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# METHOD FOR CONTINUOUSLY REARING *LIXADMONTIA FRANKI* (DIPTERA: TACHINIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF *METAMASIVUS CALLIZONA* (COLEOPTERA: DRYOPHTHORIDAE)

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## ABSTRACT

*Lixadmontia franki* Wood & Cave kills bromeliad-attacking weevils in the genus *Metamasius*. This parasitic fly is being investigated for its potential as a classical biological control agent of *M. callizona* (Chevrolat), which is devastating the native bromeliad flora in south Florida. A continuous rearing method was developed based on the fly's native host, *M. quadrilineatus* Champion, and a bromeliad species readily obtained from the field. Levels of parasitism of 3<sup>rd</sup> instars on slices of *Tillandsia standleyi* L. B. Smith stems were equivalent to those on intact plants. Larval feeding damage ( $\geq 3$  d) was necessary for successful parasitism. Average level of parasitism per 30 weevil larvae exposed to 42 female flies for 14 d was 85%. Production of *L. franki* puparia averaged  $22.0 \pm 12.2$  (SD) daily. Most (83%) final instars of the parasitoid exited their host 13-16 d after initial exposure to the flies. Weekly inputs of 210 weevil larvae and 56 fly puparia produced, on average, 154 puparia per week for a net production rate of 2.75.

Key Words: parasitic fly, rearing, bromeliad weevils, biological control, parasitism

## RESUMEN

*Lixadmontia franki* Wood & Cave ataca a los picudos del género *Metamasius* que viven en bromeliáceas. Esta mosca está siendo investigada por su potencial como un agente de control biológico clásico de *M. callizona* (Chevrolat), que está destruyendo las poblaciones de bromeliáceas nativas en el sur de Florida. Se desarrolló un sistema de producción continua usando el hospedero nativo, *M. quadrilineatus* Champion, de la mosca y una especie de bromeliácea fácilmente obtenida del campo. Niveles de parasitismo de larvas del tercer estadio del picudo en pedazos de tallo de *Tillandsia standleyi* L. B. Smith fueron equivalentes a los que se observaron en plantas enteras. Daño a la planta ( $\geq 3$  d) por la larva del picudo fue necesario para parasitismo exitoso. Los niveles promedios de parasitismo por 30 larvas de picudo expuestas a 42 moscas hembras por un periodo de 14 d fue de 85%. La producción diaria de puparios fue en promedio de  $22.0 \pm 12.2$  (DE). La mayoría (83%) de las larvas del parasitoide en el último estadio emergió de su hospedero 13-16 d después de la exposición inicial a las moscas. Ingresos semanales de 210 larvas del picudo y 56 puparios de la mosca produjeron, en promedio, 154 puparios por semana para una tasa neta de producción de 2.75.

Translation by authors.

The parasitic fly *Lixadmontia franki* Wood & Cave (Diptera: Tachinidae) was discovered in 1993 parasitizing larvae of a bromeliad weevil, *Metamasius quadrilineatus* Champion, collected from native bromeliads in cloud forests of Honduras (Cave 1997; Wood & Cave 2006). *Metamasius quadrilineatus* feeds primarily on bromeliads in the genera *Tillandsia*, *Catopsis*, and *Vriesea* growing at elevations of 1,600-2,000 m (Alvarez del Hierro & Cave 1999). In its natural habitat, *M. quadrilineatus* is not considered a pest due to the presence of natural enemies such as *L. franki*, predatory ants, and predatory beetles in the genus *Platynus* (Alvarez del Hierro & Cave 1999). Moreover, its larval development is apparently restricted to fallen bromeliads that are no longer

part of the breeding population. Its congener *Metamasius callizona* (Chevrolat) was first detected in Florida in 1989 (Frank & Thomas 1991, 1994; Frank 2000) and is now a serious pest. Since its arrival, *M. callizona* has spread through most of south Florida feeding on native species of bromeliads (Frank & Thomas 1991, 1994; Frank 2000). The effects of *M. callizona* on the south Florida bromeliad flora have been devastating and 12 of the region's 16 species are now threatened or endangered (Frank & Cave 2005). An effort to save these bromeliad species has been undertaken by organizations including the Florida Council of Bromeliad Societies and the Florida Park Service through the collection of seeds from these species to reproduce plants for future re-col-

onization of affected areas, and through research aimed at the implementation of classical biological control to suppress weevil populations.

The suitability and feasibility of *L. franki* for possible introduction into Florida to control *M. callizona* are currently being studied by the University of Florida in conjunction with the Pan-American School of Agriculture (Zamorano) in Honduras. We have observed that *L. franki* can parasitize larvae of *M. callizona* and, therefore, could be used as a classical biological control agent. However, lack of basic biological information and an efficient method to produce sufficient numbers of *L. franki* were limitations to implementing a *L. franki*-based biological control program for *M. callizona*. Large numbers of flies are needed for research efforts oriented towards better understanding the fly's biology and behavior. Therefore, we developed a continuous and cost-effective procedure to multiply *L. franki* under laboratory conditions as a first step towards the development of a classical biological control program for *M. callizona*.

