### Allium cepa L. responses when Gaeolaelaps aculeifer Canestrini and Parasitus bituberosus Karg are used to control Thrips tabaci Lindeman

Respuesta de *Allium cepa* L. en presencia de *Gaeolaelaps aculeifer* Canestrini y *Parasitus bituberosus* Karg para el control de *Thrips tabaci* Lindeman



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*G. aculeifer* **preying on** *T. tabaci* **pupa (40x)** Photo: M.A. Castro

### ABSTRACT

Thrips tabaci Lindemann is the main insect pest of Allium cepa L., causing both direct and indirect damage to crops. T. tabaci is controlled by applying chemically synthesized products; however, this insect has already developed resistance to organophosphates, carbamates, and pyrethroids. This study evaluated the effect of soil predatory mites (Gaeolaelaps aculeifer Canestrini and Parasitus bituberosus Karg) on the population density of T. tabaci in potted bulb onion (Allium cepa L) plants and on the response of physiological variables related to photosynthesis and plant development. Seven treatments were evaluated that released 50, 75 or 100 G. aculeifer or P. bituberosus adults, along with a control without predatory mites. Significant differences ( $P \le 0.05$ ) in the population density of T. tabaci were observed for 13 weeks, with a 78% reduction of individuals in the presence of G. aculeifer and a 72% reduction with P. bituberosus, regardless of mite density, as compared to the control. In addition, the relative chlorophyll index, foliar area, dry leaf weight and fresh bulb weight increased, as compared to the control. The application of 100 individuals of the two species recorded the highest values in the evaluated variables. These results indicate that G. aculeifer and P. bituberosus controls should be explored as an option for integrated T. tabaci management.



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#### RESUMEN

La principal limitante del cultivo de *Allium cepa* L. es *Thrips tabaci* dado que genera daños directos e indirectos en el cultivo. El principal método de control es mediante productos de síntesis química, no obstante, este insecto ya presenta resistencia a ingredientes activos como organofosforados, carbamatos y piretroides. Por tal razón, este estudio consistió en evaluar la densidad poblacional de *T. tabaci* y la respuesta de variables fisiológicas relacionadas con la fotosíntesis y desarrollo de plantas de cebolla de bulbo a la presencia de ácaros depredadores del suelo como *Gaeolaelaps aculeifer* Canestrini y *Parasitus bituberosus* Karg, los cuales pueden alterar el ciclo de vida de los trips, especialmente la fase de pupa presente en el suelo. Se evaluaron siete tratamientos que consistieron en la liberación en plantas de cebolla de 50, 75 y 100 adultos de *G. aculeifer*, 50, 75 y 100 adultos de *P. bituberosus* y un testigo sin ácaros depredadores. Se observaron diferencias significativas ( $P \le 0,05$ ) en la densidad poblacional de *T. tabaci* al final del experimento, con una reducción de individuos de 78% en presencia de *G. aculeifer* y 72% con *P. bituberosus*. Así mismo, hubo reducción en el índice relativo de clorofila, área foliar, peso foliar seco y peso fresco del bulbo en comparación con el testigo. La aplicación de 100 individuos de las dos especies de ácaros, registró los valores más altos en las variables evaluadas. Estos resultados indican que *G. aculeifer* y *P. bituberosus* puede ser una opción de control a explorar más a fondo dentro de un esquema de manejo integrado de *T. tabaci*.

Palabras clave adicionales: ácaros depredadores; trips; cebolla; Laelapidae; Parasitidae.

Received: 13-05-2020 Accepted: 03-12-2020 Published: 13-01-2021

### INTRODUCTION

Predatory mites of the Mesostigmata order represent potential biological control agents for agricultural pests (Carrillo *et al.*, 2015). Previous studies have found species of the families Laelapidae and Parasitidae in soils of bulb onion (*Allium cepa* L.) crops in the Department of Boyaca (Castro-Lopez, 2018), including *Gaeolaelaps aculeifer* (Canestrini) (Laelapidae), which is used commercially in European and North American crop production for controlling thrips, mites, and flies (van Lenteren, 2012). Likewise, *Parasitus bituberosus* (Karg) (Parasitidae) is a promising species for managing fly larvae, (Szafranek *et al.*, 2013) thrips, and nematodes (Rueda-Ramírez *et al.*, 2019).

