Biological Control 59 (2011) 319–325

Contents lists available at SciVerse ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Release and monitoring of a potential biological control agent, *Lixadmontia franki*, to control an invasive bromeliad-eating weevil, *Metamasius callizona*, in Florida

T.M. Cooper [a,*], J.H. Frank [a], R.D. Cave [b], M.S. Burton [b], J.S. Dawson [b], B.W. Smith [b]

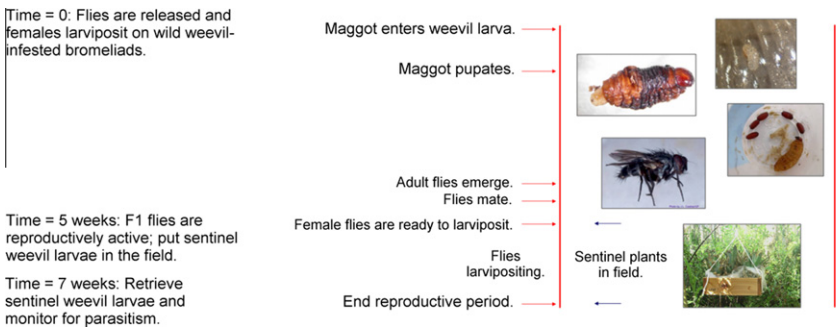
[a] Entomology and Nematology Dept., University of Florida, Bldg. 970, Natural Area Drive, Gainesville, FL 32611-0620, USA
[b] Indian River Research and Education Center, University of Florida, 2199 S. Rock Road, Ft. Pierce, FL 34945-3138, USA

HIGHLIGHTS

- A tachinid fly was released in Florida to control a bromeliad-eating weevil.
- Twenty-four releases were made at four sites, six per site and covering all seasons.
- Post-release monitoring recovered F2 flies after the first release.
- Lack of other recoveries may be for ecological or methodological reasons.
- Suggestions to improve chances of an F2 recovery are discussed.

GRAPHICAL ABSTRACT

Lixadmontia franki: post-release monitoring using sentinel weevil larvae in pineapple tops.



ARTICLE INFO

Article history:
Received 15 January 2010
Accepted 18 August 2011
Available online 28 August 2011

Keywords:
Lixadmontia franki
Metamasius callizona
Tillandsia
Biological control
Host plant–herbivore–parasitoid interactions

ABSTRACT

Metamasius callizona is an invasive bromeliad-eating weevil that has been destroying native bromeliads in Florida. A potential biological control agent, *Lixadmontia franki*, was released at four sites on six occasions. 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. Monitoring following the first release resulted in an F2 fly recovery which demonstrated that *L. franki* is capable of surviving and reproducing in Florida. Since then, no other recoveries have been made. 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 differences between the fly's native range and Florida or limited nectar sources may have limited the fly's ability to survive. Poorly located and/or too few traps or traps with less attractiveness than wild bromeliads may have caused a failure to capture flies that did survive. The loss of sentinel weevil larvae may have resulted in the loss of parasitized larvae. Recommendations are given for future release and monitoring methods.

1. 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 one 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

* Corresponding author. Fax: +1 352 392 0190.
E-mail address: tmcooper@ufl.edu (T.M. Cooper).

1049-9644/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2011.08.005

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). *L. 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 four 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 describes a method for releasing and post-release monitoring of *L. franki* and provides recommendations for release and monitoring.

2. Materials and methods

2.1. Insects and plants

M. 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). *M. 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. *T. 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. *M. 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). *L. franki*, a parasitoid of a related bromeliad-eating weevil, *M. quadrilineatus*, was discovered in Honduras in 1993 (Cave, 1997; Wood and Cave, 2006). *L. franki* was shown to parasitize *M. callizona* at least as readily as it will parasitize its native host (Frank and Cave, 2005). Florida has one 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 2–4 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 5–6 weeks.

