ANDRÉ COSTA CARDOSO

POTENTIAL OF PREDATORY MITES TO CONTROL THE WESTERN FLOWER THRIPS *Frankliniella occidentalis* (THYSANOPTERA: THRIPIDAE) IN BRAZIL

Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Angelo Pallini

Co-advisers: André Lage Perez
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Angelo Pallini
Adviser

To my great grandmother and godmother
Dinah Alves Corgosinho.

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ABSTRACT

CARDOSO, André Costa, D.Sc., Universidade Federal de Viçosa, February, 2023. **Potential of predatory mites to control the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) in Brazil**. Adviser: Angelo Pallini. Co-advisers: André Lage Perez and Madelaine Venzon.

Western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is a major invasive pest worldwide and a vector of plant viruses of economic importance. Several biological control agents are efficient in control *F. occidentalis* and many of them already commercialized by biological control companies around the world. Phytoseiid mites (Acari: Phytoseiidae) are among the most used natural enemies applied in biological control programs of *F. occidentalis*. Despite the richness of Brazilian phytoseiid fauna, no species are been used in biological control of this pest in Brazil. Therefore, the aim of this study was to select potential native phytoseiid mites to be used in biological control of this pest in Brazil. In the first chapter, it was evaluated the potential of three predatory mites, *Amblyseius herbicolus* Chant and *Amblyseius tamatavensis* Blommers and *Proprioseiopsis ovatus* Garman to control *F. occidentalis*. *Proprioseiopsis ovatus* showed low predation and oviposition rate on *F. occidentlais* first instar larvae and, therefore, this species has a low potential to be used in biological control of this pest. In the other hand, *A. herbicolus* and *A. tamatavensis* were capable to develop and reproduce preying on *F. occidentalis* larvae and, therefore, are promising agents to be used in biological control of *F. occidentalis*. In the second chapter, it was evaluated the potential of the two predatory mite species, *A. tamatavensis* and *A. herbicolus*, to control *F. occidentalis* on isolated bell pepper plants using pollen as alternative food. Both predatory mites were able to reduce pest population and significantly reduce pest damage. In the third chapter, it was evaluated the potential of *A. tamatavensis* to control *F. occidentalis* and simultaneously also other key pest of bell pepper crop, the whitefly *Bemisia tabaci*. Experiments were conducted on bell pepper plants and were also evaluated plants yield with and without *A. tamatavensis*. *Amblyseius tamatavensis* was able to control both pests at the same time and at the end of the experiment, plants that received predatory mites also produced heavier fruits. In conclusion, it was showed that the predatory mites *A. tamatavensis* and *A. herbicolus* have potential to be used in biological control programs of the western flower thrips in Brazil. Phytoseiid mites are known to be

efficient control agents for this pest in several countries, in addition of being cheap to produce them on a large scale. Therefore, the use of these predators may represent an advance in the management of this pest in the country.

Keywords: Biological control. *Capsicum annuum. Bemisia tabaci.* Alternative food. Pollen.

RESUMO

CARDOSO, André Costa, D.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Potencial de ácaros predadores para controle do tripes ocidental das flores *Frankliniella occidentalis* (Thysanoptera: Tripidae) no Brasil**. Orientador: Angelo Pallini. Coorientadores: André Lage Perez e Madelaine Venzon.