#### MATERIALS AND METHODS

##### Stock Material from the Field

Larvae of *M. quadrilineatus* were collected from fallen bromeliads from three cloud forests near El Zamorano, Honduras: Cerro Uyuca (N14°00' W87°09', 1400-1800 m elevation), Cerro Monserrat (N13°56' W86°54', 1780 m elevation), and Cerro Apalagua (N14°02' W87°04', 1500 m elevation). Larvae were brought to the laboratory, placed in 30-ml plastic cups, and provided with portions of stems of *Tillandsia standleyi* L.B. Smith as needed. The source of the stems was fallen bromeliads in the same three cloud forests. Flies emerging from parasitized weevil larvae were used as stock material to start a fly colony in the laboratory. About 300 flies constituted the original stock material for initiation of the colony, but variable numbers of flies were added at irregular intervals throughout the rearing period.

##### Parasitism of *M. quadrilineatus* by *L. franki* in the Laboratory

All experiments were conducted in a room with a constant temperature of 21°C and 70% RH under natural light conditions (approximately 12:12 L:D) supplemented with overhead fluorescent lights from 0700 to 1600 h. In preliminary experiments, adult fly emergence from puparia misted twice daily with water from a hand sprayer was seven times higher (90%) than from puparia left dry (13%). Therefore, all puparia were misted at this rate afterwards. Before conducting experiments, newly-emerged flies, ten males and ten females, were placed in a screen cage (1.5 × 1.5 × 1.2

m) and left for 1 week to reach sexual maturity and mate under the same conditions of temperature light and humidity. All plants used for these experiments were healthy *T. standleyi* (free of mechanical or bacterial damage) collected from the same three cloud forests and checked to ensure no weevil larvae were present before use. Weevil larvae used in experiments were laboratory-reared and all were in the 3<sup>rd</sup> instar at the time of exposure to flies.

The first experiment was designed to investigate fly preference for weevil larvae feeding on intact plants versus excised stems. The treatments were as follow: (1) weevil larvae were placed individually in the stem of an intact plant by using a 15-cm nail to cut a hole (0.5 cm diameter, 1 cm deep) into the side of the base of the plant and gently inserting the larva into the hole; (2) weevil larvae were placed individually in bare stems (no leaves or roots) by using a nail to cut a hole (0.5 cm diameter, 1 cm deep) into one end of the stem and gently inserting the larva into the hole (Fig. 1). Twenty-eight weevil larvae in each treatment were exposed to >8-day-old flies for 7 d immediately after being put in the plant material. After this exposure period, weevil larvae were removed from the plants or stems and placed individually in 30-ml clear plastic cups fitted with screened lids. Larvae were fed portions (2 cm thick) of *T. standleyi* stem as needed (usually every 3-4 d) to ensure larvae always had healthy plant tissue on which to feed until parasitoid emergence or weevil pupation. Differences in proportions of parasitized and unparasitized weevil larvae in each treatment were analyzed with a 2



Fig. 1. Third-instar *Metamasius quadrilineatus* in a portion of *Tillandsia standleyi* stem in which a 0.5 cm diam. hole was bored to accommodate the insect.

$\times 2$  *G*-test of independence with Williams' correction (Sokal & Rohlf 1995).

A second experiment was designed to determine the influence of length of larval feeding time in stems on level of parasitism. Insects, plants, and cages were as described in the first experiment. Three treatments were tested for this experiment: (1) one third-instar *M. quadrilineatus* within a freshly excised portion of *T. standleyi* stem and immediately exposed to flies for 1 d; (2) one third-instar *M. quadrilineatus* allowed to feed inside a freshly excised portion of *T. standleyi* stem for 3 d and then exposed to flies for 1 d; (3) excised portion of *T. standleyi* stem left without weevil larva for 3 d, then one third-instar *M. quadrilineatus* placed in the stem and immediately exposed to flies for 1 d. Sections of stems began to decompose from the time they were excised. Cutting of holes may have hastened the rate of decomposition, and this may have been compounded by feeding by weevil larvae. Thirty larvae were exposed in each treatment. Each treatment was exposed to a different set of flies to avoid any effects of fly preconditioning or neonate larva depletion. After exposure to flies, weevil larvae were extracted from stems, placed individually in 30-ml clear plastic cups fitted with screened lids and provided food as needed until pupation or parasitoid emergence. Differences in proportions of parasitized and unparasitized weevil larvae in each treatment were analyzed with a  $3 \times 2$  *G*-test of independence with Williams' correction (Sokal & Rohlf 1995). Larvae lost during the experiment were not included in the analysis.

