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Metamasius callizona (Coleoptera: Curculionidae): fertility and larval survival to the third instar in the laboratory

Teresa M. Cooper^{1,*}, and Ronald D. Cave¹

Abstract

Metamasius callizona (Chevrolat) (Coleoptera: Curculionidae) is an invasive bromeliad-eating weevil destroying native bromeliad populations in Florida. We measured the weevil's fecundity and fertility with pineapple leaf pieces as food. Eighty percent of females that paired with males laid eggs. Average adult longevity for egg-layers was 210.6 ± 7.5 d (SE). Average adult longevity for non-egg-layers was 23.8 ± 3.8 d. Average pre-oviposition period was 26.6 ± 1.3 d. The average number of eggs per female per d laid inside the leaf was 0.4 ± 0.03 eggs, and laid outside the leaf was 0.1 ± 0.01 eggs. The number of eggs laid inside the leaf declined with age, whereas the number of eggs laid outside the leaf increased with age. Egg-laying females began dying on d 80, and continued to die regularly until 305 d after adult emergence. The hatch rate of eggs laid inside leaves was 79%. Daily hatch rate was consistently around 80%. Ninety-seven percent of the larvae that emerged from eggs laid inside leaves survived to the third instar. The hatch rate of eggs laid outside leaves was 3.9%, and no larvae survived beyond second instar.

Key Words: fecundity; bromeliads; weevil; egg hatch rate; invasive species

Resumen

Metamasius callizona (Chevrolat) (Coleoptera: Curculionidae) es un picudo invasivo que se alimenta de las bromeliáceas y está destruyendo las poblaciones nativas de bromeliáceas en Florida. Nosotros medimos la fecundidad y fertilidad del picudo con pedazos de hojas de piña como comida. Ochenta por ciento de las hembras apareadas con machos ovipositaron. El promedio de longevidad de las hembras que ovipositaron fue 210.6 ± 7.5 d. El promedio de longevidad de las hembras que no ovipositaron fue 23.8 ± 3.8 d. El promedio del período de pre-oviposición fue 26.6 ± 1.3 d. El promedio del número de huevos por hembra por d depositados dentro de la hoja fue 0.4 ± 0.03 huevos y los depositado fuera de la hoja fue 0.1 ± 0.01 huevos. La cantidad de huevos depositados dentro de la hoja disminuyó con edad, mientras que la cantidad de huevos depositados fuera de la hoja aumentó con edad. Las hembras que ovipositaron empezaron a morir en el d 80 y continuaron muriendo hasta 305 d después de eclosión del adulto. La tasa de eclosión de los huevos dentro de la hoja fue 79%. La tasa diaria de eclosión de los huevos fue consistentemente alrededor de 80%. Noventa y siete por ciento de las larvas que emergieron de los huevos dentro de la hoja sobrevivieron hasta el tercer estadio. La tasa de eclosión de los huevos fuera de la hoja fue 3.9% y ninguna larva pasó el segundo estadio.

Palabras Clave: fecundidad; bromeliáceas; picudo; tasa de eclosión de huevos; especie invasiva

Metamasius callizona (Chevrolat) (Coleoptera: Curculionidae) is an invasive bromeliad-eating weevil destroying native bromeliad populations in Florida (Frank & Thomas 1994; Frank & Cave 2005). The weevil was found established on native bromeliad populations in Broward County in 1989. Since then, it has spread to 22 counties (Frank 1996). Florida has 16 native species of bromeliads, and 12 of them, as well as 2 natural hybrids, are threatened by the weevil. The weevil also attacks cultivated and ornamental bromeliads that are commonly grown in Florida (Frank & Thomas 1994). All life stages of *M. callizona* live on the host bromeliad (Frank & Cave 2005). The weevil adult consumes leaf tissue, but the herbivory is not fatal to the host plant. Eggs are deposited in leaf tissue by females. The larva, which mines the meristematic tissue, kills the plant (Frank & Thomas 1994; Frank & Cave 2005). The weevil pupates in pupal chambers created by the fifth instar from chewed plant material. Mean generation time is 13 to 17 wk, and the insect is multivoltine (Salas & Frank 2001).