The bulb onion is one of the more important vegetables worldwide (Pinzón, 2009). The presence of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in bulb onion crops is a challenge for production (Vergel *et al.*, 2017) because it reduces bulb size by up to 60% (Rueda *et al.*, 2007). This insect reduces the photosynthetic capacity by feeding on onion leaves (Jensen *et al.*, 2003), accelerating leaf senescence with the physiological stress that results from the foliar damage (Kendall and Bjostad, 1990). It is also a vector of the *Iris yellow spot virus* (Bunyaviridae: Tospovirus), which reduces bulb size and quality (Muñoz *et al.*, 2014).

Management strategies for T. tabaci include the application of chemically synthesized products. However, T. tabaci populations have been reported to be resistant to several insecticides, including organophosphates, carbamates and pyrethroids in the United States (Shelton et al., 2006), spinosad in Israel (Lebedev et al., 2013), and deltrametrin and diazinon in New Zealand (Martin et al., 2003). In South America, resistance to these chemicals is unknown. In addition, not all insect stages of development are equally affected by chemical treatments (Damte et al., 2017). Prepupae and pupae are protected by the soil or the base of onion plants and may escape contact with insecticides (Gill et al., 2015). Therefore, management strategies that include biological control should be explored.

*Gaeolaelaps aculeifer* is an edaphic mite that is used commercially for biological control in Europe and North America. This mite is a generalist predator that can feed on a wide range of prey, including astigmatic mites (Rueda-Ramírez *et al.*, 2018), nematodes (Moreira and Moraes, 2015), fly larvae (Sardar and Murphy, 1987), and collembolans (Heckmann *et al.*, 2007). A Colombian population of this predator was found to consume approximately 2.6 *Frankliniella occidentalis* (Pergande) pre-pupae or pupae daily per adult



female (Rueda-Ramírez *et al.*, 2018). In bean plants, a predation rate of 80.5% for *F. occidentalis* was reported for 14 d (Berndt *et al.*, 2004); in citrus, consumption reached for 12.3 $\pm$ 0.8 *Pezothrips kellyanus* (Bagnall) over 3 d (Navarro-Campos *et al.*, 2012). There are no studies on this predatory mite for *T. tabaci*.

Parasitus bituberosus is another edaphic mite that has been reported as a potential biological control agent, feeding 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 forpigarous mites (Szafranek *et al.*, 2013). Recently, a study observed the consumption of 2.5  $\pm 0.8$  *F. occidentalis* pre-pupae and pupae daily per adult female (Rueda-Ramírez *et al.*, 2019). This result indicated that this predator may prey on other thrips species, including *T. tabaci*.

Given these findings and the fact that pre-pupae and pupae stages of *T. tabaci* develop in the soil, the objective of this research was to evaluate the response of *A. cepa* plants to applications of *G. aculeifer* and *P. bituberosus* to control *T. tabaci* under greenhouse conditions.

### **MATERIALS AND METHODS**

#### Geographical location of the study area

This research was carried out in a greenhouse at the Grupo Manejo Biologico de Cultivos (GMBC), Universidad Pedagógica y Tecnológica de Colombia (UPTC), in the municipality of Tunja, Department of Boyaca, Colombia, with an average daily temperature of  $21\pm3^{\circ}$ C and a relative humidity of  $60\pm15\%$ .

In the greenhouse, 60 A. *cepa* onion seedlings, acquired in the municipality of Tibasosa in the Department of Boyaca, were seeded. Six plants were placed in a 1 m<sup>2</sup> square. Conventional management was used for the irrigation, fertilization, and other agronomic tasks to achieve good development of the plants.