O tripes ocidental das flores *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) é uma das principais pragas invasoras em todo o mundo e vetor de viroses de plantas de importância econômica. Vários agentes de controle biológico são eficientes no controle de *F. occidentalis* e muitos deles já comercializados por empresas de controle biológico ao redor do mundo. Ácaros fitoseídeos (Acari: Phytoseiidae) estão entre os inimigos naturais mais utilizados em programas de controle biológico de *F. occidentalis*. Apesar da riqueza da fauna brasileira de fitoseídeos, nenhuma espécie vem sendo utilizada no controle biológico desta praga no Brasil. Portanto, o objetivo deste estudo foi selecionar potenciais ácaros fitoseídeos nativos para serem utilizados no controle biológico desta praga no país. No primeiro capítulo foi avaliado o potencial de três ácaros predadores, *Amblyseius herbicolus* Chant e *Amblyseius tamatavensis* Blommers e *Proprioseiopsis ovatus* Garman para controlar *F. occidentalis. Proprioseiopsis ovatus* apresentou baixa taxa de predação e oviposição em larvas de primeiro ínstar de *F. occidentlais* e, portanto, esta espécie tem baixo potencial para ser utilizada no controle biológico desta praga. Por outro lado, *A. herbicolus* e *A. tamatavensis* foram capazes de desenvolver e reproduzir larvas predadoras de *F. occidentalis* e por isso mostraram-se promissores agentes de controle dessa praga. No segundo capítulo foi avaliado o potencial de duas espécies de ácaros predadores, *A. tamatavensis* e *A. herbicolus*, no controle de *F. occidentalis* em plantas isoladas de pimentão com pólen como alimento alternativo. Ambos os ácaros predadores foram capazes de reduzir a população do tripes e reduziram significativamente os danos causados por essa praga. No terceiro capítulo foi avaliado o potencial de *A. tamatavensis* para controlar *F. occidentalis* e simultaneamente outra praga-chave da cultura do pimentão, a mosca-branca *Bemisia tabaci*. Foram conduzidos experimentos com plantas de pimentão e também avaliada a produção de plantas com e sem *A. tamatavensis. Amblyseius tamatavensis* foi capaz de controlar as duas pragas ao mesmo tempo e ao final plantas que receberam ácaros predadores também produziram frutos mais pesados. Em conclusão, foi demonstrado que os

ácaros predadores *A. tamatavensis* e *A. herbicolus* têm potencial para serem utilizados em programas de controle biológico do tripes ocidental das flores no Brasil. Os ácaros fitoseídeos são conhecidos por serem eficientes agentes de controle dessa praga em diversos países, além de serem baratos para produção em larga escala. Portanto, a utilização desses predadores pode representar um avanço no manejo dessa praga no país.

Palavras-chave: Controle biológico. *Capsicum annuum*. *Bemisia tabaci.* Alimento alternativo. Pólen.

SUMMARY

GENERAL INTRODUCTION....11

References....13

Chapter 1 – The potential of three predatory mite species (Acari: Phytoseiidae) to control the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) 15

Abstract....16

Introduction 17

Materials and Methods....19

Results 23

Discussion....25

References....27

Figure legends 34

Figures 35

Chapter 2 - *Amblyseius tamatavensis* and *Amblyseius herbicolus* (Acari: Phytoseiidae) reduces *Frankliniella occidentalis* (Thysanoptera: Thripidae) population on bell pepper plants....38

Abstract....39

Introduction 40

Materials and Methods....41

Results 44

Discussion....45

References....48

Figure legends 52

Figures 54

Chapter 3 – Efficiency of *Amblyseius tamatavensis* to reduce simultaneously two key pests on bell pepper plants....59

Abstract 60
Introduction 61
Materials and methods 63
Results 66
Discussion 67
References 69
Figure legends 75
Figures 76
General Conclusions 78

GENERAL INTRODUCTION

The thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), commonly referred to as the western flower thrips, is a globally significant invasive pest that affects a wide range of crops including vegetables, fruits, and ornamentals (Reitz et al. 2020; He et al. 2020). Furthermore, this species has been identified as a vector for multiple economically significant plant viruses (Gilbertson et al. 2015). Its initial description dates back to 1895 in California, USA, and since the late 1970s, it has spread to at least 57 countries across nearly every continent of the world (Kirk and Terry 2003; Reitz et al. 2020).