2.2. 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. Fig. 1 is a map that shows the general locations and gives the latitudes and longitudes for the four 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 one 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 *Tillandsia fasciculata* and *T. balbisiana*. *T. fasciculata* was the predominant species. Both species grew on the trunks and upper branches of the trees. *M. 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 75–80% 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*. *M. callizona* was first seen at the Enchanted Forest in August 2006 (Frank, 1996). *T. 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 (Cooper, 2009). 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*. *T. 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 the water line, and up into the canopy. The weevil was first seen in Big Cypress in February 2005 (Frank, 1996).

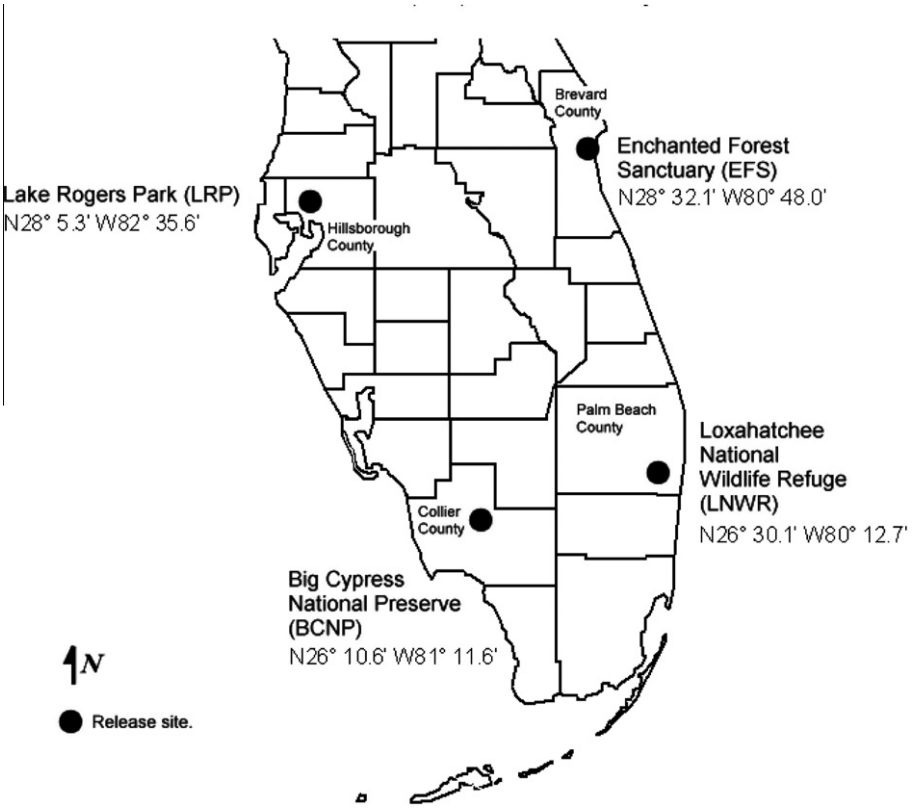


Fig. 1. Map of *L. franki* release sites in Florida: Lake Rogers Park (Hillsborough County), Enchanted Forest Sanctuary (Brevard County), Loxahatchee National Wildlife Refuge (Palm Beach County), and Big Cypress National Preserve (Collier County).

2.3. 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). *M. callizona* larvae growing in pineapple tops 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 afterward.

L. franki was released at four sites on six occasions. The first five rounds of releases were made from June 2007 to September 2008 and the 6th 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 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.

2.4. Monitoring

About 5 weeks after a release, sentinel pineapple tops infested with weevil larvae were put out in the field and retrieved at about 7 weeks. We used sentinel weevil larvae (rather than collecting wild weevil larvae) to monitor establishment of *L. franki* because we did not want to alter the weevil populations at the release sites. Also, weevil larvae in the field are difficult to find in adequate numbers for meaningful analysis. We also did not want to destroy live bromeliads while looking for wild weevil larvae. Pineapple tops were used as the sentinel plant because they are a readily-available, suitable host plant for *M. callizona* (Salas and Frank, 2001) and *L. franki* since we have been using pineapple tops successfully to grow weevils and flies in the laboratory for many years.