#### Continual-rearing Procedure for *L. franki*

Livestock for initiating the mass-rearing procedure originated from field-collected, parasitized *M. quadrilineatus* larvae provided with *T. standleyi* stems as described above until mature fly larvae emerged (Fig. 2A) and formed puparia (Fig. 2B). Eight newly formed puparia were placed in a Petri dish each day for 7 consecutive days. Puparia in each dish were sandwiched between two layers of moistened paper towel, which were misted twice daily to maintain the level of humidity required for successful fly emergence. This procedure was repeated weekly. Dishes with puparia were kept in a room maintained at 21°C with 70% RH. Two weeks after the end of each weekly collection of puparia, the Petri dish holding the puparia was introduced into a screened exposition cage (1.5 × 1.5 × 1.2 m) (Fig. 3) where adult flies emerged. The exposition cage was maintained under the same environmental conditions described for the puparia. Flies normally emerged 21 d after they pupated. Considering an average of 75% emergence and an adult fly life-time of 2 weeks, six flies on average were expected to emerge daily for a total of 42 in 1 week (21

males and 21 females assuming a 1:1 male:female sex ratio). Flies were provided with commercially available hummingbird instant nectar (Perky Pet Brand, Denver, CO) and water.

One week after first adult fly emergence, a tray containing 30 30-ml clear plastic cups, each with a 5-cm piece of *T. standleyi* stem containing one third-instar *M. quadrilineatus*, was placed in the exposition cage. A new tray of infested stems was then added daily and each tray was exposed to flies for 14 d. Fresh food was provided to larvae as needed. Portions of old food were left in the cups while the trays were inside the exposition cage, but were removed at the end of the exposition period.

After removal from the exposition cage, exposed weevil larvae were maintained under the same environmental conditions as the fly colony. Weevil larvae were provided fresh food as needed and checked for emergence of *L. franki* larvae or puparia every 3 d. To determine the efficacy of this rearing method, percent parasitism of weevil larvae and number of puparia recovered were recorded for three trays. Additionally, time in days from first day of weevil exposure to emergence of parasitoids was estimated.

#### RESULTS AND DISCUSSION

When exposed for 7 d, parasitism of *M. quadrilineatus* larvae feeding in intact plants (82%) was not different ( $G = 0.41$ ,  $\chi^2_{0.05[1]} = 3.84$ ) from larvae feeding in only a portion of the stem (75%). This is relevant because use of excised portions of stems for weevil feeding substantially reduces the number of plants used, and therefore, the labor needed to maintain the laboratory colony. A difference was detected between levels of parasitism of larvae in stems fed on for  $\geq 3$  d versus larvae in stems fed on for 1 day ( $G = 87.37$ ,  $\chi^2_{0.05[2]} = 5.99$ ). When weevil larvae were allowed to feed on the stem for  $\geq 3$  d and then exposed to the flies for 1 d, parasitism was 100%, which contrasts sharply with no parasitism of larvae in fresh stems and 8% parasitism of larvae in partially decomposed stems with only 1 day of feeding damage. This suggests that flies are possibly attracted to chemical cues produced by larval feeding; the accumulation of frass and/or masticated plant tissue may be required for the attraction to occur. Therefore, it is essential to allow weevil larvae to feed in plant stems for at least 3 d before exposing them to female *L. franki* to obtain a high level of parasitism.

An average of 6 adult flies emerged daily from this rearing system. In this manner, a stable population of approximately 42 male and 42 female flies could be maintained at all times by the regular addition of new puparia into the exposition cage, considering an average lifespan of 2 weeks for adult flies. Fly puparium production levels were consistent when a stable population of flies

