PAOLA ALEJANDRA FLORES CARBAJAL

EVALUATION OF TWO PREDATORY MITES AS POTENTIAL BIOLOGICAL CONTROL AGENT OF AN INVASIVE THRIPS SPECIES

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

Orientador: Angelo Pallini Filho
Coorientadores: Adriano Cavalleri
Henry Vacacela Ajila

VIÇOSA - MINAS GERAIS
2019

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

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Flores Carbajal, Paola Alejandra, 1984-

F632e
2019

Evaluation of two predatory mites as potential biological control agent of an invasive thrips species / Paola Alejandra Flores Carbajal. – Viçosa, MG, 2019.

41f. : il. (algumas color.) ; 29 cm.

Texto em inglês.

Orientador: Ângelo Pallini Filho.

Dissertação (mestrado) - Universidade Federal de Viçosa.

Inclui bibliografia.

1. Ácaros no controle biológico de pragas. 2. *Echinothrips caribeanus*. 3. Pimentão . I. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. II. Título.

CDD 22 ed. 595.758

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APROVADA: 16 de dezembro de 2019

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“No meio da dificuldade está a oportunidade”

(Albert Einstein)

ACKNOWLEDGEMENTS

A Deus por ser tudo na minha vida.

A minha família por ser meu motor para seguir.

Ao Professor Angelo por acreditar em mim, por me dar a oportunidade de fazer parte de sua equipe e abrir as portas do seu laboratório mesmo sem me conhecer. Muito obrigada por me ajudar neste novo começo.

Ao Professor Arne pela paciência, pela ajuda incondicional quando solicitado, mesmo sem ser sua orientada.

Ao Marcossi pela amizade e ajuda infinita para fazer este trabalho.

A meus vizinhos do laboratório de Interação inseto planta Camila, Clebson, Silvana, Thadeu, Adelson e Morgana pela amizade, por me suportar e por sempre me apoiar quando precisei, obrigada por todos os bons momentos!!

As Meninas Viviana, Maribel, Camila, Silvana, Vanessa e Josie pela amizade, pelo apoio de sempre, por fazer minha vida em Viçosa muito mais linda e tranquila.

Aos colegas do laboratório de acarologia Adriana, Morgana, Aldo, Jose, Vanessa, Julia, Andre, Pedro, Vinicius e Milena por todo o seu apoio.

À Universidade Federal de Viçosa por abrir as portas para estudantes estrangeiros e culminar em outro sucesso em nossa vida profissional.

Ao Programa de Pós-Graduação em Entomologia pela oportunidade.

A organização dos Estados Americanos (OEA), pela concessão da bolsa de estudo.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001, Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).

ABSTRACT

CARBAJAL, Paola Alejandra Flores, M.Sc., Universidade Federal de Viçosa, December, 2019. **Evaluation of two predatory mites as potential biological control agent of an invasive thrips species.** Adviser: Angelo Pallini Filho. Co-advisers: Adriano Cavalleri and Henry Vacacela Ajila.

Amblyseius herbicolus and *Amblyseius tamatavensis* are two predatory mites native from Brazil to the Phytoseiidae family that have been used in different studies to assess their potential as biological control agents of different types of pests. The aim of this study was to determine the potential of these two predatory mites to control *Echinothrips caribeanus*, which is an invasive thrips species, reported in Brazil. The study was done in sweet pepper (*Capsicum annuum*) plants under laboratory and greenhouse conditions. We evaluated (1) predation rates of the predatory mites feeding on immatures stages of thrips, (2) oviposition, development and survival rates of the predators mites feeding on immatures thrips and on cattail pollen and (3) population dynamics of *E. caribeanus* on sweet pepper plants in the presence of *A. herbicolus* and *A. tamatavensis*. Our results showed that under laboratory conditions, *A. herbicolus* show a higher predation rate of *E. caribeanus* immature compare with *A. tamatavensis*. The highest oviposition and survival rates of both species were found with pollen, as the diet offered. Data on development was not presented because neither species of predatory mites reached adulthood feeding on immature of thrips. Under greenhouses conditions, the thrips population dynamics was lower on *A. tamatavensis* treatment compare with *A. herbicolus*. Population dynamics of the predatory mite *A. tamatavensis* was higher than the other predatory mite due to an increase at the end of the evaluation period. Nevertheless, the results found in laboratory and greenhouse experiments are not promising to indicate these two predatory mites as potential biological control agents of this invasive thrips species.

Keywords: Predatory mites. Biological control. Thrips. Sweet pepper.

RESUMO

CARBAJAL, Paola Alejandra Flores, M.Sc., Universidade Federal de Viçosa, dezembro de 2019. **Avaliação de dois ácaros predadores como potenciais agentes de controle biológico de uma espécie de tripes invasiva.** Orientador: Angelo Pallini Filho. Coorientadores: Adriano Cavalleri e Henry Vacacela Ajila.

Amblyseius herbicolus e Amblyseius tamatavensis são dois ácaros predadores nativos do Brasil da família Phytoseiidae que foram utilizados em diferentes estudos para avaliar seu potencial como agentes de controle biológico de diferentes tipos de pragas. O objetivo deste estudo foi determinar o potencial desses dois ácaros predadores no controle de *Echinothrips caribeanus*, uma espécie invasora de tripes reportada no Brasil. Este estudo foi realizado na cultura de pimentão (*Capsicum annuum*) em condições de laboratório e em casa de vegetação. Foram avaliadas a (1) taxa de predação de ácaros predadores se alimentando de estágios imaturos de tripes, (2) taxa de oviposição, desenvolvimento e sobrevivência de ácaros predadores que se alimentam de tripes imaturos e de pólen de *Typha* sp. e a (3) dinâmica populacional de *E. caribeanus* em plantas de pimentão na presença de *A. herbicolus* e *A. tamatavensis*. Nossos resultados mostraram que, em condições de laboratório, *A. herbicolus* mostra uma maior taxa de predação de imaturos de *E. caribeanus* comparado com *A. tamatavensis*. A maior taxa de oviposição e sobrevivência para ambas as espécies foi encontrada com o tratamento de pólen como dieta. Os dados de taxa de desenvolvimento não foram apresentados porque nenhuma espécie de ácaro predador atingiu a idade adulta, alimentando-se de imaturos de tripes. Nas condições de casa de vegetação, a dinâmica populacional de tripes foi menor no tratamento com *A. tamatavensis* comparado com *A. herbicolus*. A dinâmica populacional do ácaro predador *A. tamatavensis* foi maior que o outro ácaro predador devido a um aumento no final do período de avaliação. No entanto, os resultados encontrados em experimentos de laboratório e estufa não são promissores para indicar esses dois ácaros predadores como potenciais agentes de controle biológico dessa espécie invasora de tripes.

Palavras-chave: Ácaros predadores. Controle biológico. Tripes. Pimentão.

SUMMARY

Introduction

Thrips are among the most important agricultural pests and present significant challenges to growers of a wide range of crops around the word (Stuart et al. 2011). Several thrips species can cause economic damage to greenhouse crops by feeding or ovipositing in plant tissues. Some damages caused by thrips is due to feeding by pressing their mouthparts against plant tissue and using their single mandible to puncture epidermal cells and then sucking cell liquid contents through a pair of maxillary stylets, resulting a cell dehydration and silvering (Parker et al. 1995; Heinz et al. 2004). This pest can also transmit plant viruses as *Tospovirus, Ilarvirus, Carmovirus* (Jones 2005) leading to severe disease outbreaks.