Six pineapple tops each were placed in 0.6 × 0.6 × 0.1 m cedar boxes with mesh bottoms. The pineapple tops were inoculated with two to three weevil larvae per 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 3rd instars when the pineapple tops 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 2 shows the dates that the sentinel pineapple tops were placed in the field then retrieved following the first five 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 sentinel pineapple tops 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 4 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. Table 2

Table 1
Dates and numbers of female and male *L. franki* released at four sites in Florida.

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			1198	1081
Average			50	45
Range			22–84	19–80

shows the dates that sentinel pineapple tops were placed in the field then retrieved following the 6th round of releases. The recovery rate was not calculated for this set.

The sentinel pineapple tops 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 gravid F1 females to the sentinel pineapple tops in the cedar boxes; the females would deposit neonate maggots (F2 generation) which would then locate and parasitize sentinel weevil larvae inside the pineapple tops. The cedar boxes with the sentinel pineapple tops 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 pineapple tops. When possible, the cedar boxes were hung near wild bromeliads (infested with the weevil or not). The cedar boxes were suspended from the same location for each monitoring episode.

The sentinel pineapple tops 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 20 mm × 60 mm Petri dishes on pineapple leaves. Parasitism was determined by the emergence of a maggot from a sentinel weevil larva.

3. Results

3.1. Fly releases

In total, 2279 flies were released, 1198 females and 1081 males (Table 1). The average number of flies released per release was 50 females (range 22–84) and 45 males (range 19–80). Analysis of variance showed no difference between the number of flies released at the four release sites and, for each site, similar numbers of females and males were released ($\alpha = 0.05$; P-value = 0.455). Greatest variance in the number of released flies was at Lake Rogers and the least variance was at the Enchanted Forest. Analysis of variance showed a difference between the number of flies released per season ($\alpha = 0.05$; P-value = 0.032). Tukey's method of multiple comparisons show that statistically similar numbers of flies were released in the summer, fall, and winter and in the summer, winter, and spring but 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.

3.2. 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 sentinel pineapple top with the parasitized weevil larva was in a cedar box that was suspended near the large *T. fasciculata* brome-

Table 2
Dates and numbers of sentinel *M. callizona* larvae placed in the field and recovered and percent weevil recovery following releases of *L. franki* at for sites in Florida from June 2007 to May 2008.

Location	Release #	Date traps out	Date traps retrieved	# Weevils out	# Weevils recovered	% Weevil recovery
Lake Rogers Park	1	7 Aug 07	21 Aug 07	96	94	98
	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
	6	5 May 09	19 May 09	–	–	–
	Total			522	497	95
Loxahatchee National Wildlife Refuge	1	30 Aug 07	13 Sep 07	72	68	94
	2	20 Nov 07	5 Dec 07	51	42	82
	3	12 Feb 08	27 Feb 08	48	34	71
	4	19 May 08	2 Jun 08	144	138	96
	5	21 Aug 08	4 Sep 08	144	141	98
	6	26 May 09	9 Jun 09	–	–	–
	Total			459	423	92
Enchanted Forest Sanctuary	1	9 Sep 07	23 Sep 07	144	138	96
	2	2 Dec 07	16 Dec 07	120	118	98
	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
	6	8 Jul	22 Jul	–	–	–
	Total			617	584	95
Big Cypress National Preserve	1	2 Oct 07	23 Oct 07	96	85	88
	2	19 Dec 07	14 Jan 08	96	71	74
	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
	6	6 Jun 09	30 Jun 09	–	–	–
	Total			572	485	85
Grand total				2170	1989	92

liad 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 sentinel pineapple tops 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 2). Statistically, there was no difference in the number of sentinel weevil larvae put out in the field and retrieved for the four release sites ($\alpha = 0.05$; P-value = 0.582) or for the seasons ($\alpha = 0.05$; P-value = 0.369).