This pest is characterized by its diminutive size and is known to undergo a distinctive type of hemimetabolic development referred to as neometaboly. This life cycle is marked by the presence of quiescent pupal-like stages that precede adult metamorphosis (Belles 2011). The deposition of eggs occurs within the plant tissue and the immature stages of development pass through two distinct feeding phases (1st and 2nd instar larvae) and two non-feeding stages (prepupae and pupae). Pupae exits the host plants to undergo pupation in the soil before the emergence of the winged adults (Reitz et al. 2020). Both the adult and larval stages of the pest have a propensity to aggregate in flowers or other concealed regions on host plants, including developing fruits, leaves, and flower buds (Hansen et al. 2003). Additionally, the pest exhibits an extremely polyphagous nature and has been documented feeding on over 250 crop species belonging to more than 60 families (Lewis 1997). All these characteristics pose significant challenges to its interception during commercial transportation of plant materials, thereby facilitating its rapid dissemination and establishment worldwide (Reitz et al. 2020)

Directly feeding and oviposition damage result in quantitative yield losses by reducing plant photosynthesis or by reducing quality of fruits and flowers (Reitz 2009). Losses incurred on high-value horticultural and ornamental crops can be particularly severe because even slight aesthetic damage is often deemed unacceptable, thereby rendering the products unmarketable (Childers and Achor 1995; Reitz et al. 2020). Extensive feeding causes abortion of flowers and fruits (Childers 1997). Furthermore, the pest serves as a vector for a multitude of orthotospoviruses (Parker et al. 1995), several of which have the potential to cause huge economic losses (Kirk and Terry

2003; Pappu et al. 2009; Gilbertson et al. 2015). The tomato spotted wilt virus (TSWV), as an example, is identified as the most economically significant disease on several plant species, particularly those in the families Solanaceae (tomato, pepper, potato), Fabaceae (peanut), Asteraceae (lettuce), and Cucurbitaceae (cucumber, squash, melon) (Schneweis et al. 2017). It has a wide host range of over 1000 plant species belonging to more than 85 families (Gupta et al. 2018), and, in certain crops, this virus can inflict severe losses (Pappu et al. 2009).

Worldwide, generalist phytoseiid mites (Acari: Mesostigmata) are widely recognized as one of the most commonly used natural enemies in the implementation of biological control programs for this pest (van Lenteren et al. 2018; He et al. 2020). Despite the abundance of phytoseiid fauna in Brazil, no commercialized predatory mite species are currently available for controlling *F. occidentalis* in the country (MAPA 2021; Demite et al. 2021). Therefore, the aim of this study was to evaluate the potential of phytoseiid mite species that occurs naturally in Brazil to control this pest in the country.

In the first chapter, it was evaluated the capacity of three predatory mite species, *Amblyseius tamatavensis* (Blommers), *Amblyseius herbicolus* (Chant) and *Proprioseiopsis ovatus* (Garman) to prey and reproduce on first instar larvae of *F. occidentalis.* Because *P. ovatus* had a very low predation and oviposition rate on the first experiment, it was excluded for the others experiments. A second experiment was conducted to evaluate the predation and oviposition of the predatory mites *A. herbicolus* and *A. tamatavensis* species on first and second instar larvae of *F. occidentalis* with pollen as a control for oviposition. Finally, we accessed the survival and development time of *A. herbicolus* and *A. tamatavensis* fed with *F. occidentalis* first instar larvae. In the second chapter, it was evaluated the potential of *A. tamatavensis* and *A. herbicolus* to control the western flower thrips on bell pepper plants. For this, a population dynamics experiment was conducted on isolated plants inside cages to verify the capacity of these predators to reduce *F. occidentalis* population. In the third chapter, it was evaluated the potential of the predatory mite *A. tamatavensis* to control the western flower thrips *F. occidentalis* and other important pest of bell pepper plants, the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). For that, a population dynamics experiment was conducted on bell pepper plants to verify the capacity of this predatory mite to control both pests simultaneously. The damage of thrips on fruits and leaves were also evaluated

Both the predatory mites *A. tamatavensis* and *A. herbicolus* showed potential to be used in biological control programs of the western flower thrips in Brazil. Phytoseiid mites are known to be efficient control agents for this pest in several countries, in addition of being cheap to produce them on a large scale. Therefore, the use of these predators may represent an advance in the management of this pest in the country.

