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Predation and oviposition rates of *Gaeolaelaps aculeifer* and *Parasitus bituberosus* (Acari: Laelapidae and Parasitidae) on pre-pupae/pupae of *Thrips tabaci* (Thysanoptera: Thripidae)

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Original research

ABSTRACT

Thrips tabaci Lindeman is one of the main insect pests of onion (*Allium cepa* L.) in Colombia and several other countries. Strategies for its control are currently based on the use of chemical products. In a recent survey, *Gaeolaelaps aculeifer* (Canestrini) and *Parasitus bituberosus* Karg, two soil-dwelling predatory mite species (Acari: Mesostigmata), were found associated with this crop at Boyacá department, Colombia. Given that *T. tabaci* spends its pre-pupal and pupal stages in the soil, this study was conducted to evaluate the predation and oviposition of *G. aculeifer* and *P. bituberosus* on these developmental stages under laboratory conditions. The predators consumed up to 6.8 ± 0.52 and 6.9 ± 0.45 *T. tabaci* pre-pupae/ pupae, respectively, when offered 6–10 prey a day. The maximum oviposition rates were 4.4 ± 0.25 eggs/female/day for *G. aculeifer* and 6.9 ± 0.26 eggs/female/day for *P. bituberosus*, with a mean egg viability higher than 91% for both predators. These results suggest that the evaluated predators may affect the population of *T. tabaci* under field conditions, and that the conduction of complementary studies on those predators is warranted, envisioning their practical use for *T. tabaci* control in Colombia.

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Keywords edaphic mites; pupal stage; biological control; Colombia

Introduction

Thrips tabaci Lindeman is one of the main onion (*Allium cepa* L.) pests in Colombia as well as in several other countries (Rueda *et al.* 2007; Riley *et al.* 2011; Pal *et al.* 2019), due to its invasive capacity, high mobility and polyphagous behavior (Smith *et al.* 2011). The damage is caused by the feeding of nymphs and adults, which result in the appearance of silvery spots on the leaves that turn into white blotches, followed by the development of silvery patches and leaf curling (Waiganjo *et al.* 2008; Munoz *et al.* 2014). These injuries result in reduced photosynthesis (Jensen *et al.* 2003) and decreases in bulb size (Waiganjo *et al.* 2008). In addition, this species, as well as *Frankliniella fusca* (Hinds) (tobacco thrips), a less common species, have been reported as vectors of the Iris Yellow Spot Virus (IYSV) in onions, garlic, chives, leeks and several ornamentals (Srinivasan *et al.* 2012; Bag *et al.* 2015).

Worldwide, *T. tabaci* is controlled mainly by sprays of synthetic insecticides (Foster *et al.* 2010; Wu *et al.* 2014). However, given the pupal developmental stages are normally found in the litter or the topsoil layer (1.5–2.0 cm) (Tommasini and Maini 1995), these can be hidden and not affected by the applied insecticide (Cannon *et al.* 2007). This has led growers to intensify insecticide use, negatively affecting the environment, human health and promoting the selection of resistant thrips populations (Herron *et al.* 2008; Nazemi *et al.* 2016). In an attempt to break that cycle, alternative control strategies have been evaluated, as the use of biological control.

Soil Mesostigmata are a diverse mite group, where numerous species are predators of small arthropods and nematodes (Lindquist *et al.* 2009; Carrillo *et al.* 2015). Mesostigmatid mites have been shown to prey on pre-pupae and pupae thrips (Wiethoff *et al.* 2004; Wu *et al.* 2014; Rueda-Ramírez *et al.* 2018, 2019), which would interrupt the cycle of these organisms, thereby preventing their return to the plants. Recent studies reported species of soil-dwelling predaceous mesostigmatids of the families Laelapidae and Parasitidae in onion growing areas of the Colombian Department of Boyacá (Castro-López 2018). Hence, it is presumable that some of these species play a natural role in thrips control in that region or could be utilized for that purpose.