4. 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. *L. 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 40–164 flies (Table 1) with a 50:50 female to male ratio. Seasonal availability of the fly was nearly consistent, with slightly fewer flies available in the spring months compared to the fall.

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 3–5 maggots are artificially larviposited on pineapple mash with a 3rd instar weevil (Cooper, 2009). 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 et al., 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). Though the flies readily parasitize *M. callizona* larvae in pineapple tops in a cage in the laboratory, it is unknown how close a fly must be to notice infested pineapple tops, or if the fly has a greater preference for other species of infested bromeliads. The monitoring method in this study could have failed because the sentinel pineapple tops were not advantageously situated or because the sentinel pineapple tops were less attractive to the fly compared to wild, infested bromeliads.

Only eight cedar boxes (with a total of 48 sentinel pineapple tops) were used around each release site following each release, thus limiting the area that could be monitored. In the Enchanted Forest and Loxahatchee, the sentinel pineapple tops were hung distantly from the wild bromeliads because the bromeliads were located high in the canopy. F1 flies may have ignored the pineapple tops 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 sentinel pineapple top 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 sentinel pineapple tops 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 cedar boxes 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 sentinel pineapple tops 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 sentinel pineapple tops 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 2). Increased time in the field increases the chance that evidence of a captured fly may become lost or destroyed.

The sentinel pineapple tops were recovered from the field after 2–3 weeks intact and in good condition. Weevil larvae were recovered at 92% (Table 2). The recovery rate was similar for the release sites and the seasons. 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 three to four 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 three to four 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 identifying and understanding the cues that attract the fly and in trap design. Trap design may or may not use weevil larvae, but if the larvae are used as sentinel organisms, then the trap design should include a mechanism for separating the weevil larvae.
5. Keep the traps in the field for a week (or less) and use several sets of traps for 5–10 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. *L. 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.

Acknowledgments

We thank our supporters, the South Florida Water Management District and the Florida Council of Bromeliad Societies. We also thank the biologist, rangers, and volunteers at Lake Rogers Park, the Enchanted Forest Sanctuary, Loxahatchee National Wildlife Refuge, and Big Cypress Natural Preserve for their help with this project.

References

Bloem, S., Hight, S.D., Carpenter, J.E., Bloem, K.A., 2005. Development of the most effective trap to monitor the presence of the cactus moth *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Florida Entomologist 88, 300–306.

Cave, R.D., 1997. *Admontia* sp., a potential biological control agent of *Metamasius callizona*. Journal of the Bromeliad Society 47, 244–249.

Cave, R.D., 2008. Biological control of the Mexican bromeliad weevil. Biocontrol News and Information 29, 1N–2N.

Cave, R.D., Duetting, P.S., Creel, O.R., Branch, C.L., 2006. Biology of *Metamasius mosieri* (Coleoptera: Dryophthoridae), with a description of larval and pupal stages. Annals of the Entomological Society of America 99, 1146–1153.

Chu, C.C., Ciomperlik, M.A., Chang, N.T., Richards, M., Henneberry, T.J., 2006. Developing and evaluating traps for monitoring *Scirtothrips dorsalis* (Thysanoptera: Thripidae). Florida Entomologist 89, 47–55.

Cooper, T.M, 2009. 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. [Doctoral dissertation]. [Gainesville (FL)]: University of Florida. pp. 104.

Frank, J.H., 1996. Bromeliad biota: History of *Metamasius callizona* in Florida [online]. Gainesville (FL): University of Florida [cited 2010 Jan. 01]. Available from: <http://www.entnemdept.ufl.edu/frank/bromeliadbiota/wvbrom6.htm>.

Frank, J.H., 1983. Bromeliad phytotelmata and their biota, especially mosquitoes. In: Frank, J.H., Lounibos, L.P. (Eds.), Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities. Plexus, Medford, (NJ), pp. 101–128.