Frank et al. (2006) reported on the fecundity of *M. callizona*. The average adult longevity was 156 d. The average pre-oviposition period was 29 d. Females had an average lifetime production of 40 eggs, and the number of eggs per female per d did not decline with age. Fertility was not measured because the pineapple leaves with the eggs were placed under a dissecting scope, and poked or sliced or otherwise opened, exposing the eggs and resulting in their destruction. Since the publication of Frank et al. (2006), the method for rearing the weevil in the laboratory has transitioned from rearing weevils on pineapple tops in greenhouses and cages to rearing them on pieces of pineapple leaves in Petri dishes and vials (described in Materials and Methods, Weevil Rearing). By using this method, we were able to quantify fertility. This study was performed to complete the reproductive profile of *M. callizona* and to provide information that will be useful in developing bromeliad conservation methods, as well as laboratory and field studies.

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Materials and Methods

Environmentally controlled growth chambers set at 25 °C, 65% RH, and a 12:12 h (L:D) photoperiod were used to rear the weevil laboratory colony, and to do the fertility and fecundity experiments.

WEEVIL REARING

Weevils used in the experiments were taken from a laboratory colony maintained at the Hayslip Biological Control Research and Containment Laboratory at the Indian River Research and Education Center, University of Florida, Ft. Pierce, Florida, USA. Pineapple leaves were used to feed weevil larvae and adults, and as an oviposition substrate. Leaves were taken from pineapple tops freshly cut from the fruit; from each leaf an approximately 4 cm long piece was cut from the base of the leaf. The outermost leaves on the pineapple tops were not used because they are too small and tough, and therefore dry out quickly. The central leaves were not used because they are too thin and soft, and therefore tend to rot. Mated females were kept individually in 18.5 mL, clear plastic vials with white plastic snap caps with a slit cut in the middle. Twice a week, pineapple leaves were removed from the vials and replaced with fresh leaves. The leaves that were removed were checked for eggs by holding the leaf in front of a bright light. Females were given clean vials every 4 wk.

Leaf pieces with usually 1 egg, sometimes 2 or rarely 3, were placed individually in 20 × 60 mm, clear plastic Petri dishes with a piece of moistened paper towel, cut in squares approximately 7 × 7 cm that were folded and pressed against the side of the Petri dish. A fresh leaf piece was slipped under the leaf piece bearing an egg 3 to 4 d later. Eggs were checked for eclosion 3 to 4 d after the first fresh leaf piece was added (7–8 d after the egg was collected).

Neonate larvae mined the original leaf piece or the first fresh leaf piece. A second fresh leaf piece was slipped under the leaf piece that the larva was mining, and the leaf piece without a larva was discarded. If both leaves had a larva, a fresh leaf piece was placed underneath each leaf. One larva and its leaf pieces remained in the Petri dish, whereas the other larva and its leaf pieces were removed to a clean Petri dish with moist paper towel. If a leaf piece had 2 or more larvae, the leaf was carefully cut to separate the larvae without cutting into the channels mined by the larvae, and the larvae were placed in separate Petri dishes, each with a fresh leaf piece. For those eggs that eclosed (almost all eggs hatch in 8–10 d at 25 °C, 65% RH; Salas and Frank 2001), the emergent larvae were given a third leaf piece under the leaf piece with the larva, and the leaf piece without the larva was discarded. Fresh leaf pieces were added twice per wk by slipping them under the masticated leaf mash that was created by larval feeding. At 1 to 2 wk after egg hatch, the moistened paper towels were removed from the Petri dishes because the masticated leaf mash provided adequate humidity for the remainder of the larva's development. At pupation, pupae were removed from their pupal chambers, and placed individually in clean Petri dishes with moistened paper towel. The adults that emerged from these pupae were used in the experiments.

FECUNDITY

Adults from the laboratory colony were sexed the d they emerged from the pupa, and 60 mating pairs were placed in 18.5 mL, clear plastic vials with slit snap caps, and given a piece of pineapple leaf approximately 4 cm long, cut from the base of the leaf. Each pair was given a unique number. Date of adult emergence and pairing was recorded. The pineapple leaves were removed from the vials daily and checked for eggs by holding the leaf in front of a bright light. If there were no

eggs, the leaf was discarded, and the pair was given a fresh piece of pineapple leaf and returned to the growth chamber. If there was an egg in the leaf, the leaf was placed in a Petri dish to test for fertility (see Materials and Methods, Fertility and Larval Survival to the Third Instar), the male was returned to the weevil colony, and the female was given a fresh piece of pineapple leaf. Pairs and egg-laying females were given clean vials every 4 wk.