One of the mesostigmatid species collected, *Gaeolaelaps aculeifer* (Canestrini) (Laelapidae), has been mass produced and commercialized in Africa, Asia, Europe, North America and Oceania for applied biological control of pest species, including thrips (Knapp *et al.* 2018; van Lenteren *et al.* 2018). It has been reported that each female of a Colombian population of this predator (collected in the Department of Cundinamarca) is able to consume daily 2.6 pre-pupae/pupae of the thrips *Frankliniella occidentalis* (Pergande) (Rueda-Ramírez *et al.* 2018). Previously, Berndt *et al.* (2004b) reported predation of *G. aculeifer* on *F. occidentalis* on substrate soil, and Navarro-Campos *et al.* (2012) on the thrips *Pezothrips kellyanus* (Bagnall) in citrus.

Parasitus bituberosus Karg (Parasitidae), found in Europe, Africa, Asia (Karg, 1972), and recently in Colombia (Rueda-Ramírez *et al.* 2019, 2021), is also a potential biological control agent. This species is reported to prey on fly larvae, (Al-Amidi and Downes 1990; Al-Amidi *et al.* 1991; Szafranek *et al.* 2013) nematodes (Szafranek *et al.* 2013; Rueda-Ramírez *et al.* 2019) and pygmephorid mites (Szafranek *et al.* 2013). In Colombia it was recently reported to prey daily 4.4 pre-pupae and pupae of *F. occidentalis* (Rueda-Ramírez *et al.* 2019), suggesting that it could be evaluated on other thrips species.

As the predation of *G. aculeifer* and *P. bituberosus* on *T. tabaci* has not been evaluated, the objective of this study was to determine their predation and oviposition rates on *T. tabaci* pre-pupae/pupae under laboratory conditions.

Material and Methods

This study was conducted between August of 2017 and February of 2018, at the Entomology Laboratory of the Biological Crop Management Group (GMBC), Universidad Pedagógica y Tecnológica de Colombia, Tunja, Boyacá, Colombia.

Stock colonies

About 10 months before starting the experiments, specimens of *G. aculeifer* and *P. bituberosus* were collected from the rhizosphere of onion plants in the municipalities of Duitama (5°48'40.75'' N, 73°0'46.908'' W), Tibasosa (5°44'52.15'' N, 72°59'28.18'' W) and Nobsa (5°47'56.33'' N, 72°58'39.11'' W) in the Department of Boyacá, and in soil of rose crops in the municipalities of Cogua (05°03'23.3'' N, 073°55'44.4'' W), Guasca (04°50'38.3'' N, 073°53'07.9'' W), Nemocón (05°07'03.1–03.2'' N, 073°51'31.7–31.9'' W) and Tocancipa (04°59'19.3'' N, 073°54'15.9'' W), in the Department of Cundinamarca, Colombia.

These mites were used to establish stock colonies in rearing units modified from Abbatiello (1965) and Freire and Moraes (2007). Each unit consisted of a plastic container (10 cm diameter x 7 cm high), whose bottom was covered with a layer of a plaster made of a mixture of nine parts gypsum and one part activated charcoal. *Gaeolaelaps aculeifer* was fed with a mixture of all developmental stages of the mite *Aleuroglyphus ovatus* (Troupeau) (Sarcoptiformes, Astigmatina, Acaridae) reared on crushed commercial dog food (Purina®; nutritional content: 9% fat, 12% moisture, 8% ash and 25% protein). *Parasitus bituberosus* was fed with *Rhabditella axei* nematodes reared on decomposing bean pods (*Phaseolus vulgaris* L.) and a mixture of all developmental stages of *A. ovatus*. The units were maintained in a growth chamber, in the dark, at 19 ± 3 °C, $60 \pm 10\%$ RH. The units were closed with a piece of plastic film and maintained permanently moist by daily addition of distilled water. *Thrips tabaci* pre-pupae and pupae were obtained from a colony started with adult specimens collected from onion plants. The identification of the thrips species was confirmed by a Thysanoptera specialist, Dr. Everth Ebrat Ravelo. The colonies were maintained as described by López *et al.* (2007), in transparent plastic containers (11 cm diameter x 9 cm high), with bottom covered with a thin layer of cotton overlaid by a sheet of paper towel maintained humid daily with distilled water. Germinated faba bean (*Vicia faba* L.) seeds were placed onto the towel to serve as a substrate for insect feeding and oviposition. Each container was sealed with a transparent plastic film, to prevent thrips from escaping, and maintained in a growth chamber in the dark, at 19 ± 3 °C and $60 \pm 10\%$ RH.

