of the maggots in the brood chamber. Further research should include observation of pharate maggots in neatly dissected, intact brood chambers as well as natural larviposition by living flies.

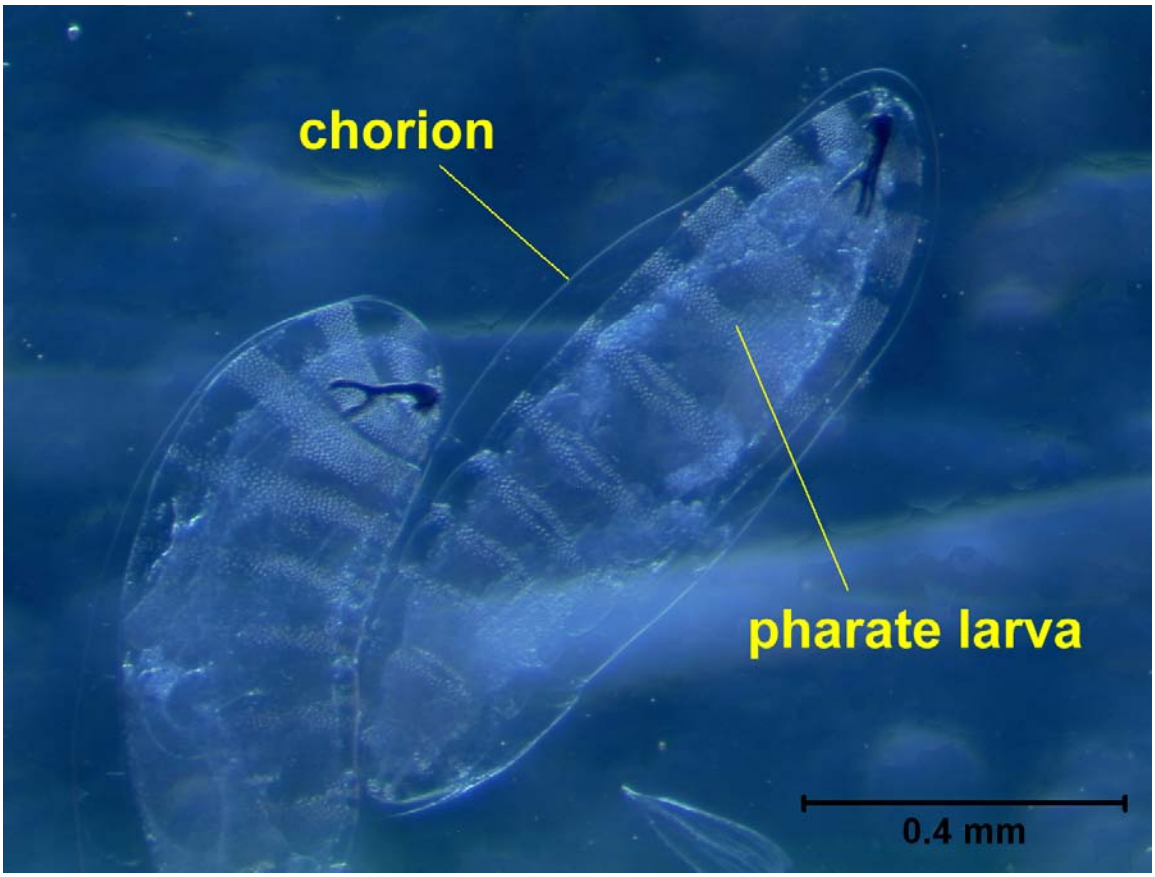



Figure 6-1. First instar maggots in chorion, dissected from female *L. franki*.