The mite colonies were kept in breeding units, which consisted of an incubator chamber with a  $19\pm3^{\circ}$ C average temperature and  $60\pm15\%$  relative humidity, in the absence of light. This methodology was adapted from Freire and Moraes (2007). *Gaeolaelaps aculeifer* was fed a mixture of all developmental stages of *Aleuroglyphus ovatus* (Troupeau) (Sarcoptiformes,

Astigmatina, Acaridae), which was reproduced and reared on crushed commercial dog food (Purina®; nutritional content: 9% fat, 12% moisture, 8% ash and 25% protein). *Parasitus bituberosus* was fed with *A. ovatus* and Rhabditidae nematodes maintained on bean pods (*Phaseolus vulgaris* L.).

# Evaluation of *G. aculeifer* and *P. bituberosus* for the control of *T. tabaci* in onion plants

The onion plants were infested with 50 *T. tabaci* adults that were collected from *A. cepa* crops in the municipalities of Tibasosa and Nobsa. After 60 d, they were expected to have completed four life cycles. Afterwards, ethe prepupae and pupae were used as food for the predatory mites; different amounts of the mite predator species were released.

A completely randomized experiment design was used that included seven treatments: 50 (G50), 75 (G75), or 100 (G100) f *G. aculeifer* adults, 50 (P50), 75 (P75), or 100 (P100) *P. Bituberosus* adults, and a control without applications of the predator (control). Each treatment was replicated six times, for a total of 42 experiment units. Two releases of G50, G75, G100, P50, P75 and P100 were carried out for the treatments, at the beginning of assay and in the 8<sup>th</sup> week.

Every 2 weeks, the *T. tabaci* population was assessed, recording all developmental stages (excluding the eggs of the thrips) found on three leaves in the central third of six plants per treatment. This method was used from the  $5^{\text{th}}$  week until the  $13^{\text{th}}$  week, which was the end of the growing cycle.

# Effects on bulb onion plants from the *G. aculeifer* and P. bituberosus

To determine the effects on onion plant growth, five leaves were selected from the central third of each plant and treatment to measure the chlorophyll content (Minolta SPAD 502 plus chlorophyllmeter) every 15 d, recording the average per plant, expressed in SPAD Units. At week 14, when maturity was reached, the foliar area was measured, expressed in  $cm^2$  were done, with a CI 202, Bio-Science Inc. The fresh and dry plant weights were quantified using an electronic balance (Acculab VIC 612 - 0,01 g precision). The plants were dried for 48 h at 70°C in a Memmert oven. In addition, soil samples were taken with a metal cylinder,10 cm diameter and 5 cm depth. The samples were transported in plastic bags to the laboratory, where the mites were extracted with a modified Berlesse funnel (Oliveira *et al.*, 2000). Each sample was observed with a stereomicroscope (Leica microsystems) to confirm the presence of the Mesostigmata mites. The variables were measured in the laboratories of Biological control and Plant physiology of the UPTC.

The data were subjected to normality and variance homogeneity tests using Shapiro-Wilk and Bartlett tests, respectively. When the assumptions were verified, the variables that had statistical differences were tested with Tukey's comparison of means ( $P \le 0.05$ ). The analyses were carried out in R (version 3.3.2).

### **RESULTS AND DISCUSSION**

## Population estimate of *T. tabaci* in onion plants with *G. aculeifer* and *P. bituberosus*

The population of *T. tabaci* in the onion plants declined, with significant differences ( $P \le 0.05$ ) between the six treatments with *G. aculeifer* (G50, G75, and G100,) and the control after week seven. The Tukey test corroborated the differences, with  $10\pm3.6$ ;  $26\pm4.3$  and  $17\pm2.6$  *T. tabaci* individuals on the leaves, which indicated a 78, 63 and 48% population decrease for the treatments with G100, G75 and G50 *G. aculeifer*, respectively (Fig. 1A).

A similar response was observed in the onion plants with *P. bituberosus* after the 9<sup>th</sup> week, with counts of  $13\pm2.0$ ;  $23\pm2.4$  and  $19\pm2.6$  individuals, indicating a reduction of 72, 62, and 55% in the *T. tabaci* population, as shown in figure 1B.