For each ovipositing female, the date of first egg produced was recorded. The pineapple leaf pieces were removed from the vials daily and replaced with fresh leaf pieces. The inside wall of the vials and the leaves that were removed were checked for eggs. Eggs on the wall of the vials were removed and destroyed. The only way to measure fertility of eggs laid on the wall of the vial was to remove everything but the egg from the vial and watch for egg hatch. This would have created variation in conditions among vials and introduce unnecessary variability in the experiment. The method for testing fertility of eggs laid outside of leaves is described in Materials and Methods, Fertility and Larval Survival to the Third Instar.

The percentage of paired females that became egg-layers was calculated, as well as means and standard deviations ($\alpha = 0.05$) for adult longevity, pre-oviposition period, and number of eggs laid inside and outside of leaves per female per d. These data were compared with fecundity data from Frank et al. (2006). The means and standard deviations ($\alpha = 0.05$) were calculated and plotted for the number of eggs laid inside and outside the leaf per female per d from 10 d to 230 d after the first egg was laid.

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Eggs Inside Leaf

All leaves with eggs that were collected from ovipositing females from the fecundity experiment were placed individually in 20 × 60 mm, clear plastic Petri dishes with a piece of moistened paper towel, cut in squares 7 × 7 cm that were folded and pressed against the side of the Petri dish. At 3 to 4 d, a fresh leaf piece was slipped under the egg-bearing leaf. Eggs were checked for larval eclosion 3 to 4 d after the fresh leaf piece was added. If the egg did not hatch, the leaf piece was removed, a fresh leaf piece was slipped under the egg-bearing leaf, and the Petri dish was returned to the growth chamber. This procedure was repeated until a larva emerged from an egg or an egg had been held for 18 d, well over the expected egg incubation time (Salas & Frank 2001).

Larvae that emerged from the eggs were given a fresh leaf piece under the leaf piece with the larva (as described in Weevil Rearing). Larvae were fed twice per wk until they died or reached the third instar (resource constraints prohibited monitoring to adulthood), at which time the larvae were returned to the laboratory colony. A larva was considered dead if it did not move when gently poked with a finger. Date of larval death or date when the larva reached the third instar was recorded.

Eggs Outside Leaf

To measure fertility of eggs laid outside the leaf, we took a random sample of 20 females from the egg-laying population in the laboratory colony. The females were given fresh leaves every 3 to 4 d, and clean vials every 4 wk; eggs in leaves were returned to the weevil colony. For 68 d, we checked daily for eggs on the wall, floor, and lid of each vial (we did not use eggs that were laid on the surface of the leaf because the leaf pieces would rot or desiccate during egg incubation). When an egg was detected, the female was removed, a small piece of moistened paper towel, approximately 2 cm², was added to the vial (without contacting the egg), and the vial was closed with a slit snap cap and

returned to the growth chamber. The eggs were monitored every 24 h for eclosion.

Larvae that emerged were given a fresh leaf piece under the leaf piece with the larva. The larvae were fed twice per wk until they died or reached the 3rd instar, at which time the larvae were returned to the laboratory colony. A larva was considered dead if it did not move when gently poked with a finger.

Data Analysis

Rates of egg eclosion per 10 d and confidence intervals ($\alpha = 0.05$) were calculated for eggs inside and outside leaves. Confidence intervals were calculated using Wilson score intervals (Wilson 1927). Means are reported with their standard deviation. Averages were calculated for the number of eggs females laid in the first half of their oviposition period, and the second half of their oviposition period for eggs laid inside + outside the leaves, inside the leaves, and outside the leaves. Means are reported with their confidence intervals. Percentage larval survival to the third instar was calculated for eggs inside and outside leaves.

Results

FECUNDITY

Among the 60 paired females, 80% laid at least 1 egg. Females that did not lay an egg lived on average 23.8 ± 13.1 d (SE), ranging from 5 to 48 d. Egg-laying females had an average longevity of 210.6 ± 51.7 d, ranging from 80 to 305 d. Average pre-oviposition period was 26.6 ± 8.9 d, ranging from 12 to 54 d. The average number of reproductive d (= number of days from first egg to death) was 184.4 ± 51.2 d, ranging from 54 to 283 d.