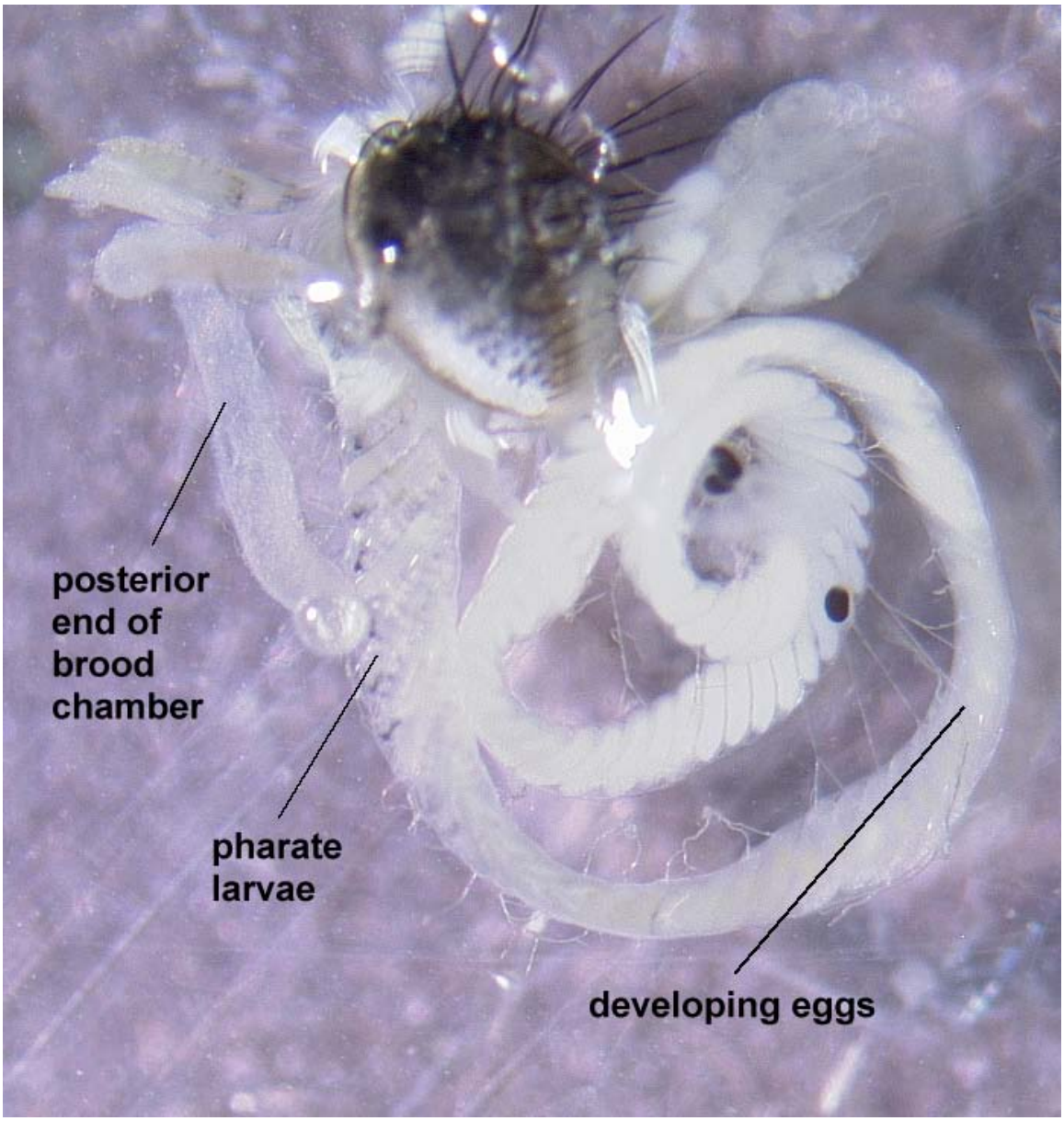



Figure 6 -2.  Brood chamber of female L. franki less than 12 days after mating.

