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Source: Florida Entomologist, 96(3) : 859-863

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.096.0320

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PREDATION OF *GYNAIKOTHRIPS UZELI* (THYSANOPTERA: PHLAEOTHRIPIDAE) BY *ANDROTHRIPS RAMACHANDRAI* (THYSANOPTERA: PHLAEOTHRIPIDAE)

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ABSTRACT

The galling thrips *Gynaikothrips uzeli* Zimmermann (Phlaeothripidae) has attained pest status by attacking the leaves of the weeping fig, *Ficus benjamina*. Another thrips species, *Androthrips ramachandrai* Karny (Phlaeothripidae), was recently reported feeding on *G. uzeli* immatures. Here we conducted choice tests with adults of *A. ramachandrai* to evaluate any preference for the immature stages of *G. uzeli* (egg, larva or pupa) in artificial arenas. No-choice experiments were also conducted to measure the rate of feeding on each immature stage of the galling thrips by adults of *A. ramachandrai* in a period of 24 h. There was no significant preference by the predators for the different immature stages of *G. uzeli,* but adults consumed more eggs than larvae or pupae of the gall thrips. Here we discuss the predatory behavior of this thrips and its potential as a biological control agent against *G. uzeli*.

Key Words: Biological control, feeding behavior, *Ficus benjamina*, gall-inducing insects, Thysanoptera

RESUMO

O tripes galhador *Gynaikothrips uzeli* Zimmermann (Phlaeothripidae) adquiriu status de praga por atacar as folhas da figueira-benjamim, *Ficus benjamina*. Recentemente outro tripes, *Androthrips ramachandrai* Karny (Phlaeothripidae), foi registrado se alimentando de imaturos de *G. uzeli*. Nós conduzimos testes de escolha utilizando adultos de *A. ramachandrai* a fim de verificar se este possui preferência por algum estágio imaturo de *G. uzeli* (ovo, larva ou pupa) em arenas artificiais. Experimentos sem a opção de escolha também foram realizados para medir a taxa de consumo de cada estágio de *G. uzeli* por adultos de *A. ramachandrai* em um período de 24 h. Os testes de escolha não mostraram nenhuma preferência pelo predador por diferentes estágios imaturos do tripes galhador. Entretanto, os testes de taxa de consumo sem a chance de escolha indicaram que adultos de *A. ramachandrai* consumiram mais ovos do que larvas e pupas. Discutimos o comportamento predador deste tripes e o seu potencial como controlador biológico de *G. uzeli*.

Palavras Chave: Controle biológico, comportamento alimentar, *Ficus benjamina*, insetos galhadores, Thysanoptera

The genus *Gynaikothrips* (Phlaeothripidae) comprises about 40 species, mainly from the Oriental region (ThripsWiki 2013). They are all phytophagous thrips inducing leaf-fold and leaf-roll galls, where they breed and develop. Among them, 2 species have been referred to as important pests of decorative *Ficus* (Moraceae) trees: *Gynaikothrips ficorum* (Marchal) and *Gynaikothrips uzeli* (Zimmermann) (Mound et al. 1995; Held et al. 2005). The first infests *Ficus microcarpa* L., whereas the second species is apparently associated only with *Ficus benjamina* L. leaves (Mound et al. 1995). These plants have been cultivated in almost all regions of the world and both thrips species are now widely distributed (ThripsWiki 2013). Feeding by these insects permanently damages the newly developing leaves of their hosts, causing cell hypertrophy and tissue hyperplasia. In spite of these changes, the resulting structures have been considered rudimentary galls, since there is no tissue differentiation (Souza et al. 2000).