In greenhouse vegetables production, species as *Frankliniella occidentalis* (Pergande), *Thrips palmi* Karny and *Thrips tabaci* Lindeman are widely distributed, highly polyphagous and are recognized by their economic importance as pests(Lenteren 1995; Parker et al. 1995; Weintraub 2007; Viteri et al. 2010). Their host plants include vegetables and ornamentals. Among greenhouses vegetables, the main crops attacked are peppers, cucumbers, strawberries and tomatoes. In the case of ornamentals the most common host are chrysanthemums, gerberas, carnations, cyclamens and roses (Castañé et al. 1999).

In Brazil, it was recorded a new and invasive thrips species found feeding on a wide spectrum of host plants, including greenhouse crops. *Echinothrips caribeanus* Hood, 1955 (Thysanoptera: Thripidae) is a thrips species that was first described from Guadeloupe, French island in the Caribbean. Adult thrips were found on ornamental plants such as *Asclepias* (Asclepiadaceae) and wild plants as *Phyllanthus niruri* (Phyllanthaceae) (Cavalleri et al. 2016). E. caribeanus is a thrips species relatively new and with few studies carried out, so some the information on its biology presented in this document will be illustrated and described according to observations made during the establishment of the colony and the development of the experiments.

E. caribeanus has six developmental stages, eggs, first and second instar larva, prepupa, pupa and adult (Fig. 1). This thrips is a leaf-dwelling thrips species, it is not expected to feed on pollen. In addition, *E. caribeanus* are slow- moving and complete all life stages on the green tissue of plants compare with other thrips species.

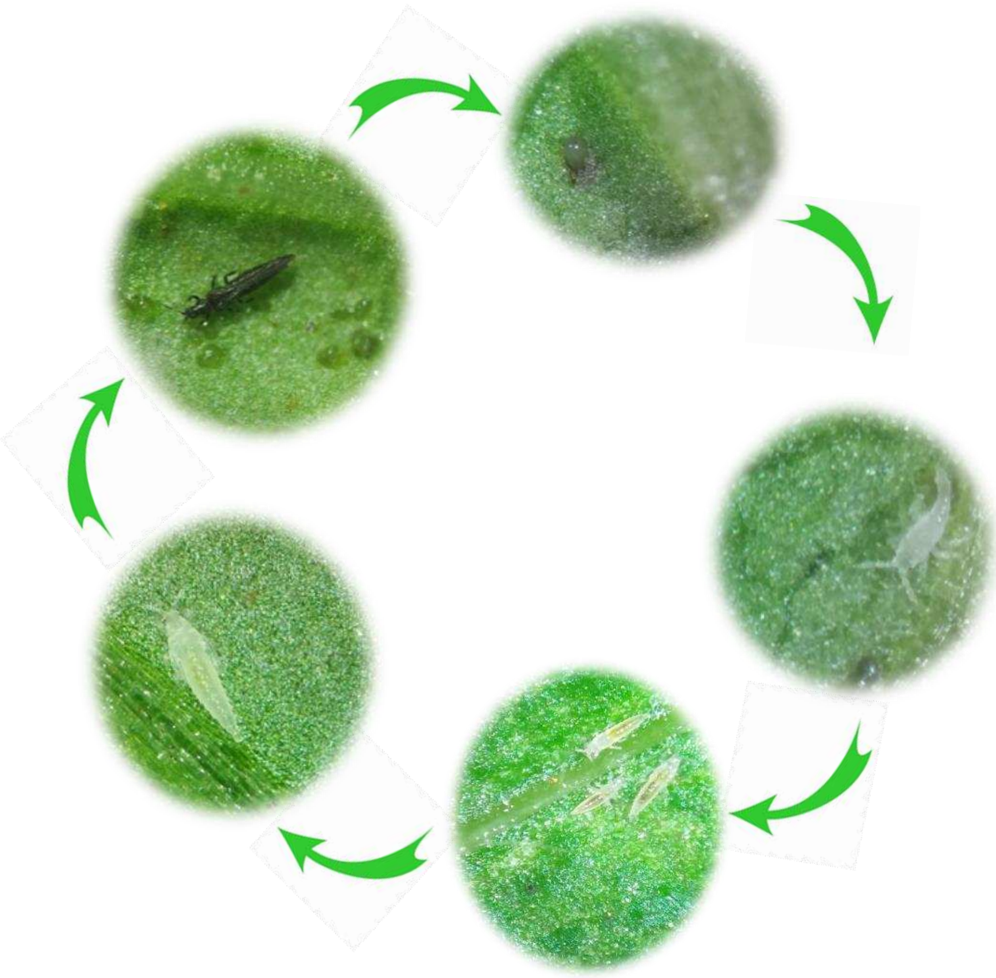

Figure 1. Life cycle of *E. caribeanus*. Credits Paola A. Flores

Adults of E. caribeanus have a dark brown body with red bands between abdominal segments; uniformly darkened anterior wings. Head strongly reticulated, with three pairs of ocellar bristles; antenna with 8 segments, III & IV with simple and curved sensory cones (Cavalleri et al. 2016) (Fig. 2). The eggs of *E. caribenaus* are inserted by a adult female inside the plant tissue on the underside of the leaf. (Fig. 3) Development time of the egg could be around 8 to 10 days at 28 °C on sweet pepper plant. The first instar larva is white and second instar larva is yellow and bigger. (Fig. 4). The duration of the larva stage is approximately 2 days to first instar and 6 days to second instar. Pupal stages are white, inactive and without mobility, E. caribeanus pupa stay in the leaf or in the plant stem during five days approximately until reaches adulthood.

Figure 2. Adult *Echinothrips caribeanus* Hood

Figure 3. Eggs of *E. caribeanus*

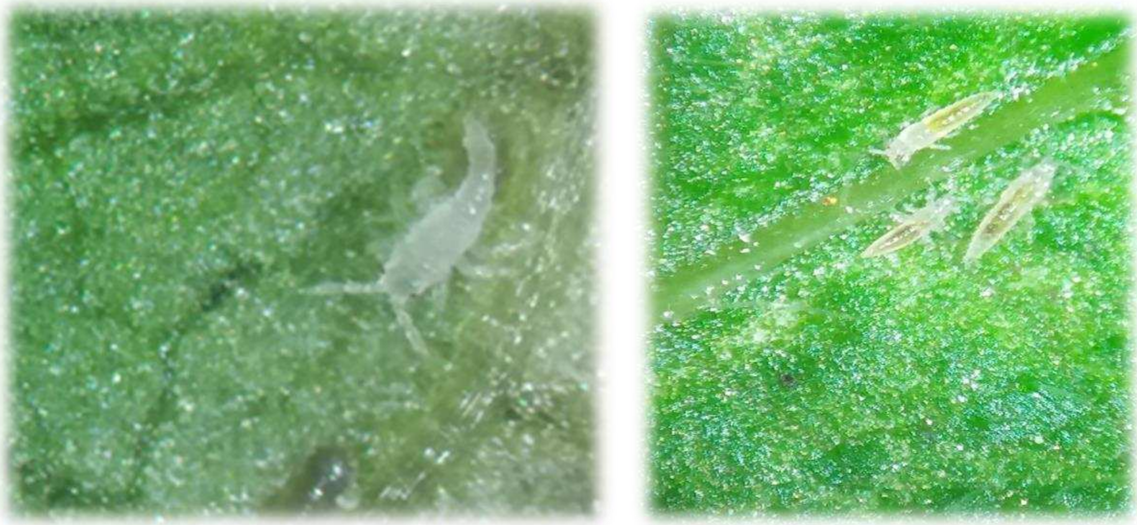

Figure 4. First and second instar larva of *Echinothrips caribeanus*

Figure 5. Pupa of *Echinothrips caribeanus*

The fact that this thrips were found in ornamental plants and crops in protected systems might be a reason to become an important agricultural pest. An example that we can mention is the case of *Echinothrips americanus*, which is a native species of eastern North America. It was reported at the first time in 1984 as a pest of greenhouse crops in Georgia (Panthi et al.), but was until the last ten years that has a high impact as a pest in Europe, Asia and United states (Ghasemzadeh et al. 2017). There are no records of economic damage caused by E. caribeanus. However, unsuitable use of some agricultural practices could trigger this kind of thrips as a pest of economic importance (McLaughlin and Mineau 1995; Kaur and Garg 2014).