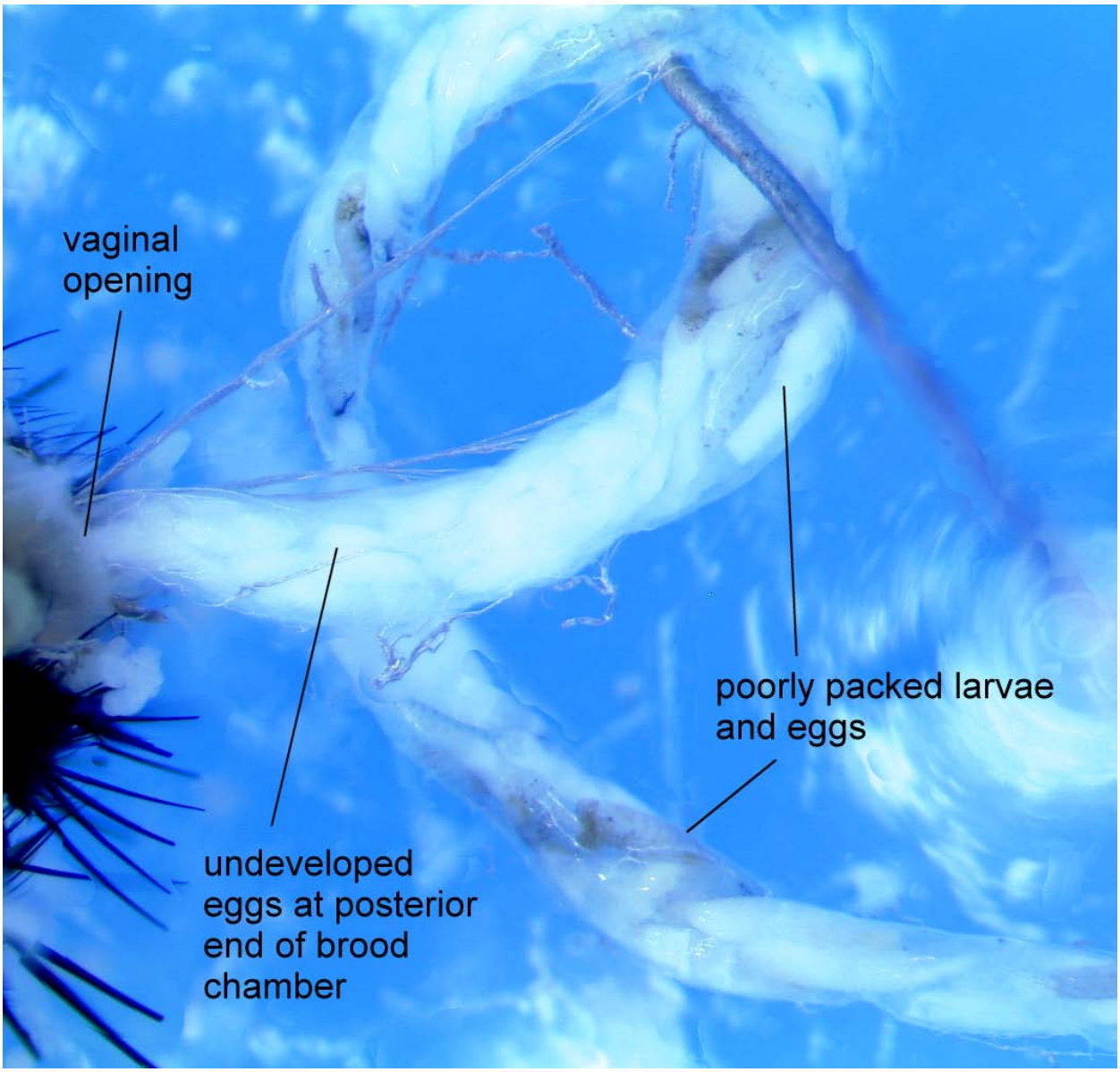



Figure 6-3. Brood chamber of female *L. franki* more than 12 days after mating.

Figure 6-4. Close-up of degraded eggs at posterior end of *L. franki* brood chamber at more than 12 days post-mated.

Figure 6-5. Dead *L. franki* pharate larva in chorion.

# CHAPTER 7
# CONCLUSIONS

## *Metamasius callizona* Infestation on a *T. utriculata* Population

*Tillandsia utriculata* is the bromeliad species in Florida that is most susceptible to being attacked by *M. callizona* (Frank and Cave 2005; Cooper 2006). In the Enchanted Forest Sanctuary, *M. callizona* reduced a large *T. utriculata* population by 87% in 6 months and by 97.4% in 2 years (Chapter 2). This was more rapid and severe than expected, probably because the *T. utriculata* population was large, dense, and contiguous and because *T. utriculata* was the only available host plant for *M. callizona*.

*Metamasius callizona* was seasonal on the *T. utriculata* population with activity happening from March to August and peaking in June. This is contrary to *T. fasciculata*, which supports an aseasonal *M. callizona* population (Cooper 2008). As well, *T. utriculata* supports (when in season) an epidemic weevil infestation, whereas *T. fasciculata* maintains a more endemic population. Seasonality became less pronounced as the weevil infestation progressed and the bromeliad population declined. Releases of a biological control agent, monitoring strategies, and analyses should consider the size, density, and continuity of a bromeliad population as well as the number and type of host bromeliad species and the stage of the infestation.

## *Lixadmontia franki* Releases and Post-release Monitoring

The experimental releases and post-monitoring at Lake Rogers Park, the Enchanted Forest Sanctuary, Loxahatchee National Wildlife Refuge, and Big Cypress National Preserve resulted in only a single recovery of 2 F2 generation flies (Chapter 3). This recovery was from the 1$^{st}$ release, at Lake Rogers. Sentinel plants infested with host weevil larvae were placed in the field to capture F2 flies. The lack of captures,

other than at Lake Rogers, may be because no other flies survived to parasitize the sentinel weevil larvae, or flies did survive but either did not parasitize sentinel weevil larvae or did but the parasitism went unnoticed.

Failure of a biological control agent may be caused by an inappropriate climate (Samways 1989; Goolsby *et al*. 2005) or the absence or reduction of a nectar source (Walker *et al*. 1996; Wäckers 2004). Both of these may apply to *L. franki*, particularly the first mentioned cause. *Lixadmontia franki* comes from cloud forests in Honduras, where the climate is much cooler and more humid than in Florida (Frank and Cave 2005). Very little is known about *L. franki*'s preferred nectar sources.