Experimental procedure

For each predatory mites, three treatments were evaluated: a daily density of six, eight and ten of *T. tabaci* pre-pupae or pupae as prey. The experimental unit consisted of a plastic Petri dishes (4 cm diameter, 1.3 cm high), whose bottom was covered with plaster as previously described for the mite rearing units. Thirty gravid females of 3–8-day-old each mite species per treatment were individually transferred from the stock colony to each experimental unit, creating 30 repetitions for each treatment and species.

Experimental units with *P. bituberosus* were evaluated daily for eight days, while units with *G. aculeifer* for ten days. The differences in the evaluation time of these two species were due to differences in their longevity (Rueda-Ramírez *et al.* 2018, 2019). Units were evaluated counting the number of consumed prey and laid eggs. Consumed and non-consumed prey were replaced by new ones after each evaluation. Daily eggs laid were transferred into new containers and maintained until larval emergence, to assess egg viability. Experiments were conducted at controlled laboratory conditions identical to those previous described for mite colony maintenance.

Statistical analysis

Mean daily predation and oviposition rates, and mean egg viability (proportion of eggs hatched/female) were calculated. The predation rate was analyzed with a two-way (2×2) analysis of variance (ANOVA) in a factorial design, with three prey densities (densities of six, eight and ten *T. tabaci* pre-pupae/pupae provided daily per unit) and predators (*G. aculeifer* and *P. bituberosus*) as factors, as normality and homoscedasticity assumptions were met. Oviposition and egg viability were analyzed with a generalized linear model (GLM) with treatment and predator as factors and a quasi-binomial distribution. Post hoc Tukey's test was used for testing differences between means considering the best fit model. Statistical analyses were performed using the R program (Packages ExpDes.pt, lme4, multcomp and ggplot2, version 3.6.2, The R foundation for Statistical Computing, 2019-12-12).

Results

Predation rate

Significant differences in predation rates were observed between prey densities ($F = 311.6$, d.f. = 2, $P < 0.0005$) but not between predator species at any prey density ($F = 1.3$, d.f. = 1, $P = 0.25$). Number of consumed preys was highest when ten prey were offered daily to *G. aculeifer* and to *P. bituberosus*.

For both species, predation rate increased with increasing prey density. The number of consumed individuals of *T. tabaci* pre-pupae / pupae by *G. aculeifer* was 6.8 ± 0.52 , 6.4 ± 0.39 and 5.1 ± 0.33 and by *P. bituberosus* was 6.9 ± 0.45 , 6.2 ± 0.35 and 5.1 ± 0.28 when ten, eight and six prey were offered daily, respectively. However, statistically predation rate of both predators did not tend to increase above a density of eight pre-pupae/pupae of *T. tabaci*, with a smaller difference between predation at eight and ten than between six and eight (Figure 1).

Oviposition rate

Significant differences were observed between predator species ($\text{Chi}^2 = 1871.8$, d.f. = 1, $P < 2 \times 10^{-16}$) and between prey densities ($\text{Chi}^2 = 286.3$, d.f. = 2, $P < 2 \times 10^{-16}$) in relation to oviposition (Figure 2). No significant differences were observed for the interaction of the factors.