Biological control is one of the strategies used to control several kinds of pests. Van Lenteren et al. (2018a) define biological control as the use of one organism population to reduce the population of another organism. There is a long history in the application of biological control in protected systems and natural ecosystems, which provides a valuable alternative as a long-term management for exotic pests and weeds (Mills and Heimpel 2018). The use of biological control agents in greenhouse production systems has grown in parallel with the size of this type of industry, with the aim of improving some agricultural practices and reducing the excessive use of pesticide (Pilkington et al. 2010). Augmentative biological control is based on periodical releases of natural enemies (van Lenteren 2012; Messelink et al. 2014) used as an alternative to pest control in protected crop systems due to resistance that pests have developed by the intensive use of pesticide.

In Brazil, several successful cases of biological control has been reported (Parra et al. 2002; Parra and Zucchi 2004; Parra, 2014)).The use of biological control agents is also one of the alternatives used to control thrips. There are various natural enemies and microorganisms that can be used for this purpose. This includes insects predators, parasitoids, predatory mites and entomopathogenic fungi (Sabelis and van Rijn 1997). Examples of predators most successfully used for biological control of thrips in various parts of the world include Hemiptera of the Anthocoridae family, especially those belonging to the genus *Orius* sp. Among the species that stand out are *Orius* *albidipennis* (Reuter), *Orius laevigatus* (Fieber), *Orius majusculus* (Reuter), *Orius niger* (Wolff), *Orius insidiosus* (Say) and *Orius tristicolor* (White) (Castañé et al. 1999). Other organisms that have been successfully used to control thrips are predatory mites, the Phytoseiidae family (Mcmurtry et al. 2013). Those are used as natural enemies in the augmentative biological control of thrips. Predatory mites could be a better option to use as a biological control of thrips, due some of their morphological and behavior characteristics and the high cost of rearing of *Orius* sp. compare with predatory mites rearing (Shipp and Wang 2003; Skirvin et al. 2007; Weintraub et al. 2011).

Neoseiulus barkeri (Hughes) was the first species of predatory mites used for thrips control in greenhouses (Heinz et al. 2004). However, there have been many species of mites evaluated to thrips control in greenhouses. Among the most common examples are *Neoseiulus cucumeris* (Oudemans), *Iphiseius degenerans* (Berlese) (van Houten and van Stratum 1995) and *Amblyseius swirskii*(Athias-Henriot) (Parker et al. 1995; van Houten and van Stratum 1995; Messelink et al. 2008; Wimmer et al. 2008; Arthurs et al. 2009; Xu and Enkegaard 2010; Calvo et al. 2011; Kakkar et al. 2016; Ghasemzadeh et al. 2017).

Some native species of phytoseiidae mites in Brazil may have potential for biological control of thrips. *Amblyseius herbicolus* Chant is associated with pepper plants and some research has shown its potential for controlling the broad mite *Polyphagotarsonemus lattus* (Banks) (Rodríguez-Cruz et al. 2013; Duarte et al. 2015) and immatures of *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae) (Lam et al. 2019). *Amblyseius tamatavensis* Blommers is another native species associated with sweet pepper (*Capsicum annuum*) that shows promising results as a candidate for biological control of *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae) (Cavalcante et al. 2015, 2017b). The importance in the use of native species is because in the last 100 years many exotic natural enemies have been imported, mass reared and released as biological control agents. Negative environmental effects of these releases have rarely been reported (Lenteren et al. 2003). Moreover, risk assessment for biological control is difficult because of how hard it is to predict community- and ecosystem-wide impacts of introduced species and because introduced species disperse and evolve (Simberloff and Stiling 1996).

The aim of this study was to determine the potential of these two species of predatory mites as biological control agent of invasive species of thrips as *E. caribeanus.* We evaluated (1) predation rates of *A. herbicolus* and *A. tamatavensis* feeding on immatures stages of *E. caribeanus*, (2) oviposition, development and survival rates of the predatory mites on immatures thrips and on cattail pollen, (3) population dynamics of *E. caribeanus* on sweet pepper plants in the presence of the predators *A. herbicolus* and *A. tamatavensis* and pollen as alternative food in greenhouse condition.

Materials and methods

Rearing methods

Plants

Beans (*Phaseolus vulgaris*) seeds were sowed every week in plastic pots filled with a commercial substrate (Mecplant®) and after 10 days were transferred and maintained in a cage (88x44x44cm) in the greenhouse at 28 ± 2°C, 60± 10% relative humidity. Sweet pepper (*Capsicum annuum*) seeds were sown in polystyrene trays (50.2x35.5x5cm) filled with a commercial substrate (Mecplant®) in the greenhouse. Plants of 35 days old were transplanted to plastic pots (2L) filled with commercial substrate. The plants remained in the pots for two weeks, after that they were used in the experiments.

Thrips

Echinothrips caribeanus was collected from beans (*P. vulgaris*) and sweet pepper *(C. annuum)* plants on the campus of the Federal University of Viçosa and identified using Mound & Marullo (1996). Voucher specimens were mounted in microscopic slides and are deposited at the entomological collection of the Federal University of Rio Grande, RS, Brazil.

To establish the thrips rearing, it was used the same plant species from which they were collected. Synchronous cohorts of thrips were obtained by transferring adults from the beans stock rearing and sweet pepper plants to clean plants (one week old since germination). The plants were placed in a plastic cage (24x14x13cm) with holes covered with mesh on the sides to provide ventilation. After 6 days, the leaves were cut and under binocular microscope the first thrips instar were taken to use in the experiment. In the case of second thrips instars, the leaves were cut after 8 days. To distinguish the different stages of the life cycle of *E. caribeanus* we used the description of the biology and morphology of *Echinotrhips americanus* (due to the similarity of gender) (Koppert Biological System 2019).

Mite

Amblyseius herbicolus was taken from the stock colony of the Laboratory of Acarology at the Federal University of Viçosa, Minas Gerais, Brazil. This predatory mite was collected from tomato plants in vegetable gardens in the urban and rural areas of Viçosa (latitude: 20º 45 '14 "S; longitude: 42º 52' 55" W) and Prados (latitude: 21º 03 ' S; longitude: 44º 04' 47" W), both in the Minas Gerais state (Cardoso, 2019). A*mblyseius tamatavensis* was collected in greenhouse with chili pepper and sweet pepper at the Federal University of Viçosa, Mina Gerais, Brazil (latitude: 20 ° 45'37 " S; longitude 42 ° 52 '04'O). Later, these mites were taken to the laboratory and were selected with a brush and transferred to a rearing arena. Some specimens were placed in Eppendorf tubes with 70% alcohol for identification.