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Chapter 1 – The potential of three predatory mite species (Acari: Phytoseiidae) to control the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae)

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Abstract

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae), also known as western flower thrips, is a highly invasive pest worldwide that can also transmit various plant viruses. There are several biological control agents that have been effective in controlling this pest, and many of these agents are commercially available from biological control companies worldwide. Commonly, generalist phytoseiid mites (Acari: Phytoseiidae) are used as natural enemies in the biological control of this pest. Despite the richness of Brazilian phytoseiid fauna, no species are been used in biological control of *F. occidentalis* in Brazil. Therefore, the aim of this study was evaluating the capacity of three predatory mite species, *Amblyseius tamatavensis*, *Amblyseius herbicolus* and *Proprioseiopsis ovatus* to prey and reproduce on first instar larvae of *F. occidentalis.* Because *P. ovatus* had a very low predation and oviposition rate on the first experiment, it was excluded for the others experiments. A second experiment was conducted to evaluate the predation and oviposition of the first two predatory mite species on first and second instar larvae of *F. occidentalis* with pollen as a control for oviposition. Finally, we accessed the survival and development time of *A. herbicolus* and *A. tamatavensis* fed with *F. occidentalis* first instar larvae. *Proprioseiopsis ovatus* showed low predation and oviposition rate on *F. occidentalis* first instar larvae and, therefore, this species has a low potential to be used in biological control of this pest. On the other hand, *A. herbicolus* and *A. tamatavensis* preyed about four first instar larvae per day and were capable to reproduce as well as that fed on pollen. Both predators are also able to prey second instar larvae, which are much larger than newly emerged larvae. Both predators are also able to develop and survive fed on thrips larvae and, therefore, have potential to be used in biological control of this pest.

Keywords: Biological control; Predation rate. Oviposition rate. *Amblyseius tamatavensis. Amblyseius herbicolus. Proprioseiopsis ovatus.*

Introduction

The western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is an economically important pest of vegetables, fruits and ornamental crops worldwide (Kirk and Terry 2003; Reitz 2009; Reitz et al. 2020). It was first described in 1895 in California, USA and, since the late 1970s, it has dispersed to at least 57 countries in almost every continent of the world (Kirk and Terry 2003; Reitz et al. 2020). This thrips species is extremely polyphagous and has been recorded feeding on more 250 crop plant species and on many uncultivated plants (Lewis 1997; Nyasani et al. 2013).

Directly feeding and oviposition damage result in quantitative yield losses by reducing plant photosynthesis or by reducing quality of fruits and flowers (Reitz 2009). On high-value horticultural and ornamental crops losses can be even higher because aesthetic damage is unacceptable making products unmarketable (Childers and Achor 1995; Reitz et al. 2020). Extensive feeding can also result in flower and fruit abortion (Childers 1997). In addition, this pest is a vector of numerous orthotospoviruses (Parker et al. 1995) and many of them can be really devastating, leading to huge economic losses (Kirk and Terry 2003; Pappu et al. 2009; Gilbertson et al. 2015). The tomato spotted wilt virus (TSWV) is the most economically important (Schneweis et al. 2017). This virus infects more than 1000 plant species belonging to more than 85 families (Gupta et al. 2018) and, in some crops, losses can be really severe (Pappu et al. 2009) making difficult the establishment of integrated pest management systems (Morse and Hoddle 2006).

Western flower thrips is a very small insect and its life cycle is a particular type of hemimetaboly known as neometaboly where quiescent pupal-like stages precede adult metamorphosis (Belles 2011). Eggs are deposited inside plant tissue and immatures pass through two feeding stages (1st and 2nd instar larvae) and two non-feeding stages (prepupae and pupae). Adults and larvae congregate in flowers or other hidden areas on plants such as developing fruits, leaves, and flower buds (Hansen et al. 2003). Pupae drop from host plants to complete pupation in the soil before the emergence of winged adults (Reitz et al. 2020).

Insecticides remain the main strategy used to control this pest in the most of the agricultural areas (Bielza 2008). Because populations of this pests are often under constant insecticide pressure, several resistant populations have been reported

worldwide (Gao et al. 2012a). In addition, their preference for residing in tightly enclosed spaces of plants (thigmotactic behavior), and the presence of edaphic stages make its management difficult to the growers because reduce the exposure of individuals to contact insecticides (Tommasini and Main 1995; Jensen 2000). Its small size and high levels of aggregation also make its detection difficult when density is low in the crop (Sutherland and Parrella 2011) and populations of this pest can increase quickly at optimum conditions due to it high intrinsic rates of population increase (Reitz 2009).