Our results showed a reduction of 48-78% in the T. tabaci population with G. aculeifer and P. bituberosus over 13 weeks. This was comparable to reports for other species of edaphic mesostigmatid mites. For example, Stratiolaelaps scimitus (Womersley) reduced the population density of T. tabaci by 64% over 6 weeks (Wu et al., 2014), Hypoaspis miles (Berlese) saw a reduction of 44.9%, G. aculeifer (mentioned as Hypoaspis aculeifer) saw a reduction of 57.6% for 14 d (Berndt et al., 2004) and 51%, and Macrocheles robustulus (Berlese) saw a reduction of 66-70% for 13 weeks (Messelink and van Holstein-Saj, 2008), feeding on F. occidentalis. However, the edaphic phases of T. tabaci control only provide an alternative within integrated management programs because approximately 20-25% of thrip populations can complete their life cycle in the presence of predatory mites (Berndt et al., 2004).

These differences in the predation rate may be related to several factors, e.g. slight contortions that have been observed in the pupal stages of thrips (Rueda-Ramírez *et al.*, 2019), size of the pre pupa and pupa, which are usually smaller (0.91-0.96 mm) (Shaikh *et al.*, 2015) than *F. occidentalis* (1.1-1.3 mm) (Cárdenas and Corredor, 1989). This may determine the preference of G. aculeifer and P. bituberosus for some types of prey.







# Effects on bulb onion plant development with *G. aculeifer* and *P. bituberosus*

Evaluation of the relative chlorophyll index showed statistical differences ( $P \le 0.05$ ) between the treatments after the 7<sup>th</sup> week, when the bulb formation phase began (Fig. 2). The treatment with 100 individuals of predatory mites presented the highest value for this index in week 13, with 135±3.4 SPAD units.

The results in figure 2 indicated that the onion plants infested with *G. aculeifer* and *P. bituberosus* had a higher chlorophyll content than the control plants  $(97\pm2.0 \text{ SPAD} \text{ units})$ , and the treatment with 50 *P. bituberosus* individuals achieved  $103\pm3.2 \text{ SPAD}$  Units within 13 weeks, possibly because a small number of *P. bituberosus* mites preferred another type of prey rather than thrips, such as small insects, collembolans and other mites that live in the soil (Castilho *et al.*, 2015).

The leaf area showed significant differences between the treatments. According to the Tukey test ( $P \le 0.05$ ), this result was more evident with the treatments where *G. aculeifer* was added, with an average leaf area for all treatments of  $238 \pm 14.7$  cm<sup>2</sup>, while this average for *P. bituberosus* was  $220 \pm 9.7$  cm<sup>2</sup>. Both were higher than the control ( $130 \pm 16.8$  cm<sup>2</sup>) (Fig. 3).

Pineda-Mares *et al.* (2001) indicated that leaf lamina is one of the most important parts for developing photosynthesis. *T. tabaci* adults and individual nymphs that perforate the surface tissues to absorb cellular contents interfere with this process (Jensen *et al.*, 2003), directly affecting leaf area (Marschner, 2012). Likewise, the populations of *T. tabaci* were significantly higher when the crop had a growing period longer than 3 weeks, resulting in longer leaves that can be more attractive for thrips than in seedlings (Hsu *et al.*, 2010). This behaviour was observed here, especially in the control plants.

The dry leaf weight had significant differences between the treatments ( $P \le 0.05$ ). The higher values were 52.9±2.29 and 55.2±2.59 g when 100



Figure 3. Leaf area in bulb onion plants (A. cepa) exposed to different concentrations of the predatory mites G. aculeifer and P. bituberosus and a control without application. Means with different letters indicate a significant statistical difference according to the Tukey test ( $P \le 0.05$ ). The vertical bars indicate  $\pm$ standard error.





individuals of *G. aculeifer* or *P. bituberosus* were added, respectively (Fig. 4). In comparison, the control treatment presented the lowest value with  $28.2 \pm 1.78$  g, indicating a negative effect when onion plants do not have any control for *T. tabaci* because the nutrient supply is directly related to the accumulation of dry matter (Hernández *et al.*, 1996).