Considering the predatory mite species and the prey density, the highest daily oviposition was observed for *P. bituberosus* (6.9 ± 0.26 eggs/female/day) when the offered prey density was 10 pre-pupae/pupae, and the lowest for *G. aculeifer* (3.6 ± 0.36 eggs/female/day) when the offered prey density was 6 pre-pupae/pupae of *T. tabaci*. The highest daily oviposition for *G. aculeifer* (4.4 ± 0.25 eggs/female/day) was also observed when the offered prey density was 10 pre-pupae/pupae of *T. tabaci*. A significant positive correlation was observed between daily oviposition rate and the number of *T. tabaci* pre-pupae/pupae preyed by both predators ($r = 0.8$, $P < 0.001$ and $r = 0.53$, $P < 0.001$ for *P. bituberosus* and *G. aculeifer*, respectively).

Mean egg viability

Significant differences between the viability of eggs produced by females offered different prey densities were recorded ($\text{Chi}^2 = 22.02$, d.f. = 2, $P = 1.65 \times 10^{-5}$), but no significant differences

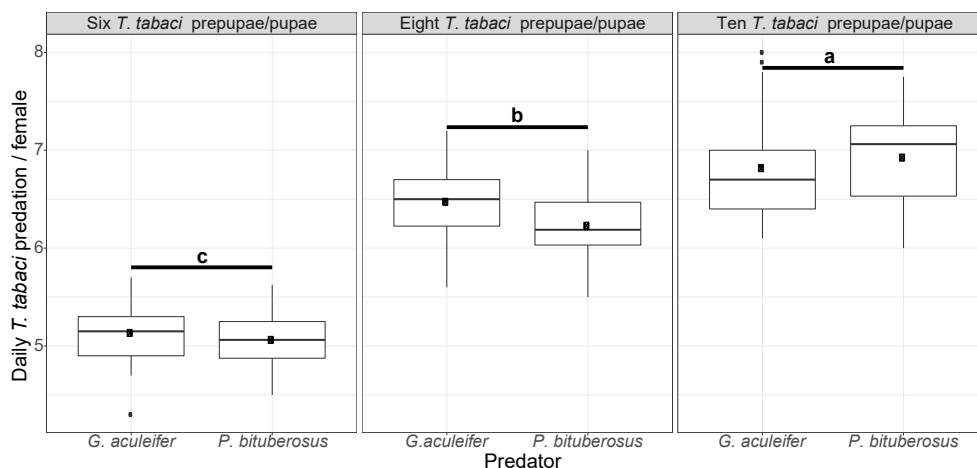


Figure 1 Daily predation rate of *Thrips tabaci* pre-pupae/pupae per female of *Gaeolaelaps aculeifer* and *Parasitus bituberosus* when offered 6, 8 and 10 pre-pupae/pupae. Different letters indicate significant differences in daily predation rate between amounts of prey offered consumed, according to the Tukey test ($P \leq 0.05$).

were observed between predator species (Figure 3). Viability was lowest ($86 \pm 5.8\%$) for *G. aculeifer* offered six prey day compared to other combinations of predator and prey densities, which did not differ significantly among themselves ($> 91\%$).

Discussion

Results of this study contributed to assess the predation potential of *G. aculeifer* and *P. bituberosus* on *T. tabaci* pre-pupae/pupae. Predation rates of *G. aculeifer* and *P. bituberosus* on *T. tabaci* were higher than those reported for laelapid species feeding on *F. occidentalis*. Specifically, *Stratiolaelaps scimitus* (Womersley) consumed 2.13 ± 0.1 prey/day (Park *et al.* 2021) and 4.5 ± 0.42 prey/day (Wu *et al.* 2014), *G. aculeifer* 2.93 ± 0.1 prey/day (Park *et al.* 2021) and 3.5 ± 0.5 prey/day (Berndt *et al.* 2004b), *G. aculeifer* (the same strain used in the present study) 2.6 ± 0.1 prey/day (Rueda-Ramírez *et al.* 2018), *Stratiolaelaps miles* (Berlese) 1.6 ± 0.3 prey/day (Berndt *et al.* 2004a) and *Cosmolaelaps jaboticabalensis* (Moreira, Klompen and Moraes) 2.6 ± 1.1 prey/day (Moreira *et al.* 2015). In addition, Park *et al.* (2021) reported predation rate of *G. aculeifer* and *S. scimitus* of 2.4 ± 0.1 and 2.0 ± 0.1 prey/day when these species were fed with *F. intonsa* (Tryborn), and of 3.3 ± 0.1 and 3.4 ± 0.1 when fed on *Thrips palmi* (Karny), respectively. The species *G. aculeifer*, *S. scimitus* and *S. miles* are commercialized for the control of *F. occidentalis* (Knapp *et al.* 2018). Rueda-Ramírez *et al.* (2019) reported a daily predation of 4.4 ± 0.2 *F. occidentalis* pre-pupae/pupae per female of the same population of *P. bituberosus* used in the present study.