Several biological control agents are efficient controlling *F. occidentalis* and many of them are already commercialized by biological control companies around the world (van Lenteren et al. 2018; Van Lenteren et al. 2019). Generalist phytoseiid mites (Acari: Mesostigmata) are among the most used natural enemies applied in biological control programs of this pest (van Lenteren et al. 2018; He et al. 2020). They are cheap to be mass reared and most of them can feed on others pests like whiteflies and phytophagous mites which help to reduce the number of natural enemies to be introduced in the crop (Messelink et al. 2008; Van Lenteren et al. 2019). Moreover, many generalist phytoseiid mites, can feed on pollen of several plant species and astigmatid mites (McMurtry and Scriven 1966; Leman and Messelink 2015; Pirayeshfar et al. 2021) making possible to establish populations of these predators in a crop before pest invasions (Janssen and Sabelis 2015; Kumar et al. 2020; Pijnakker et al. 2020).

Despite the abundance of phytoseiid fauna in Brazil, there is currently a lack of research exploring the potential of native predatory mites for controlling *F. occidentalis* within the country (MAPA 2021; Demite et al. 2021). Therefore, the aim of this study was to evaluate the potential of three predatory mite species that occurs naturally in Brazil to control this pest. The predatory mites tested were *Amblyseius tamatavensis*, *Amblyseius herbicolus* and *Proprioseiopsis ovatus*. The two first was selected because others predatory mites of the same genus are among the predatory mites most commercialized in other countries to control *F. occidentalis*. *Amblyseius swirskii* is probably the most famous being capable to control several thrips species as well whiteflies and other harmful phytophagous mites (Calvo et al. 2012, 2015; Buitenhuis et al. 2015; Janssen and Sabelis 2015). *Amblyseis tamatavensisis* is already commercialized in Brazil to control the whitefly *Bemisia tabaci* (MAPA 2021) and *A. herbicolus* also showed a good predation rate on this whitefly species (Cavalcante et al. 2015b) and showed potential to control the broad mite *Polyphagotarsonemus latus*

(Duarte et al. 2015) and one of the most important pest of citrus orchards, the psyllid *Diaphorina citri* (Kalile et al. 2020). *Proprioseiopsis ovatus*, in turn, was selected because it was found occurring naturally on strawberry plants that is one of the crops that *F. occidentalis* is a key pest. Additionally, this species developed high populations in laboratory rearings which indicate that this species can be easily mass-produced.

Materials and Methods

Cultivation of clean plants

Bell pepper plants were obtained by transplant commercial seedlings (var. Itamara) to 3-liter pots with moist substrate (Mecplant, MecPrec®). When the plants reached 20 cm high, they were supported with bamboo sticks tied with a string and grown for two more weeks until they were used in the rearings and experiments (average of 15 leaves and 40 cm high). Bell pepper plants were fertilized three times a week with a solution with 0,060 g of calcium nitrate $Ca(NO_3)_2$; 0,060 g of potassium nitrate (KNO_3) and 0,070 g of Monoammonium phosphate (MAP; 2% N, 61% P_2O_5). Plants were cultivated inside a greenhouse with automatic drip irrigation and equipped with exhaust fans, which regulated temperature to remain below 27ºC.

Pollen

Pollen used in *F. occidentalis* rearing and in experiment was collected from *Typha sp.* plants (Viçosa, Minas Gerais, Brazil) and dried in an oven at 40℃ for 12 h and later stored in a sealed 15-ml polypropylene falcon tubes in the freezer (-16℃). Small amounts were periodically placed in 1.5-ml eppendorf tubes and stored in the refrigerator (8℃) for use during few weeks.