These leaf galls commonly enclose many other arthropods, and frequently they are accompanied by non-gall-producing thrips. Most of these invaders enter the galls for protection, but predatory thrips such as some *Androthrips* species may be feeding on the original galler. *Androthrips*

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genus includes 12 species (Mound 2013) and they are known to be predators on other gall-inducing thrips species in Asia (Varadarasan & Ananthakrishnan 1981; Sureshkumar & Ananthakrishnan 1987). Some recent surveys have recorded *Androthrips ramachandrai* Karny (Phlaeothripidae) breeding inside *F. benjamina* galls inhabited by *G. uzeli* in the Americas, from USA to Southern Brazil (Boyd & Held 2006; Cabrera-Asencio et al. 2008; Sepúlveda et al. 2009; Cambero-Campos et al. 2010; de Borbón & Agostini 2011; Cavalleri et al. 2011). *Androthrips ramachandrai* was described from India and found in association with galls of *Austrothrips cochinchinensis* (Phlaeothripidae) on *Calycopteris floribunda* (Combretaceae) (Anantakrishnan 1978). This thrips has been referred as a predator of *Gynaikothrips*, and Cavalleri et al. (2011) observed adults and larvae of *A. ramachandrai* feeding on eggs, larvae and pupae of *G. uzeli* in *F. benjamina* galls in Brazil.

Several techniques such as sticky traps and insecticides, which are useful against other pest thrips, are not appropriate or have limited effect against *G. uzeli* (Held & Boyd 2008). Although many predators of *G. uzeli* have been referred to in literature (see Held et al. 2005), only a few were implicated as active successful biological control agents (Arthurs et al. 2011). Ríos-Velasco (2011) showed that some entomopathogenic fungi can be effective against this thrips, although further research is needed to develop methods and equipment for application under field conditions. *Montandoniola confusa* (Heteroptera: Anthocoridae) was also evaluated for the control of *G. uzeli* and seems to be efficient, but its use is limited by the lack of commercial availability (Arthurs et al. 2011).

One of the first steps in applying any kind of pest control using other living organisms is to identify and understand the life history and behaviors exhibited by the control species. Even though *A. ramachandrai* is apparently an important predator, little is known about its ecology and biology in *F. benjamina* galls (Boyd & Held 2006). *Gynaikothrips* eggs are laid intermittently so each gall may contain adults and immature in different stages of development (Varadarasan & Ananthakrishnan 1982), and here we test if there is any preference of *A. ramachandrai* toward the different developmental stages of *G. uzeli*. We also compare the feeding rates on different life stages of the prey and describe the predatory behavior of *A. ramachandrai*.

Materials and Methods

Feeding Preferences of *Androthrips ramachandrai* (Choice Tests)

We collected galls of *F. benjamina* infested with *G. uzeli* and *A. ramachandrai* in Porto Alegre, southern Brazil, between Mar 2012 and Jan 2013. Females of *A. ramachandrai* were separated using a thin brush and submitted to 96 h of fasting in plastic containers before the tests. We thereafter placed a single *A. ramachandrai* female in an arena created with a 4 cm-diam watch glass at room temperature to simulate similar conditions inside the galls. We offered simultaneously 1 egg, 1 larva and 1 pupa of *G. uzeli*, all alive and displayed at equal distances (n = 37). In order to exclude the effect of the minute size of the eggs, which could lead to a difficulty for detection by the predator, a complementary experiment was developed with a cluster of 5 eggs instead of 1 (n = 19). All observations were conducted under natural lighting. The larvae used varied from late instar I to early instar II. Each *A. ramachandrai* female remained 15 min at most in the arena, regardless of making a choice, and then replaced by another individual. Predation events were registered when *A. ramachandrai* successfully immobilized its prey (for larvae and pupae) for a few seconds and started feeding. The choice was then recorded and the predator was removed from the arena.