The average number of eggs per female per d laid inside the leaf was 0.4 ± 0.03 eggs, ranging from 0.1 to 0.8 eggs, whereas the average number laid outside the leaf was 0.12 ± 0.10 eggs, ranging from 0.0 to 0.4 eggs. The average number of eggs per female per d laid inside + outside the leaf was 0.48 ± 0.17 eggs, ranging from 0.09 to 0.88 eggs.

Oviposition rates of eggs laid inside leaves and outside leaves were fairly consistent for the first 120 d (Fig. 1; plotted only to d 230, because at this time there were too few remaining egg-laying females for

statistical analysis), and the average number of eggs laid inside leaves remained much higher than those laid outside leaves (consistently around 80% inside the leaf and 20% outside the leaf). The number of eggs laid inside leaves then began to gradually decline, and the number of eggs laid outside leaves began to increase. At d 160, the number of eggs laid inside leaves (50% of total eggs laid) was equal to the number of eggs laid outside leaves (50% of total eggs laid). By d 190, the number of eggs laid outside leaves (78% of total eggs laid) was significantly greater than those laid inside leaves (22% of total eggs laid).

Results from our study when we compared the number of eggs laid in the first half of the oviposition period compared to the number of eggs laid in the second half are shown in Figure 2. The average total number of eggs laid (inside and outside the leaves) in the first half (47.3 ; CI = 41.6 – 53.0) was similar to the total number of eggs laid during the second half (41.2 ; CI = 35.0 – 47.3). However, the average number of eggs laid inside leaves was much higher in the first half (43.2 ; CI = 36.7 – 49.6) than in the second half (22.2 ; CI = 16.8 – 27.7), and the average number of eggs laid outside leaves was much lower in the first half (4.1 ; CI = 1.8 – 6.3) than in the second half (18.9 ; CI = 14.5 – 23.4).

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For eggs laid inside leaves, average egg hatch rate per female was 79%. The egg hatch rate per d from initiation of oviposition to d 120 was consistently about 80% (Fig. 3). Fertility then gradually declined, increased to a peak on d 190, and then declined sharply. On d 230, fertility was 0% ($N = 4$ eggs). After d 230, too few eggs were collected for statistical analysis.

The total number of larvae that emerged from 4,131 eggs laid inside leaves was 2,496 (60.4% eclosion rate). Of these, 2,421 (97%) survived to the third instar. The total number of larvae that emerged from 132 eggs laid outside of leaves was 5 (3.8% eclosion rate). None of these larvae survived beyond the second instar.

Discussion

Our parameters for percentage egg-layers, longevity, and pre-oviposition period are similar to those reported by Frank et al. (2006): percentage egg-layers = 80.6%; egg-layer longevity = 156.4 ± 96.7 d, ranging from 26 to 387 d; and pre-oviposition period = 28.9 ± 17.8 d,