Frankliniella occidentalis *rearing*

Frankliniella occidentalis individuals were collected from commercial rose greenhouses in Barbacena, Minas Gerais, Brazil and taken to Federal University of Viçosa, Viçosa, Minas Gerais, Brazil. The thrips rearing was adapted from the method described by DeGraaf and Wood (2009). They were reared inside 6-L plastic food storage boxes with two rectangles (12 x 7 cm) cut out of 2 opposite walls in the box

that were covered with a fine mesh (160 µm). The open top of the box was sealed with clingwrap pulled tight and secured with a rubber band. Each box contained two plastic dishes (ø = 5.5 cm; 1.4 cm high) with wet cotton inside, and one dish with 200 mg of *Typha* sp. pollen and a drop of honey solution (10% v/v). Two absorbent paper towels (19x21cm, Elegante®) were placed in the base of the box for thrips pupation. Adults were allowed to oviposit in two Japanese cucumber fruits with the ends sealed with paraffin for 3-4 days and then, the fruits were removed and put into a new plastic box as described above with two new fruits. Pollen was provided twice a week until the pupae emerged into adults. Newly emerged adults were then aspirated into a pipet tip and returned to the oviposition box.

Predatory mite rearings

Amblyseius tamatavensis population was obtained from *F. occidentalis* and *Tetranychus urticae* rearings of the Laboratory of Acarology at the Federal University of Viçosa. *Amblyseius herbicolus* was collected from orange jasmine plants*, Murraya paniculata,* (L.) Jack*,* Rutaceae, in the rural area of Viçosa, Minas Gerais, Brazil. *Proprioseiopsis ovatus* was collected from strawberry (Fragaria X ananassa Duch) cultivated in a greenhouse at the Federal University of Viçosa. These predators were reared on plastic arenas (10 x 15 cm²) placed on foam, which were placed in a tray with about 2 cm of water to prevent the escape of the mites and to serve as a source of water. The method was adapted from that described by McMurtry and Scriven (1965). Predators were feed twice a week. *Amblyseius* sp. predators were fed with 2 mg of *Typha sp*. pollen 200 mg of wheat germ containing *Thyreophagus cracentiseta* Barbosa, OConnor & Moraes (Acari: Acaridae) and, *P. ovatus* was fed with wheat germ containing *Tyrophagus putrescentiae* Schrank (Acari, Astigmata)*.* Wheat germ with prey mites was offered inside a cap of a 1.5ml Screw Cap Microtube. Rearing arenas were kept in a room at 25 ± 2° C with 70 ± 10% RH and 12 hours photophase.

Obtaining first and second instar larvae of thrips for the experiments

To obtain first and second instar larvae of *F. occidentalis* for experiments we made consecutive cohorts on bell pepper leaf discs (ø = 8 cm). Leaf discs were placed with the upper surface on a three mm of 1% (w/v) agar solution inside Petri dishes (ø = 8 cm). This dish was placed in a plastic pot (ø = 15 cm diameter; 9 cm high) covered

with a plastic lid. Ventilation was possible by a hole (3.5 cm in diameter) on the lid covered with a fine mesh (160 µm). Inside each pot, it was also offered 100 mg of pollen and a drop of honey solution (10% v/v) on a small plastic dish (ø = 3 cm). After 24 h, the adult thrips on the leaf discs were blown into the plastic boxes and a new petri dish with a new leaf disc was placed inside each box. Petri dishes with thrips eggs were kept on a wet cotton inside a tray. Larvae that emerged from the eggs were kept on the same leaf discs until they reached the stage needed for experiments.

Cohorts of predatory mites

To standardize the age of predatory mites, adult females of predatory mites were transferred to a new arena with *Typha sp.* pollen. After 24 hours, adult females were removed, and their eggs were kept with sufficient pollen until they had developed into adults. Predatory mites used in the experiments were 9±1 days old since egg.

***Predation and oviposition of* A. tamatavensis*, A. herbicolus and* P. ovatus *on first instar larvae of* F. occidentalis**