Feeding Rate by *Androthrips ramachandrai* (No-choice Tests)

About 3 h before starting this experiment, we sampled galls of *F. benjamina*, and removed all insects with a fine brush under a stereomicroscope. Then *G. uzeli* individuals were placed inside each gall, together with 1 *A. ramachandrai* female. Similar to the previous experiment, field collected females of *A. ramachandrai* were also isolated without food for 96 h. The feeding rate tests were conducted through 3 distinct laboratory experiments: (i) galls with 10 *G. uzeli* eggs (n = 23); (ii) galls with 5-10 *G. uzeli* larvae (n = 8); (iii) galls with 5-10 *G. uzeli* pupae (n = 25). These galls were left in 10 cm-diam Petri dishes for 24 h, sealed and kept in a dark place at room temperature. After that time *A. ramachandrai* females were removed and the number of immature prey remaining was recorded. Eggs, larvae and pupae that were consumed could easily be recognized under a dissecting microscope by their shriveled appearance. We disregarded the trials in which *A. ramachandrai* or *G. uzeli* individuals were not present inside the gall after the 24 h period.

Statistical Analysis

We compared the successful events of preferential predation by *A. ramachandrai* females on different prey instars using a G-test of homogeneity. For no-choice tests, we compared the number of preyed individuals in the 3 different life stages using one-way analyses of variance, followed by Tukey's pairwise comparison tests. We performed

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all statistics in PAST 2.02 software (Hammer et al. 2001).

Results

Behavioral Observations

Observations made ad libitum on *Androthrips ramachandrai* adults indicated that they use their large forelegs to grasp *Gynaikothrips* larvae and pupae and to compress the eggs as well. This predator moves freely through the galls without much resistance and is able to manipulate and kill immatures of similar size as the attacker. We did not observe predation on *G. uzeli* adults. However, the adults of *G. uzeli* usually display defensive behavior by curving their abdomen vertically, and larvae and pupae frequently exhibit similar behavior when disturbed.

Feeding Tests

During choice experiments, *A. ramachandrai* behavior varied from going directly to a specific prey to exploring the arena and touching the different options with their antenna. There was no significant difference in the preference of *A. ramachandrai* for a specific immature stage of *G. uzeli* when offered simultaneously ($n = 37$, $G = 0.22$, $P = 0.89$). Similarly, the results were also not significantly different when using a cluster of 5 eggs ($n = 19$, $G = 0.72$, $P = 0.69$). In many failed attempts, larvae and pupae of *G. uzeli* often displayed rapid lateral and vertical abdominal movements, which were able to deter *A. ramachandrai* attacks during the 15-min trials.

The number of *G. uzeli* that were attacked differed among thrips life stages in no-choice experiments ($F = 39.7$; $P < 0.005$). The number of eggs attacked was significantly higher than that observed for larvae and pupae (Tukey test, $P < 0.005$ for both), and no difference was detected between these last immature stages ($P = 0.9$). The average number of eggs consumed was 5.08 (SD = 2.82), whereas the number of larvae and pupae was 0.75 (SD = 0.71) and 0.44 (SD = 0.58), respectively. Egg predation occurred in all events, and all eggs of a cluster were eaten in 5 cases.

Discussion

Androthrips ramachandrai is capable of feeding on all immature stages of *G. uzeli*, as recorded for its congener, *Androthrips flavipes,* which feeds on other galling species (Varadarasan & Ananthakrishnan 1982). The particular morphology of their forelegs seems to be a powerful adaptation to immobilize and capture prey. This behavior contrasts with that referred for *A. flavipes*, which reportedly does not use the forelegs for feeding (Varadarasan & Ananthakrishnan 1982). The behavior displayed by *G. uzeli* in raising their abdomen in a defensive position when threatened was also reported by Suzuki et al. (1989). Moreover, *Gynaikothrips* are known to produce an allomone excreted through the anus, which is used to repel intruding insects or predators (Suzuki et al. 1989; Mound et al. 1995).