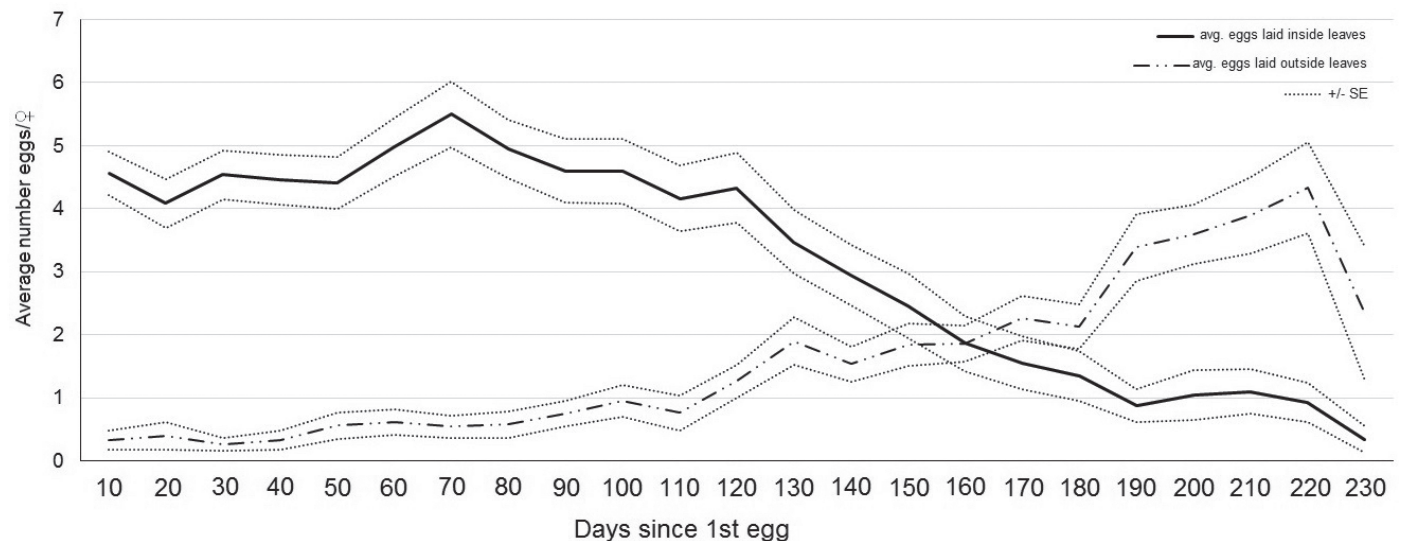


Fig. 1. Average number of eggs laid per leaf per *Metamasius callizona* female per 10 d (\pm SE) inside and outside leaves over time.

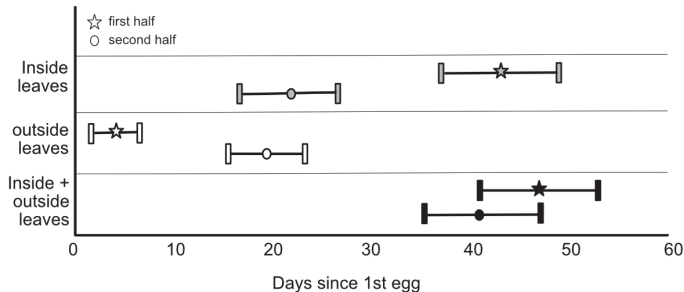


Fig. 2. Average number of eggs laid by *Metamasius callizona* inside, outside, and inside + outside the leaf by *Metamasius callizona*, with 95% confidence intervals for the first half and second half of the female reproductive periods.

ranging from 8 to 89 d. Frank et al. (2006) calculated an oviposition rate of 0.32 eggs per female per d by dividing the total number of eggs collected (inside and outside leaves = 2,973) by the total number of reproductive d (= number of d from first egg to death). Because Frank et al. (2006) did not calculate a standard deviation, a statistical comparison between our results cannot be inferred. However, the oviposition rate calculated by Frank et al. (2006) falls within 1 SD of the oviposition rate in our study. As well, both results are similar to the average oviposition rate calculated by Cooper and Cave (2016) for the weevil's optimal temperature range (0.40 ± 0.07 eggs per female per d at 22–33 °C).

Frank et al. (2006) compared the number of eggs laid during the initial and final halves of the female reproductive periods, with the midpoint defined as half the number of d from time of first egg laid to death (the midpoint d of females with an odd number of reproductive d was ignored to equalize both halves). They concluded that the oviposition rate did not decline with age. Results showed that about half of the females laid more eggs in the first half of their reproductive period, about half laid more eggs in the second half, and a few laid an equal number of eggs. Our results of number of eggs laid in the first half versus the second half of the oviposition period are consistent with the results in Frank et al. (2006).

In our study, oviposition rate does not decline with age, oviposition behavior does change with age, and more eggs are laid outside of leaves. As shown in the fertility and larval survival experiments, eggs laid outside of the leaf have extremely low percentage eclosion, and

the larvae do not survive beyond the second instar. It is unknown if the viability of the eggs diminishes as the females age or if the habit of laying the eggs outside of the leaves creates conditions that cause lower viability.

Metamasius callizona females are capable of consistently high oviposition rates throughout their lifetime, but eclosion and larval survival start to decline after a 4 mo period due to the females' habit of laying more eggs outside the leaf than inside the leaf (Figs. 1 & 3). Because the weevil requires about 2 mo to develop from egg to adult, plus another mo for a pre-oviposition period, overlapping generations of the weevil in the same deme may be ovipositing at the same time, thus greatly increasing the potential for weevil population growth.