All mites were reared on arenas as described by McMurtry and Scriven (1965) with some small modifications. Arenas consisted of plastic trays (29x22x7cm) containing foams of rectangular shape (17x11x2.5cm) moistened with distilled water with a black plastic sheet placed on its top. The edges of the plastic sheet were covered with a layer of wet Kleenex® paper tissue and another layer of wet cotton wool to prevent mite escape. Cotton wool filaments were placed on top of the black plastic sheet, which were covered with small pieces of plastic, as oviposition site. All predators were fed with cattail pollen, which was kept in a freezer (2°C). There are some research where show the use and effectiveness of cattail pollen as an alternative food in experiments with predatory mites (Pijnakker et al.; Skirvin et al. 2007; Delisle et al. 2015). Cattail pollen is a supplementary or alternative food source used to measure parameters as survival, development and fecundity and also is an alternative food source to the mas rearing of some predatory mites (Rodríguez-Cruz et al. 2013; Janssen and Sabelis 2015; Leman and Messelink 2015; Duarte et al. 2015; Vacacela Ajila et al. 2019). In greenhouse conditions, some advantages associated with supplemental food are that predatory mites as natural enemies are able to the maintenance more time in a crop in case prey densities are low (Kunkel and Cottrell 2007).

In the case of *A. tamatavensis*, the mite *Thyreophagus cracen*tiseta Barbosa (Acari: Astigmatina) was provided as additional food (Massaro et al. 2016). *Thyreophagus cracentiseta* were provided by the Laboratory of Acarology at the Escola Superior de Agricultura “Luiz de Queiroz” University of São Paulo, Piracicaba, São Paulo, Brazil. Food was provided twice a week for both predators. All mites colonies were maintained at 25 ± 2°C, 75± 10% relative humidity (RH) and 12:12 (L:D) photoperiod.

Synchronous cohorts of *A. herbicolus* were obtaining by isolating individual females from the rearing on arenas, which consisted of black plastic Petri dishes (diameter 5.5 cm, height 1.5cm) containing a piece of moist paper tissue (0.5cm^2). Pollen of cattail was provided as food source. After 24 hours, all females were removed and the arenas with eggs were provisioned with pollen every 48 hours until the mites reached adulthood.

Synchronous cohorts of A*. tamatavensis* were obtained by transferring adult females from the stock rearing to a new arena for oviposition. The arenas used for oviposition were the same ones used for rearing. After 24 hours, all females were removed and the arenas with eggs were provisioned with pollen and *T. cracentiseta* twice per week until the mites reached adulthood.

Experiments

Predation of *A. herbicolus* and *A. tamatavensis*

The predation rate of the adult female predator on thrips was evaluated in arenas consisted of black plastic Petri dishes (diameter 5.5 cm, height 1.5cm) with a piece of moist paper tissue (0.5 cm^2). The treatments were (T1.) 3 first instar thrips larvae on a piece of bean leaf (0.5cm^2) + 1 adult female predatory mite and (T2.) 3 second instar thrips larvae on a piece of bean leaf (0.5cm^2) + 1 adult female predatory mite. The same treatment distribution was done to each one of the species. For each treatment, 30 replicas were done. During 6 days, the alive and preyed thrips were recorded. Every day were replaced the preys. Predation data on the two instar thrips were compared using a Generalized Linear Model (GLM) with quasi-Poisson error distribution. All analyses were performed using statistical software R 3.5.0 (R Core Team 2019).

Oviposition of *A. herbicolus* and *A. tamatavensis*

To determine oviposition rate of both species, a single 10-12 days old female of *A. herbicolus* and *A. tamatavensis* were used. Each female of each species was placed on a black plastic Petri dish (diameter 5.5 cm, height 1.5cm) with a piece of moist paper tissue (0.5cm^2). The treatments used for each predator were (T1.) 3 first instar thrips larvae placed on a piece of bean leaf (0.5cm^2) + one predatory mite adult female, (T2.) 3 second instar thrips larvae placed on a piece of bean leaf (0.5cm^2) + one predatory mite adult female, (T3.) Pollen + one predatory mite adult female and (4) no food + one predatory mite adult female.

Oviposition was recorded daily during six days, removing the eggs from each treatment. The oviposition of the first day was not included in the analysis due to the effect of the diets of the previous days (Sabelis 1990). Food sources were replaced daily. The effect of different food source on the oviposition rate was assessed with Linear Mixed Effects model (LME) with food source and time as fixed factors and the individuals as a random factor to correct for repeated measures. Contrasts among treatments were assessed with the Tukey method with the packages lsmeans (Lenth 2016). All analyses were performed with the statistical software R 3.5.0 (R Core Team 2019).

Development and Survival of *A. herbicolus* and *A. tamatavensis*

To evaluate the development and survival rate of both species of mites feeding on thrips, newly deposited eggs were chosen from each species. They were individually placed in a separate black plastic Petri dish (diameter 5.5 cm, height 1.5cm). A small piece of moist paper tissue was also placed on each arena as a water source. The treatments for both species of predatory mites were distributed as follows: (T1.) 2 first instar thrips larvae on a piece of bean leaf (0.5 cm^2), (T2.) 2 second instar thrips larvae on a piece of bean leaf (0.5 cm^2), (T3.) cattail pollen as a food source and (T4.) no food as control.

Juvenile development and survival were monitored daily until the individuals reached adulthood. Data on the effect of different food resource on development time and survival were analyzed with Cox proportional hazard model (coxph) of the survival package (R Core Team 2019).

Population dynamics of *E. caribeanus, A. herbicolus and A. tamatavensis* on sweet pepper plants in a greenhouse

This experiment evaluated the suppression of *E. caribeanus* by *A. herbicolus* or *A. tamatavensis* using sweet pepper plants (50 days old) as a host. The treatments used were (T1.) *A. herbicolus* + *E. caribeanus*, (T2.) *A. tamatavensis* + *E.caribeanus*, (T3.) *E. caribeanus* alone (control). For each treatment were made four replicates. Treatments were distributed in twelve cages, using three plants per cage. Each cage was infested with 30 adults of *E. caribeanus*. After 7 days, thrips densities were assessed, counting all the visible stages on all the leaves of each plant using a hand-held 910 magnifying glass. Subsequently, after the evaluation, five adult females of *A. herbicolus* and *A. tamatavensis* were placed on each plant from the respective treatment. Furthermore, 0.015 grams of pollen were added to each plant in all treatments using a long bristle brush. Every three days, it was counted all the visible stages of the pest and the predators on the entire intact plants using hand-held 910 magnifying glass. Thus, every three days it was added again 0.015 grams of pollen per plant as alternative food. After fourteen days, destructive evaluation was made by cutting all the plants and taking them to the laboratory. All mobile life stages of mites and thrips were counted in each respective treatment with the help of a stereoscopic microscope (Zeiss Stemi 2000_c).

The total number of all life stages of thrips and predators were compared among treatments using generalized mixed-effect models (GLMER) with the package lme4, with treatments and time as an independent fixed factor and replicates as random factors to correct for repeated measures. In thrips analysis, the contrasts among treatments were assessed with Tukey method with the package lsmeans. All analyses were performed with the statistical software R 3.5.0 (R Core Team 2019).

Results

Predation rate

The predation rates differed significantly between species (Fig. 6). A. herbicolus showed the higher predation rate feeding on first instar thrips larvae 0.67±0.08 (mean ± standard deviation), compare with A. tamatavensis 0.33 ± 0.05 (GLM X2=71.72, p< 0.0001). In the second instar thrips larvae, A. herbicolus had a higher predation rate 0.35 ±0.06 compare to A. tamatavensis 0.20±0.04 (GLM X2=59.75, p= 0.005.

Oviposition rate

The oviposition rates of *A. herbicolus* and *A. tamatavensis* differed significantly between treatments (Fig. 7 y Fig. 8). The higher oviposition rate was found with pollen treatment to both species. This differed from oviposition rate of the other treatments for *A. herbicolus* (LME, X2 =45.12 df= 3, p < 0.0001) and *for A. tamatavensis* (LME, X^2= 23.3 df = 3, p < 0.02).