Based on our previous observations on these thrips we predicted that *A. ramachandrai* would prefer to attack *G. uzeli* eggs during choice tests. However, *A. ramachandrai* had no significant preference for any of the immature stages. The eggs were always easily consumed, whereas the larvae and pupae of *G. uzeli* were sometimes effective in repelling *A. ramachandrai* attacks. Similarly, Varadarasan & Ananthakrishnan (1981) pointed out that the larvae and adults of some gall-inducing thrips can easily deter the predaceous *Androthrips flavipes* by violent flicking of the abdomen as observed in our tests. The lack of preference found here contrasts with the results found in studies with other insect predators of this phytophagous thrips. Arthurs et al. (2011) showed that 2 species of Anthocoridae (Hemiptera) feed preferably on eggs instead of larvae and adults of *G. uzeli* in the USA. Curiously, Paine (1992) recorded another predatory anthocorid and a Chrysopidae (Neuroptera) consuming more adults than immatures of *G. ficorum* on *Ficus* galls in multiple choice tests. In general, predatory thrips find their prey by random searching and recognize it only from very near or upon contact, and even starved individuals may pass close to suitable prey and fail to locate it (Lewis 1973). Unfortunately, we were unable to reproduce the experiments inside the galls, and the prey location by *Androthrips* in the artificial arenas might also be an important factor affecting our results in choice tests.

On the other hand, no-choice tests inside the galls indicated that *A. ramachandrai* attacked a higher number of eggs than larvae or pupae when offered separately. Our extensive field observations detected the recurrent presence of *A. ramachandrai* on young galls, contrasting with the records of Ananthakrishnan (1978), which indicate that this species is rarely found on early stages of gall development. We can reasonably assume that the minute *G. uzeli* eggs are common prey of *A. ramachandrai* inside the galls of *F. benjamina*. Nutritional aspects may play an important role in *A. ramachandrai* choice as well. The nutritional value of food eaten by larvae partly determines the duration of their development, and the size and fecundity of adults. Larvae of the predatory *Haplothrips faurei* (Phlaeothripidae) reared on mite eggs develop in 8-11 days compared with 14-22 days for those fed on young mites (Lewis 1973). It is also plausible that the energy spent in capturing *G. uzeli* pupae or larvae is more than the

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energy required to capture multiple eggs, and the predator might select its prey in order to maximize their rate of energy intake and nutritional requirements. At the same time, our results on no-choice tests may just reflect different handling times, which include capture, killing and eating. *Gynaikothrips* minute eggs are easier to handle and therefore *A. ramachandrai* can consume more per unit time than the other life stages.

Androthrips ramachandrai has been spreading rapidly throughout the Americas, although its abundance seems to be highly variable geographically and even within plant individuals (see Boyd & Held 2006; Cambero-Campos et al. 2010; de Borbón & Agostini 2011; Cavalleri et al. 2011). Considering previous studies on other predatory *Androthrips*, the biology of *A. ramachandrai* might be closely related to that of its prey. For instance, Varadarasan & Ananthakrishnan (1982) showed that the females of *A. flavipes* have a singular pattern of ovariole development, which reduces the time they need to oviposit. As a result, their eggs develop faster than those laid by its prey and this pattern is likely to occur in *A. ramachandrai* as well. We also observed *A. ramachandrai* inside the galls of *F. microcarpa* induced by *G. ficorum* in southern Brazil, and laboratory observations indicated that they are also able to feed on its eggs. Similarly, *A. flavipes* also attacks several gall-inducing thrips in India, playing a key role in limiting the population of these phytophagous insects (Varadarasan & Ananthakrishnan 1982).

Although these natural enemies are not commercially available, we suggest that a supplemental release of *A. ramachandrai* on infested *Ficus* trees can potentially be a viable option for the biological control of *G. uzeli*. Indeed, the findings provided by Boyd & Held (2006) also suggest that *Gynaikothrips* populations might decline during an increased presence of *A. ramachandrai* in *F. benjamina* galls in USA. In addition, *A. ramachandrai* would have a greater impact on *G. uzeli* populations if released during early gall development because of the large number of *Gynaikothrips* eggs. However, we do not know if *Androthrips* will have very little impact on reduction of plant damage caused in *Ficus* trees. This artificial augmentation of predators in nature or cultivars will require further laboratory and field experimentation, together with detailed biological data on both thrips species and their impact on the plants.

Acknowledgments

We thank Juliana S. Silva for field support and Tiago S. Toma for his comments and suggestions. This project was partially funded by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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