How might our laboratory results be relevant in the field? A population of *Tillandsia utriculata* L. (Bromeliaceae) in its first or second yr of a weevil infestation was monitored in the Enchanted Forest Sanctuary, Titusville, Florida, USA, from Mar 2007 to Jun 2009. At the beginning of the study, there were an estimated 46,552 medium to very large *T. utriculata* in an area of 240,000 m². The weevil destroyed 87% of this *T. utriculata* population in the first 6 mo of the study (Cooper et al. 2014). At 27 mo, 97% of the population was destroyed; 99% bromeliad mortality was caused by the weevil (Cooper et al. 2014). The rapid and nearly complete destruction of the *T. utriculata* population in the Enchanted Forest was demonstrative of the fecundity and fertility capabilities measured in the laboratory.

The weevil shows variable demographics on Florida's bromeliads, including differences in the progression of a weevil infestation, percentage mortality of the bromeliads, and seasonality (Cooper 2006, Cooper 2008). The variability of the progression of a weevil infestation is likely driven by plant characteristics that affect fecundity, fertility, and survival. How likely is the female to oviposit on a species of bromeliad given the leaf texture, stem size, and fiber content? *Tillandsia utriculata* is a large, big-stemmed plant, whereas *Tillandsia fasciculata* Swartz (Bromeliaceae) is a large, small-stemmed plant; *T. utriculata* is less fibrous and contains more nutrients than *T. fasciculata* (Isley 1987, Cooper 2008). Therefore, it is not surprising that the weevil is able to rapidly increase its population on a large, dense *T. utriculata* population (Cooper et al. 2014), but does not do so on a large, dense *T. fasciculata* population (Cooper 2006). However, long-term observations indicate that the weevil can be as destructive to *T. fasciculata* populations as it is to *T. utriculata* populations, but it takes longer to

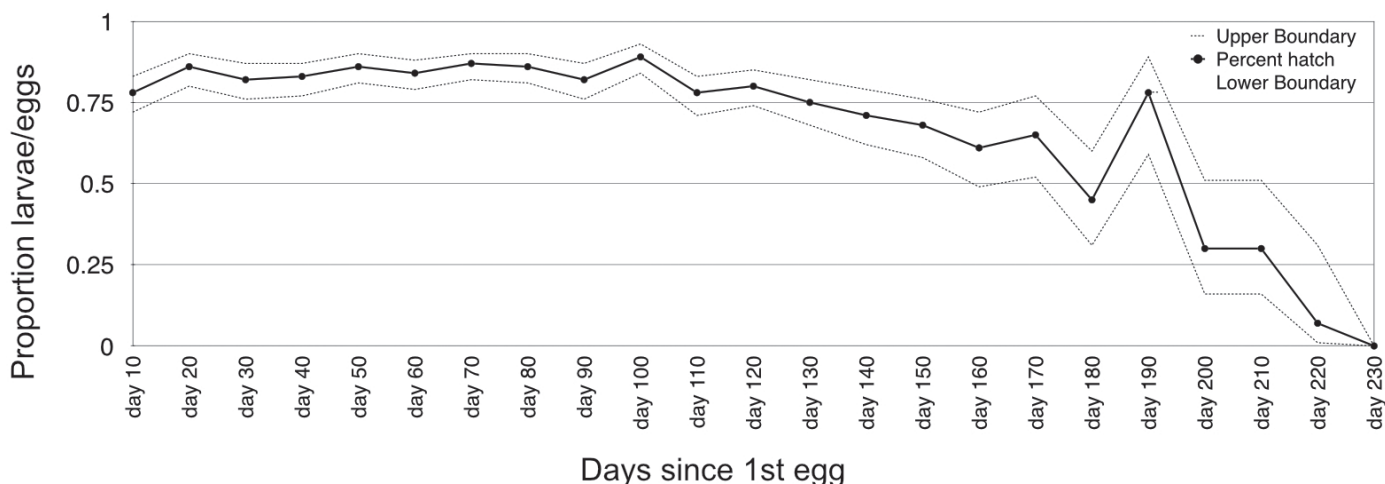


Fig. 3. Proportions and 95% confidence intervals for egg eclosion per 10 d from eggs laid inside leaves by *Metamasius callizona*. Confidence intervals were calculated using Wilson score intervals ($\alpha = 0.05$; Wilson 1927).

happen (decades rather than mo; unpublished data). More research is needed to better understand the weevil's reproductive behavior on Florida's 12 species of bromeliads, and how the host bromeliads and the conditions in which the bromeliads grow affect the weevil's realized fecundity and fertility.

Acknowledgments

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