Table 1. Means of oviposition of *A. herbicolus* accumulated in the 5 days of evaluation.

Treatment	Oviposition mean	S.D.
1st Instar larvae	1.79	±0.28
2nd Instar larvae	1.91	±0.22
Pollen	8.80	±0.21
Starvation	1.67	±0.20

Table 2. Means of oviposition of *A. tamatavensis* accumulated in the 5 days of evaluation.

Treatment	Oviposition mean	S.D.
1st Instar larvae	2.50	±0.29
2nd Instar larvae	2.34	±0.27
Pollen	8.14	±0.42
Starvation	1.64	±0.04

Development and Survival

Juvenile development data are not presented in these analyzes due to most mites died in the early days as larvae or protonymphs, specifically when the predatory mites fed on first and second thrips instar larvae. Predatory mites reached adulthood only in pollen treatment.

The survival of the predatory mites significantly differed among diets (Cox proportional hazards- Likelihood ratio test= 80.46 df= 3 $p<0.001$) (Figures 9 and 10). The treatment with highest rate of survival for *A. herbicolus* was pollen, which differed from the other treatments (Fig 9). *Amblyseius tamatavensis* showed similar results. The higher survival rate was observed when the predatory mite fed on pollen (Cox proportional hazards-likelihood ratio test= 25.6 df =3 $p<0.001$). Survival in all other treatments was similar (Fig. 10).

Population dynamics

Echinothrips caribeanus population differed significantly among the treatments (GLMER: X^2=12.02, df=2 P= 0.002). *A. tamatavensis* treatment show less population of thrips than *A. herbicolus* treatment. However, the amounts of thrips per plant at the end of the experiment were above the permitted economic thresholds in another thrips species, which indicates that neither of the two predatory mites shows a potential in the control of this species of thrips in greenhouse conditions.

Predatory mites population differed significantly between species (GLMER: X^2=147.83 df=1, P<0.001). *Amblyseius tamatavensis* showed an increase in the population over the time, which may indicate that this mite use other resources of the plant as pollen or nectar as food, this could be a reason to achieve a greater population growth.

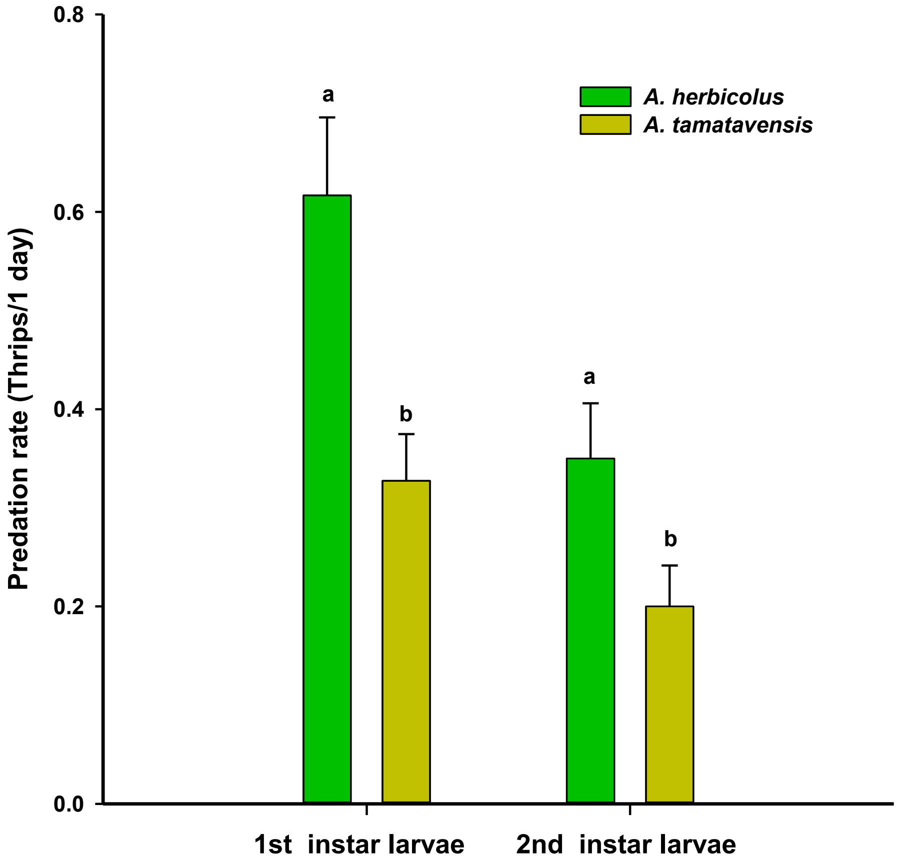

Fig 6. Average predation rates of adult females of *A. herbicolus* and *A. tamatavensis* (±SEM) on first and second instar larvae of *E. caribeanus* ($P < 0.05$).

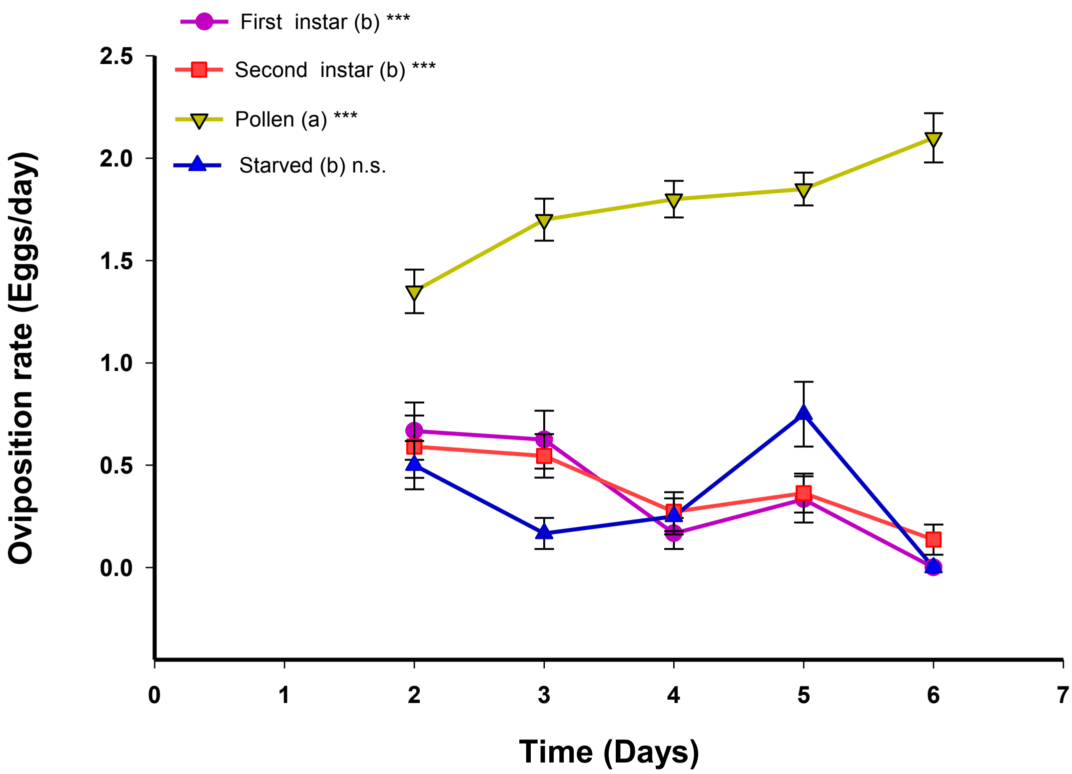

Figure 7. Average oviposition of *A. herbicolus* females (±SEM) when fed on different instar of thrips (*E. caribeanus*), pollen or no food. Different letters indicate significant differences among day treatments ($P < 0.05$).