Frank, J.H., Cave, R.D., 2005. *Metamasius callizona* is destroying Florida's native bromeliads. In: Hoddle MS, editor. USDA Forest Service publication FHTET-2005-08. Vol 1. Second International Symposium on Biological Control of

Arthropods; 2005 Sep12–16; Davos, Switzerland. Washington, DC: USDA Forest Service, pp. 91–101.
Frank, J.H., Fish, D., 2008. Potential biodiversity loss in Florida bromeliad phytotelmata due to *Metamasius callizona* (Coleoptera: Dryophthoridae), an invasive species. Florida Entomologist 91, 1–8.
Frank, J.H., Thomas, M.C., 1994. *Metamasius callizona* (Chevrolat) (Coleoptera: Curculionidae), an immigrant pest, destroys bromeliads in Florida. Canadian Entomologist 126, 673–682.
Goolsby, J.A., Jesudasan, R.W.A., Jourdan, H., Muthuraj, B., Bourne, A.S., Pemberton, R.W., 2005. Continental comparisons of the interaction between climate and the herbivorous mite, *Floracarus perrepae* (Acari: Eriophyidae). Florida Entomologist 88, 129–134.
Grevstad, F.S., 1999. Factors influencing the chance of population establishment: implications for release strategies in biological control. Ecological Applications 9, 1439–1447.
Hall, D.G., Hentz, M.G., Ciomperlik, M.A., 2007. A comparison of traps and stem tap sampling for monitoring adult Asian citrus psyllid (Hemiptera: Psyllidae) in citrus. Florida Entomologist 90, 327–333.
Manrique, V., Cuda, J.P., Overholt, W.A., Ewe, S.M.L., 2009. Influence of host-plant quality on the performance of *Episimus unguiculus*, a candidate biological control agent of Brazilian peppertree in Florida. BioControl 54, 475–484.
Meier, R., Kotrba, M., Ferrar, P., 1999. Ovoviviparity and viviparity in the Diptera. Biological Reviews 74, 199–258.
Salas, J., Frank, J.H., 2001. Development of *Metamasius callizona* (Coleoptera: Curculionidae) on pineapple stems. Florida Entomologist 84, 123–126.
Samways, M.J., 1989. Climate diagrams and biological control: an example from the areography of the ladybird *Chilocorus nigritus* (Fabricius, 1798) (Insecta, Coleoptera, Coccinellidae). Journal of Biogeography 16, 345–351.
Stephen, W.P., Rao, S., 2005. Unscented color traps for non-*Apis* bees (Hymenoptera: Apiformes). Journal of the Kansas Entomological Society 78, 373–380.
Stireman, J.O., O'Hara, J.E., Wood, M.D., 2006. Tachinidae: evolution, behavior, and ecology. Annual Review of Entomology 51, 525–555.
Suazo, A., Arismendi, N., Frank, J.H., Cave, R.D., 2006. Method for continuously rearing *Lixadmontia franki* (Diptera: Tachinidae), a potential biological control agent of *Metamasius callizona* (Coleoptera: Dryophthoridae). Florida Entomologist 89, 348–353.
Suazo, A., Cave, R.D., Frank, J.H., 2008. Reproductive biology and development of *Lixadmontia franki* (Diptera: Tachinidae), a parasitoid of bromeliad-eating weevils. Florida Entomologist 91, 453–459.
Wäckers, F.L., 2004. Assessing the suitability of flowering herbs as parasitoid food sources: flower attractiveness and nectar accessibility. Biological Control 29, 307–314.
Walker, T.J., Parkman, J.P., Frank, J.H., Schuster, D.J., 1996. Seasonality of *Ormia depleta* and limits to its spread. Biological Control 6, 378–383.
Wood, D.M., Cave, R.D., 2006. Description of a new genus and species of weevil parasitoid from Honduras (Diptera: Tachinidae). Florida Entomologist 89, 239–244.