If F2 flies did survive, they may have failed to parasitize sentinel weevil larvae because the traps were less attractive to the fly compared to wild, infested bromeliads, or because the traps were disadvantageously situated. If F2 flies did survive and larviposit on the sentinel pineapple tops, it may have gone unnoticed if the maggots failed to find and parasitize a host weevil in the plant or failed to survive after parasitism.

**Can *L. franki* Assess Host Density?**

Tachinids often use chemical cues derived from accumulated chewed host plant tissue and/or host frass to detect larvipositional sites and to induce larviposition (Roth *et al*. 1982; Kainoh *et al*. 1999; Stireman 2002; Stireman *et al*. 2006). Suazo *et al*. (2006) showed that *L. franki* requires the presence of chewed bromeliad tissue and the host weevil larva in order to induce larviposition. Can *L. franki* use these volatiles to assess host density? Data show that as the amount of weevil-chewed pineapple/frass increased per experimental unit, so did the total number of maggots larviposited by *L. franki* flies (based on the number of maggots found in parasitized host weevil larvae; chapter 4).

Further research is needed to identify and isolate the chemicals in the weevil-chewed pineapple/frass mix that attract *L. franki*, that induce the fly to larviposit, and to understand the choices the fly makes when confronted with variables such as species of host bromeliad and the density and stage of the infestation. Such knowledge would be useful in designing better traps for monitoring the fly in the field and for designing and doing laboratory research that demands observation of the fly's natural larvipositing behavior.

## Larval Stages of *L. franki*

*Lixadmontia franki* has 3 instars that are easily recognized by the size and structure of the body, and the size and shape of the mouth hooks and cephalopharyngeal skeleton (Chapter 5). Early 1$^{st}$ instars and 2$^{nd}$ instars before emerging were not attached to host tracheae. Late 1$^{st}$ instars, 2$^{nd}$ instars, and early 3$^{rd}$ instars were attached to host tracheae, usually near the host larva's anterior or the posterior spiracle, though some were found centrally attached. When 1$^{st}$ and 2$^{nd}$ instars molted, the exuviae remained attached to the respiratory funnel. Only the 3$^{rd}$ instar had anterior spiracles.

## Ovoviviparity versus Viviparity

More than 2,000 pharate larvae were examined in dissected gravid *L. franki* and all were enclosed in a chorion which supports the argument that *L. franki* is ovoviviparous but not viviparous (chapter 6). This research does not definitively determine the answer to this question. Further research should include observing flies in the act of larvipositing and recording the event with enough resolution to determine the presence or absence and/or shedding of the chorion as pharate maggots emerge from the parent.

As gravid females aged, the number of dead pharate larvae and degraded eggs increased and the packing of the larvae and eggs in the brood chamber became more chaotic. The posterior portion of the brood chamber was plugged with undeveloped eggs. It is unknown whether these changing conditions are the natural progression of egg and maggot development for this species or if it is a result of inbreeding in the fly colony.

### How Useful is *L. franki* Likely to be as a Biological Control Agent?

*Lixadmontia franki* has not fulfilled its expected potential as a biological control agent. After releasing more than 2000 flies spread over 4 locations for each season of the year, there was only a single recovery of 2 F2 flies. This indicates that *L. franki* can survive in Florida. However, none of the weevil infestations showed any signs of being affected by the presence of the parasitoid. If *L. franki* did survive for 2 or more generations at any of the release sites, it was not effective in regulating the weevil.

Post-mated females had an increase in the number of dead pharate maggots and degraded eggs in the brood chamber as the time since mating increased. Fertility is likely negatively affected by the deaths of pharate maggots and the degradation of eggs as well as the plugging that occurs at the posterior end of the brood chamber. The plugging may inhibit viable maggots from being larviposited. This would likely limit *L. franki*'s ability as a biological control agent.

*Lixadmontia franki* should continue to be considered as a potential biological control agent and releases should continue to be made. *Lixadmontia franki* is the only candidate biological control agent presently available and the potential losses from this biological invasion are too great to ignore the only possibility we have at present for controlling the weevil. Biological control agents can take several years before

establishment happens (Grevstad 1999) and *L. franki* may eventually become established. If not, there is the possibility that the fly may be used as augmentative control in suitable habitats or seasons. Searches continue to be made for alternative biological control agents to control the weevil (Frank and Cave 2005). Information gained from studying *L. franki* will be useful in understanding other parasitoids or regulatory agents that may be found.

If experimental releases and post-monitoring are continued, it is strongly recommended that the flies are released where weevil activity is high (rather than repeated at a location) and that better traps (lighter and more attractive to *L. franki* flies) are designed and used.

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