A decrease in the dry matter was observed in figure 4, indicating that there was a decrease in the uptake and efficiency of radiation, as reflected in the accumulation of dry matter in the onion plants. Since this parameter indicates the amount of photoassimilates stored in different parts for plant growth (Estrada-Ortiz *et al.*, 2011), directly affecting production, conversion of simple sugars in leaf tissue and bulb growth (Dogliotti *et al.*, 2011) (Fig. 5).

In terms of fresh bulb weight, there were significant differences between the treatments ( $P \le 0.05$ ). The higher weights were 1,341.15±29.1 g and 1,188.36±12 g with the presence of 100 individuals of *G. aculeifer* or *P. bituberosus*, respectively. Additionally, the weight with the release of 50 *P. bituberosus* mites (618.3±16.2 g) was remarkably similar to the control (620.1±29.3 g) (Fig. 5) because the number of soil predatory mites was not sufficient for managing *T. tabaci*, affecting the fresh bulb weight.

The chlorophyll content is directly related to the photosynthetic rate (Shekari *et al.*, 2017), so, direct damage by *T. tabaci* destroys the mesophyll, affecting the amount of chlorophyll in the leaf (Molenaar, 1984) and interfering with the transport of nutrients to the bulb (Parrella and Lewis, 1997; Diaz-Montano *et al.*, 2011). On the other hand, a higher production of ethylene is induced in onion plants when the salivary gland products of *T. tabaci* affect tissues (Kendall and Bjostad, 1990), inducing early maturation and senescence of the leaves (Levy and Kedar, 1970). This was observed in the present study because the control had the lowest values for the evaluated variables, such as leaf area, leaf dry weight and fresh bulb weight, as reflected in the growth and development of the onion plants.

Despite the physiological differences obtained with the predatory mite releases evaluated in the onion plants, alternative management strategies are recommended to suppress populations of T. tabaci because combined strategies result in more efficient insect pest control. For example, S. scimitus and Gaeolaelaps gillespiei (Beaulieu) (Mesostigmata: Laelapidae), when used with Metarhizium anisopliae (Metschnikoff) and Beauveria bassiana (Balsamo), achieved a mortality greater than 90% for F. occidentalis (Saito and Brownbridge, 2016). These components may be considered for future research and field verification, especially for the protection of bulb onion leaves, where juvenile and adult T. tabaci are found, playing an important role in reducing yield by damaging crops (Jandricic et al., 2016).







Figure 5. Fresh bulb weight (g) in bulb onion plants (A. cepa) exposed to different concentrations of the predatory mites G. aculeifer and P. bituberosus and a control without application. Means with different letters indicate a significant statistical difference according the to Tukey test ( $P \le 0.05$ ). The vertical bars indicate  $\pm$  standard error.



#### CONCLUSIONS

The results in this research indicated that releasing the predatory mites *G. aculeifer* and *P. bituberosus* reduced the population of *T. tabaci* by 72 and 78%, respectively. Consequently, the relative chlorophyll index, leaf area index, dry leaf weight and fresh bulb weight increased when compared to the onion plants without a control for this bulb onion insect pest.

The highest values in the physiological variables, which contribute to the yield of bulb onion crops, were obtained with the release of 100 individuals of *G. aculeifer* or *P. bituberosus,* representing a new tool for integrated management programs for *T. tabaci.* 

#### ACKNOWLEDGMENTS

This research was carried out with the support of Vicerrectoria de investigación y extension and the Grupo Manejo Biologico de Cultivos at the Universidad Pedagógica y Tecnológica de Colombia, Tunja. Research project code SGI-2335. The authors thank Diana Rueda Ramirez of Explora Agrotecnologia for providing *G. aculeifer* and *P. bituberosus* individuals when the colonies decreased.

**Conflict of interests:** The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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