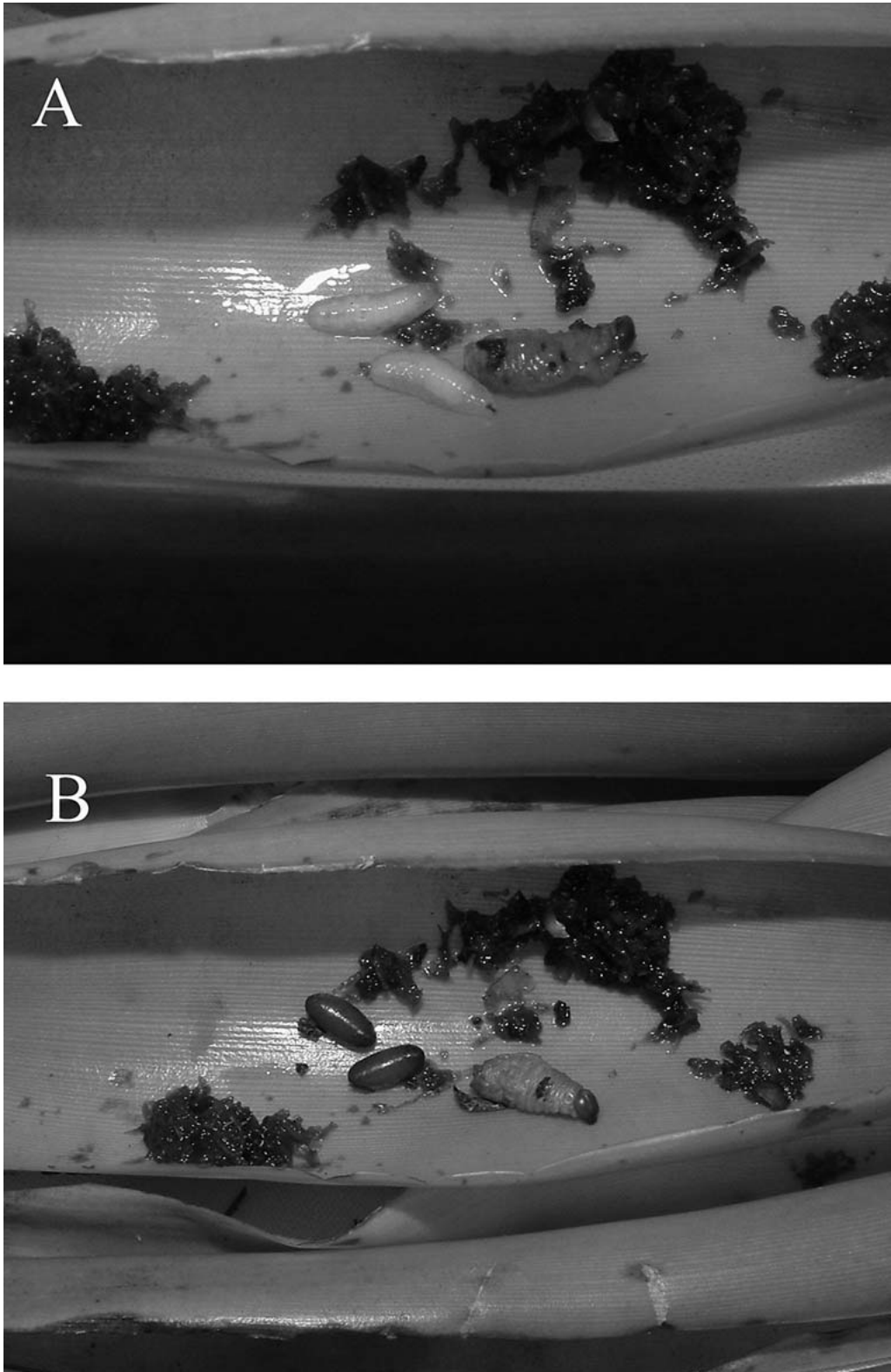


Fig. 2. Weevil carcass with mature larvae (A) and puparia (B) of *Lixadmontia franki*.



Fig. 3. Fly exposition cage holding rearing trays with 30-ml plastic cups containing a portion of *Tillandsia standleyi* stem infested with third-instar *Metamasius quadrilineatus* for parasitism. The Petri dishes containing puparia and cups with hummingbird food and water can be seen in the center of the cage.

was reached, averaging  $22 \pm 12.2$  fly puparia/day, or approximately 154 puparia weekly. Levels of parasitism averaged 85% (maximum = 90%, minimum = 75%) for 3 trays of 30 weevil larvae each. Emergence of mature fly larvae from hosts was observed as soon as 9 days and continued until 26 days after introduction into the exposition cage. However, approximately 83% of the mature fly larvae emerged 13-16 d after introduction of hosts into the exposition cage. This continuous production level is enough to supply flies for research and field releases.

Large-scale production of puparia with this rearing method is limited in Honduras by the reliance on weevil larvae and *T. standleyi* (or other suitable host plant) collected in the field. In order to establish a large-scale production system in Florida, this problem will need to be circumvented by the use of *M. callizona* larvae reared in pineapple crowns, which are available from some grocery stores. *Metamasius callizona* can be reared using pineapple crowns (Salas & Frank 2001), and a large-scale production system using pineapple crowns to mass-multiply this insect has

been developed. Fortunately, *L. franki* parasitizing *M. callizona* larvae in pineapple stems has been observed by R. D. Cave.

The method described here is effective for producing sufficient numbers of *L. franki* to facilitate further investigation of this natural enemy to control *M. callizona* in Florida. If *L. franki* is approved for release in the field, the techniques described herein can be modified as needed to increase the number of puparia produced to fit the needs. The development of an artificial diet for *M. callizona* would cut down the amount of labor and plant material used. Furthermore, the isolation and identification of fly attractants from stems with 3-d-old feeding damage may also prove useful for attracting and arresting the flies to the sites of interest. These are all areas that need further investigation.

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