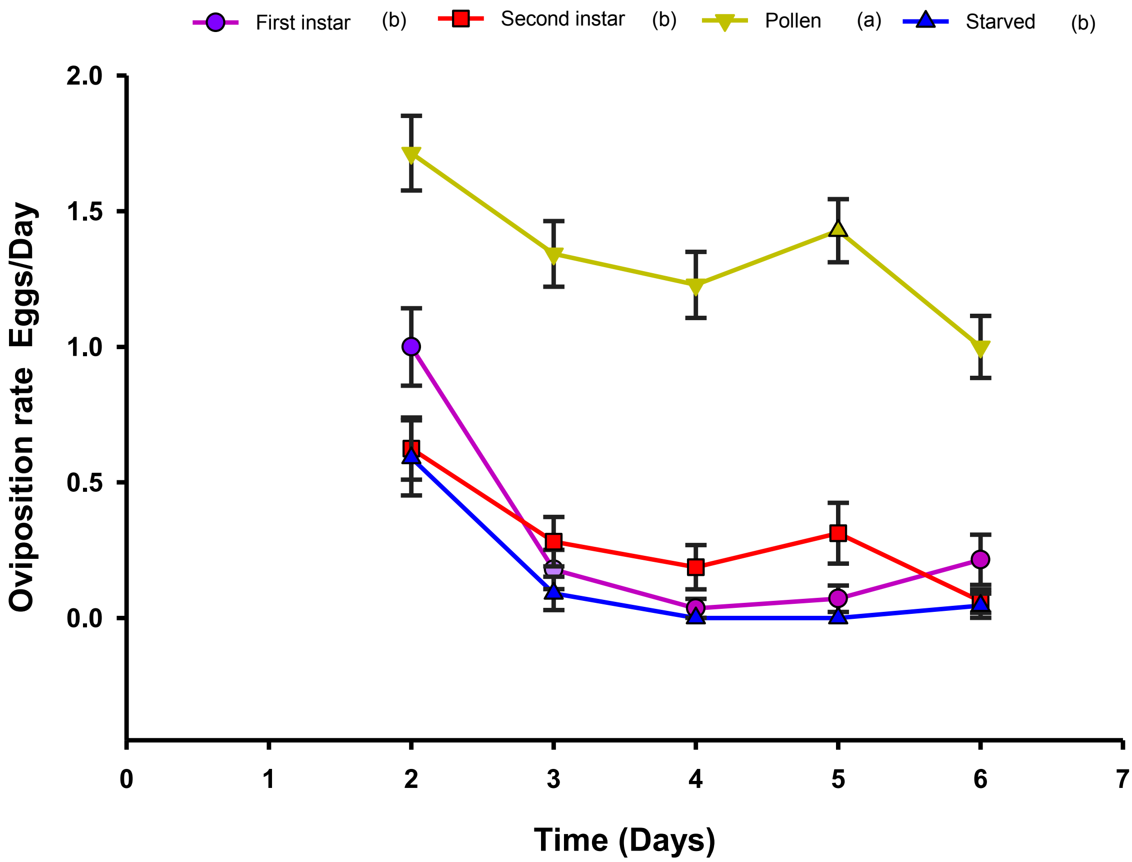

Figure 8. Average oviposition of *A. tamatavensis* females (±SEM) when fed on different instars of thrips (*E. caribeanus*), pollen and no food. Different letters indicate significant differences among treatments (P <0.05).

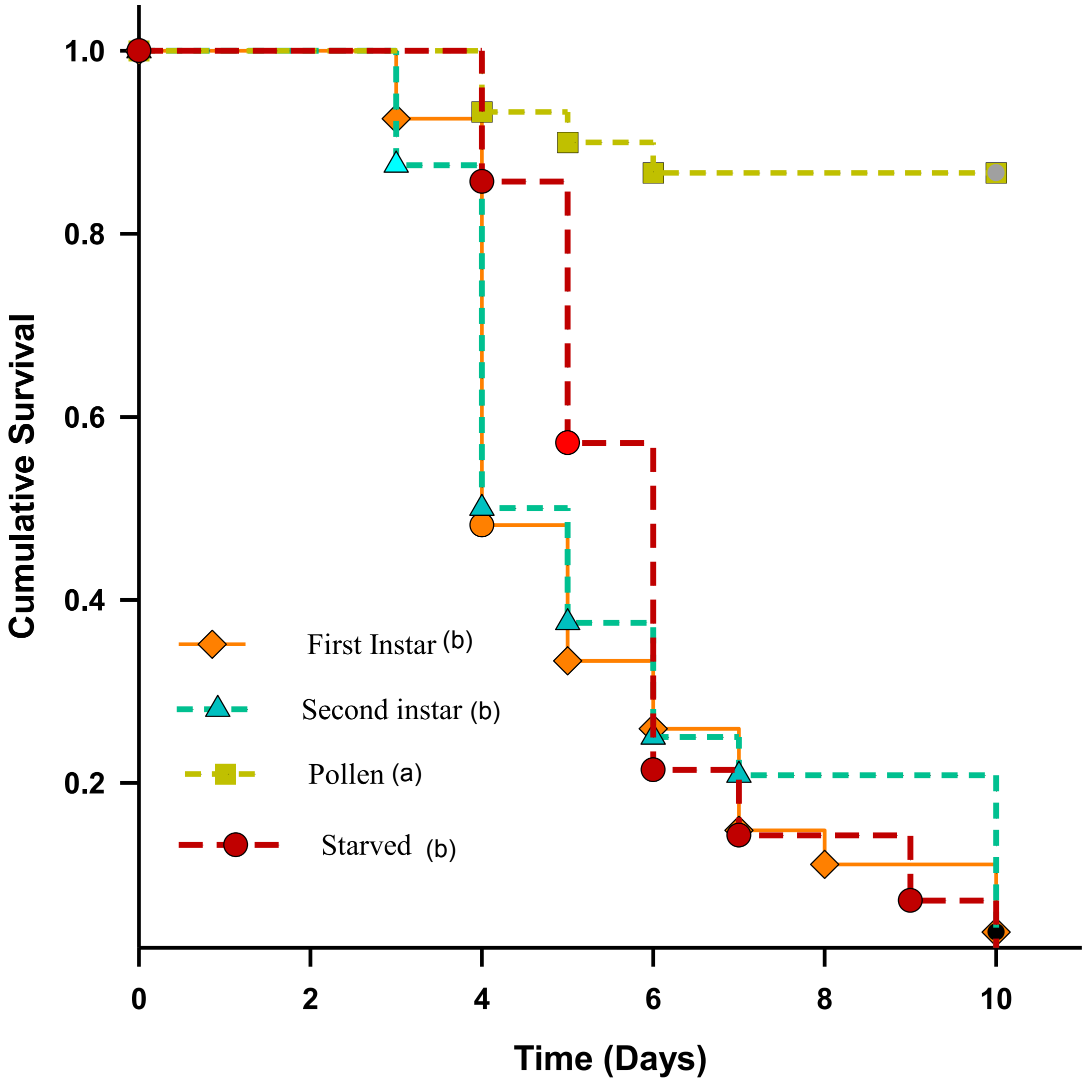

Figure 9. Survival of *A. herbicolus* juveniles fed on different food sources. Orange diamonds: First instar thrips larvae; dark cyan triangles: second instar thrips larvae; green squares: pollen; red circles: starved treatment.