In summary, for both *G. aculeifer* and *P. bituberosus*, predation in the current study on *T. tabaci* was approximately 1.6 times higher than *F. occidentalis* in the cited studies. These differences in predation rate may be related to characteristics of *T. tabaci*. Shaikh *et al.* (2015) reported the length of pre-pupae and pupae of *T. tabaci* to be respectively 0.91 ± 0.10 mm and 0.96 ± 0.12 mm, whereas Cárdenas and Corredor (1989) reported for *F. occidentalis* length of respectively about 1.1 mm and 1.3 mm. The smaller size of *T. tabaci* could account for the greater predation rate than the former. However, in addition to size, other factors could be involved, resulting from the metabolism of organic acids as glutamic, malic, citric, in onion plants (Rodríguez-Galdón *et al.* 2008). These hypotheses need to be further explored.

The oviposition rates of *G. aculeifer* were higher when fed on *T. tabaci* pre-pupae/pupae

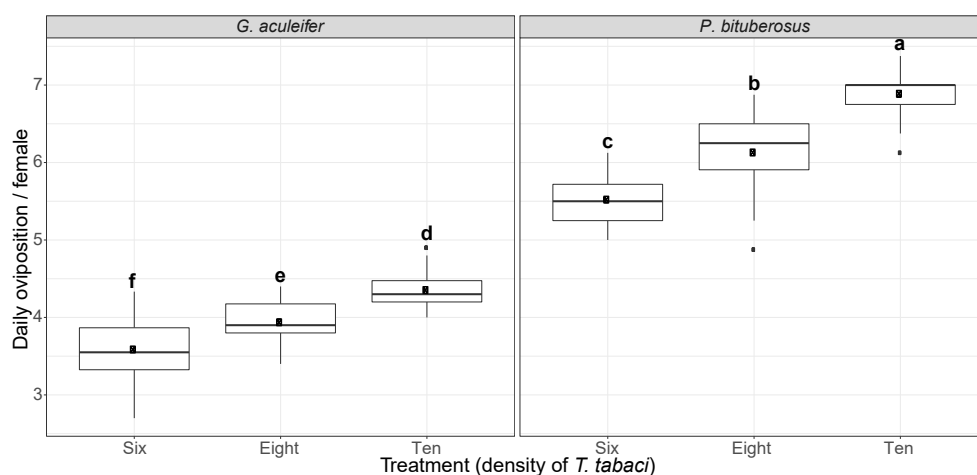


Figure 2 Daily oviposition of *Gaeolaelaps aculeifer* and *Parasitus bituberosus* on different densities of *Thrips tabaci* pre-pupae/pupae daily available for consumption per female predator. Different letters indicate significant differences in daily oviposition between amounts of prey offered for the two predators, according to the Tukey test ($P \leq 0.05$).