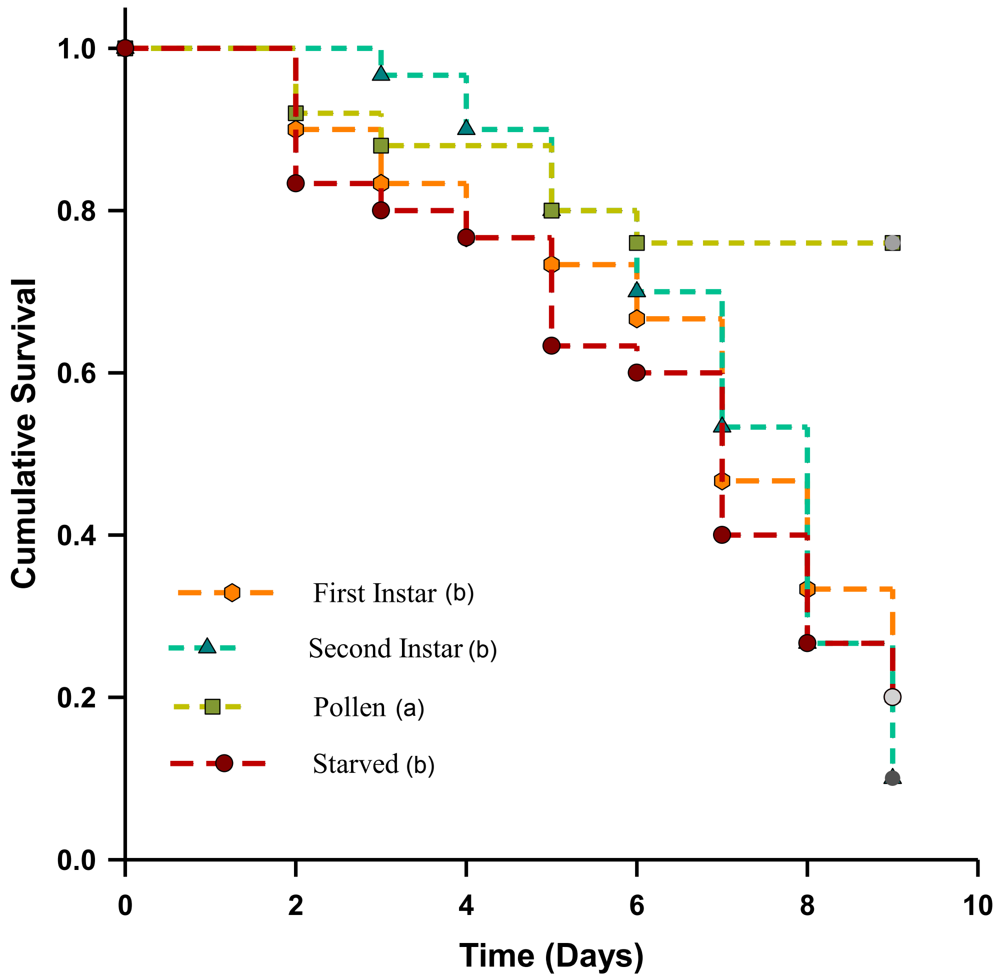

Figure 10. Survival of *A. tamatavensis* juveniles fed on different food sources. Orange diamonds: First instar thrips larvae; dark cyan triangles: second instar thrips larvae; green squares: pollen; red circles: starved treatment.

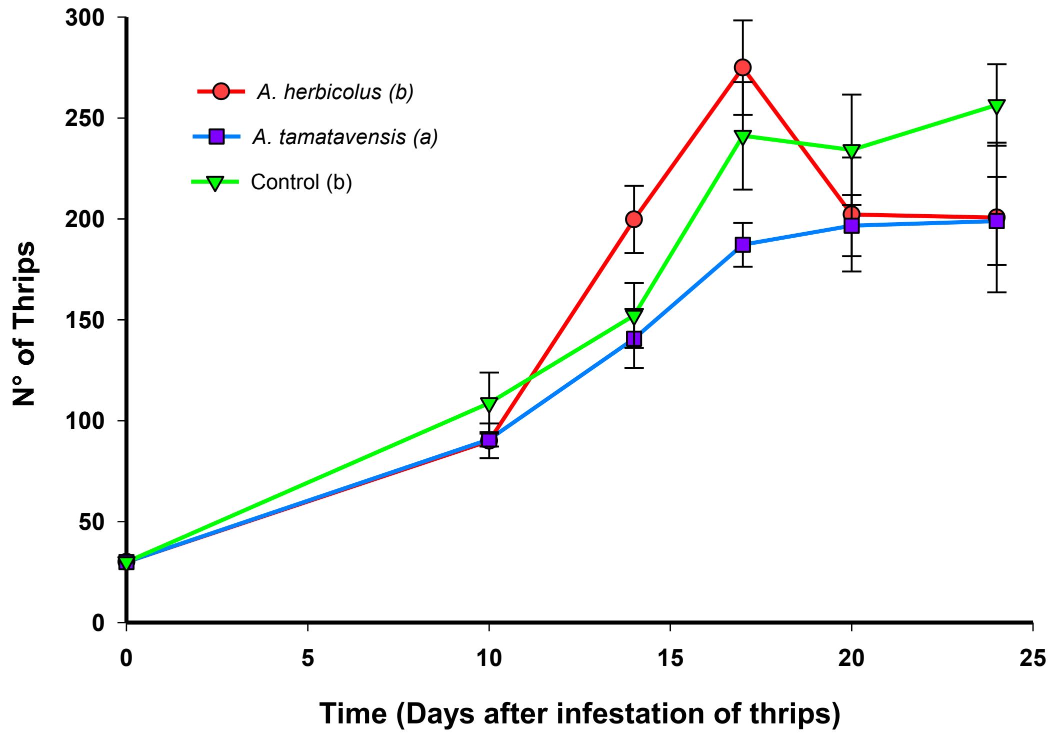

Figure 11. Numbers (Mean±SE) of *Echinothrips caribeanus* (all stages) as a function of time on sweet pepper plants with *A. herbicolus* (blue diamond), with A. tamatavensis (oranges square), and without predator (gray triangle). (P<0.05).

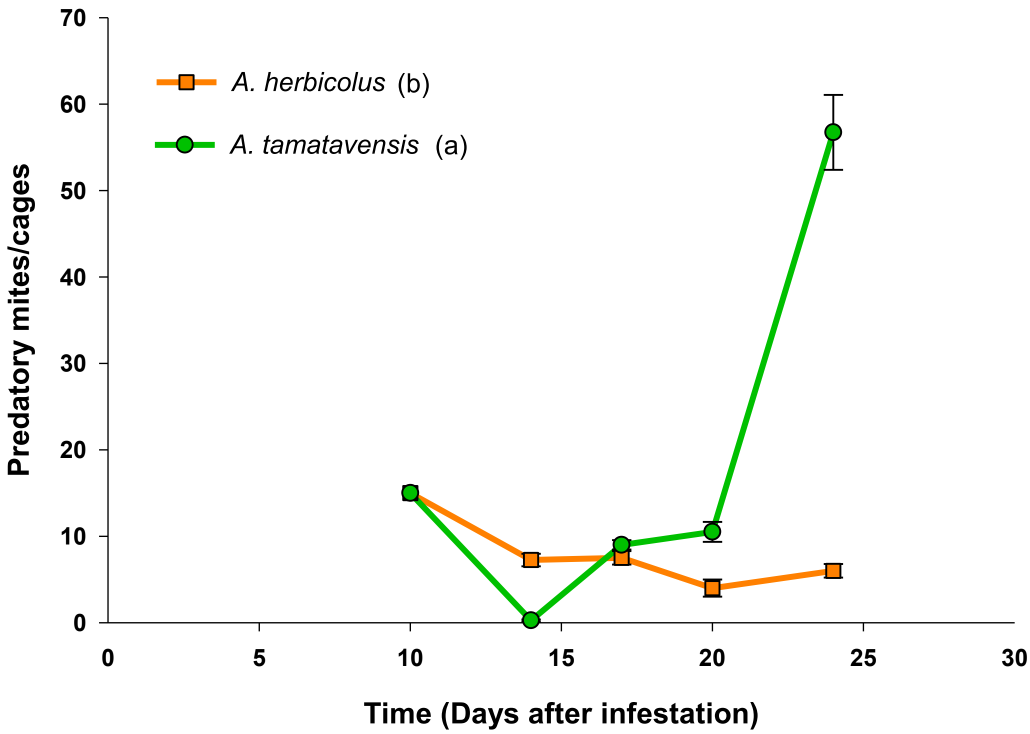

Figure 12. Number (means±SE) of predatory mites (all stages) as a function of time on sweet pepper plants after infestation with *Echinothrips caribeanus*. Plants with *A. herbicolus* (oranges square) and with *A. tamatavensis* (green circles) (P<0.05).

Discussion

We investigated whether the two generalist predatory mites *A. herbicolus* and *A. tamatavensis* are potential biological control agents of the thrips species *E. caribeanus*. Our results show that in laboratory conditions the two species of mites were able to feed on the 1st and 2nd instar larvae of thrips but not high enough predation rate to be considered a promising biocontrol agents. Ghasemzadeh et al. (2017) evaluated the rate of predation of mites such as *Amblydromalus limonicus, Amblyseius swirskii and Euseius ovalis* feeding on immature *Echinothrips americanus* obtaining a daily predation rate of at least 3 thrips per mite, which represents 80% more predation in relation to our results (Fig. 6). Similarly, Lam et al. (2019) evaluated the predation rate of *A. herbicolus* and *Neoseiulus cucumeris* feeding on immature of *Sericothrips staphylinus* finding predation rates of 73% and 68% higher than our results. Other research carried out by Zilahi-Balogh et al. (2006) showed a daily predation rate of 6 first instar thrips larvae, which represents 90% more predation compared to our results.

Oviposition rate to both species of predatory mites was highest when fed on pollen, these results indicated that immature of *E. caribeanus* are not a quality food for any of the species of predatory mites evaluated. Some research evaluated daily oviposition rate of *A. swirkii* feeding on first instar larvae of *F. occidentalis* and *T. tabaci,* the result showed rate of 1- 1.4 eggs/female/day, represent 50% more oviposition compare with our results (Wimmer et al. 2008). Even, Ghasemzadeh et al. (2017) showed oviposition rate of *A. limonicus* of 1.4 egg/female/day feeding on immature of *E. caribeanus* which is higher compare with our results (Fig. 7 and 8).

Furthermore, we found that adult females of the predatory mite *A. herbicolus* were able to lay eggs after having been without food for five days (Fig. 7). Previous studies have shown that some species of predatory mites as *N. cucumeris* have the ability to retain their eggs (Montserrat et al. 2007). However, this behavior is used to prevent predation of their eggs by other predators. Although *A. herbicolus* was not exposed to other predators, we hypothesize that this species of predatory mite exposed to situations of starvation perhaps triggers egg retention. This requires future investigations.

In our results of development and survival, we observed that both species of predatory mites developed into adulthood and survived with pollen treatment, these results indicate that larvae of *E. caribeanus* are not a suitable food source for either of two predatory mites. The predatory mites did not complete its life cycle when fed on this prey and most of them die as protonymph in the treatments with immature of thrips as their only food source (Fig. 9 and 10). Previous research showed that pollen is an excellent food source for predatory mites. It can improve survival and reproduction in the absence of their primary prey (Nomikou et al. 2010; Janssen and Sabelis 2015; Duarte et al. 2015; Vacacela Ajila et al. 2019).

Our population dynamics results showed that statistically thrips population was lower in the treatment of the predatory mite *A. tamatavensis*. However, similar previous research shows a much lower thrips density of approximately 95% less population in relation to the results obtained in this study (Messelink et al. 2008) (Fig. 11). Population dynamics of predatory mites was significantly different due an increase on *A. tamatavensis* population at the end of the evaluation period (Fig. 12). This may be due to in the last day of evaluation, adults and immatures of *A. tamatavensis* were found inside the flower near the pollen and nectar. There are some studies demonstrating how nectar and pollen can contribute to survival and reproduce of naturals enemies (van Rijn P.C.J. 1999; Lundgren and Seagraves 2011). This could be a reason why the density of A. tamatavensis was higher at the end of experiments. Additionally, it was observed that this species of predatory mites use plant structures, such as leaves and floral button in development to protect and hide itself (personal observation). Furthermore, there are some studies demonstrating how predators can use plant structure as a refuge (Walter 1996; Onzo et al. 2003). *A. tamatavensis* showed an increase in population, but did not show control of thrips populations. The density of thrips observed at the end of the experiment (Fig. 11) is above the economic threshold permitted to other thrips species, which means that this amount of thrips can cause significant damage to the crop (Shipp et al. 1998; Park et al. 2007; da S. Paes et al. 2019).

The addition of pollen in the plants was fundamental to keep on the survival of the mites. There are quite some studies demonstrating how the biological control agents released together with pollen, as a supplementary food, help the development and survivorship of natural enemies (Nomikou et al. 2003, 2010; Brown et al. 2010; Janssen and Sabelis 2015; Vacacela Ajila et al. 2019) . During the experiment, one of the limitations was the difficulty of observing the mites at the time of the evaluations, because these are small organisms. This could be one reason which the amounts of A. tamatavensis observed at the beginning of the evaluations were low. Another limitation observed was that the greenhouse used was acclimatized, so the temperature variations were greater and could interfere with the growth of the organisms evaluated.

The study of native species of predatory mites is an important issue within the biological control field since we do not know the impact on non-target organisms when an exotic biological control agent is introduced (Lenteren et al. 2003; van Lenteren et al. 2006; Cavalcante et al. 2017a). Additionally, the mites evaluated in this experiment are classified as generalists (McMurtry and Croft 1997). To our knowledge, this is the first report evaluating on *A. tamatavensis* as a potential thrips predator. It remains to be investigated whether this species is a potential control agent of other thrips species as *Frankliniella occidentalis* (Pergande), *Thrips palmi* Karny, *Thrips tabaci* Lindeman and *Echinothrips americanus* Morgan. These species are widely distributed in the world and recognized for the economic damages caused to ornamental and horticultural crops. So, future research could be emphasized to study of this species of predatory mite as biological control agents of other thrips species or another type of organism (insect or mite). Also, the use the nectar and pollen of the flowers of sweet pepper plants as an alternative food to survive and develop, as well as behaviors such as cannibalism and intraguild predation between these native species.

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GENERAL CONCLUSIONS

- In laboratory conditions, *A. herbicolus* presented a higher predation rate feeding on first and second instar of thrips compare with *A. tamatavensis*. Oviposition and survival rate from both species of predatory mites were higher in pollen treatment. Data on development was not presented because neither species of predatory mites reached adulthood feeding on immature of thrips.
- Under greenhouse conditions, the thrips population dynamics was lower on *A. tamatavensis* treatment compare with *A. herbicolus*. Population dynamics of the predatory mite *A. tamatavensis* was higher than the other predator due to an increase at the end of the evaluation period.