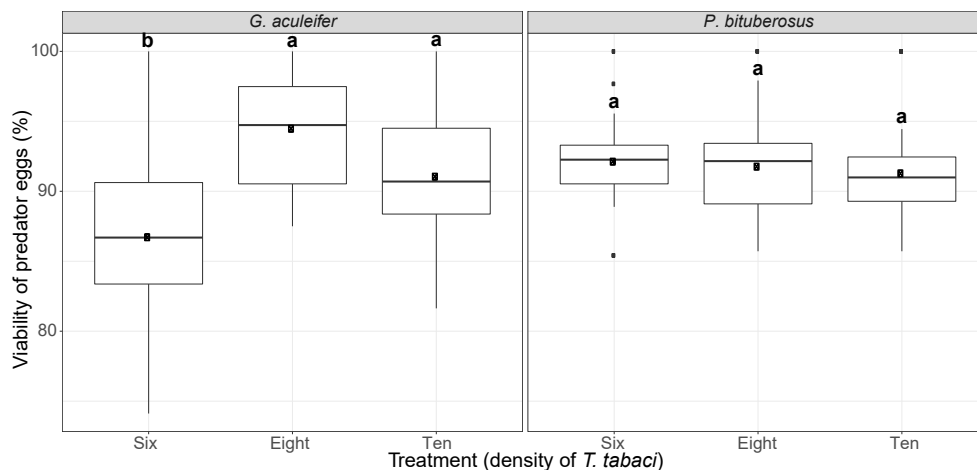


Figure 3 Viability of eggs (%) of *Gaeolaelaps aculeifer* and *Parasitus bituberosus* produced by females offered different daily prey densities (pre-pupae/ pupae of *Thrips tabaci*). Different letters indicate significant differences in viability of eggs between combinations of predator and prey density (Tukey test, $P \leq 0.05$).

than the rates reported by Rueda-Ramírez *et al.* (2018), Navarro-Campos *et al.* (2016) and Berndt *et al.* (2004a) when this species was fed with *F. occidentalis* (about 2.9 ± 0.1 , 2.2 and 2.5 ± 0.87 eggs/female/day, respectively). Another laelapid species as *C. jabolicabalensis*, only laid 0.2 eggs/female/day (Moreira *et al.* 2015) and *S. miles* with 0.8 ± 0.53 eggs/female/day when fed *F. occidentalis*. In the case of *P. bituberosus*, the oviposition rate was lower than that reported by Rueda-Ramírez *et al.* (2019) when *F. occidentalis* pre-pupae/pupae was offered as prey (8.9 ± 0.8 eggs/female/day). The oviposition capacity depends on several factors, among which the nutritional content of the food source and the use of resources to extend longevity in presence of nutrient-poor or stressful conditions for predatory species (Gotoh and Tsuchiya 2008). Both factors need to be analyzed. McMurtry (1982) indicated that the growth capacity of a population is only one feature determining the performance of a biological control agent; several other factors may influence the efficacy of predators such as intraguild predation, competition, response to abiotic environmental factors, functional and numerical response, and others (Skirvin and Fenlon 2001; Gontijo *et al.* 2012), thus further experiments should be performed. The difficulty in obtaining large numbers of *T. tabaci* to conduct this study hampered the possibility to evaluate a larger array of prey densities or a larger number of replicates. Under greenhouse conditions, a reduction of 78% and 72% were observed in the population of *T. tabaci* in the presence of *G. aculeifer* and *P. bituberosus*, respectively in onion plants (Castro-López and Martínez-Osorio 2021). However, complementary field studies should be conducted to explore other possibilities, including the association of *T. tabaci* pre-pupae and pupae with other food sources such as nematodes (Rueda-Ramírez *et al.* 2019; Azevedo *et al.* 2019, 2020), naturally found in agricultural ecosystems (Rueda-Ramírez *et al.* 2018, 2019). The use of entomopathogenic fungi in combination with predatory mites has shown good potential, as shown in the study conducted by Saito and Brownbridge (2016), in which mortality of thrips was higher than 90%. These complementary studies should lead to the practical use of *G. aculeifer* and or *P. bituberosus* in Colombia where thrips species are still controlled primarily with insecticides.

In conclusion, while previous studies reported on the predation of *G. aculeifer* and *P. bituberosus* of edaphic phases of thrips species (Berndt *et al.* 2004a; Navarro-Campos *et al.* 2012; Rueda-Ramírez *et al.* 2018, 2019), this is the first study demonstrating the predation capacity on *T. tabaci* pre-pupae/pupae.

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