The aim of this experiment was making a first selection among the tested predators. Because, first instar larva is the most vulnerable developmental stage of *F. occidentalis* to predation by predatory mites (van der Hoeven and Van Rijn 1990), we set up an experiment with the aim to compare the number of first instar larvae killed and the number of eggs laid by each predatory mite species during four days. The experiments were conducted in transparent plastic dishes (ø = 2.5 cm; 1.2 cm high) with a sweet pepper leaf disc, of the same diameter, placed inside. Leaf discs were infested with 12 first instar larvae of *F. occidentalis* from the cohorts. One adult female predatory mite from the cohort was transferred to each dish. Then, dishes were covered with a plastic lid with a small hole (6 mm diameter) covered with fine mesh (160 µm) to allow ventilation. Predatory mites were transferred to a new arena with the same number of thrips individuals of the same stage or with the same amount of pollen every 24 h for 3 days. Dishes were inspected under a stereomicroscope and the number of larvae bodies that were dead and sucked were counted as well as the number of eggs laid by the predators. The dishes were kept at a room with controlled conditions (25 ± 2 ° C, 70 ± 10% RH and 12 hours photophase) and 11 replicates were done per treatment. Oviposition of the first day was excluded from analysis because of the possible effect

of previous diet (Sabelis 1990). Predation and oviposition data were analyzed with linear mixed effects models (LME) with individual predator as random factor and treatments (predator species) and time as fixed factors. Contrasts among treatments were obtained with the package emmeans with a Tukey correction for multiple comparisons (Lenth et al. 2019). All statistical analyses were done using the software R 4.2.0 (R. Core Team 2021).

Predation and oviposition of* A. tamatavensis *and* A. herbicolus *on first and second instar* F. occidentalis *larvae

Because *P. ovatus* did not show promising results in the first experiment the second experiment was conducted only with the *Amblyseius* species. Is known that predatory mites tend to kill larger thrips larvae at a lower rate, as these larvae have developed defensive mechanisms such as counterattacks and secretion of rectal fluids to deter their predators (Bakker and Sabelis 1989; Steiner et al. 2003). Therefore, the aim of this experiment was investigating the capacity of these predatory mite species to prey and reproduce on second instar larvae of *F. occidentalis* and compare this result with the predation and oviposition on first instar of *F. occidentalis* larvae and oviposition on *Typha* sp. pollen. The experiments were conducted on bell pepper leaf discs (ø = 2.5 cm) on a wet cotton inside Petri dishes (ø = 5 cm; 1.5 cm high). A small piece of black PVC plastic was placed on each leaf disc to provide shelter to predatory mites and reduce scape of the mites. Each predatory mite species was submitted to three different treatments: (1) 10 first instar thrips larvae; (2) 10 second instar thrips larvae; and (3) 0.2 mg *Typha* sp. pollen as a control for oviposition because it is known to be a good food source to these predatory mites. One adult female predatory mite from the cohorts was transferred to each dish. The experiment was conducted exactly in the same way of the first experiment described above and eighteen replicates were done per treatment.

Survival and development of* A. tamatavensis *and* A. herbicolus *on first instar larvae of* F. occidentalis *and on* Typha sp*. pollen

This experiment was designed to: (1) verify if *A. tamatavensis* and *A. herbicolus* could reach adulthood when fed with *F. occidentalis* first instar larvae, and (2) compare developmental time and survival of predatory mites fed with *F. occidentalis* first instar

larvae with others fed with *Typha* sp. pollen, which is known to be a good food source for these predators (Cavalcante et al. 2015b). The experiments were conducted in transparent plastic dishes (ø = 2.5 cm; 1.2 cm high) with a sweet pepper leaf disc placed upside down on water agar solution. Dishes were provided with 10 first instar larvae of *F. occidentalis* from cohorts (explained above) or with 0,2 mg of *Typha* sp. pollen. After adding the thrips or pollen, one predatory mite larva, collected randomly from cohorts, was placed inside each dish. The dishes were then covered with a lid with a small hole (6 mm diameter) covered with fine mesh (160 µm) to allow ventilation. The experiment was evaluated every 24 hours. At that time, survival of individuals was recorded and alive predatory mites were transferred to a new dish with the same number of thrips larvae or amount of pollen. Evaluations were carried out until mites reached adulthood when sex was determined by morphological features. Males are typically smaller and slender than females, with a more pointed posterior end (Krantz and Walter 2009).There were 25 replicates for thrips larvae and 16 for pollen. The experiment was done in a room with controlled conditions (25 ± 2 ° C, 70 ± 10% RH and 12 hours photophase). Effects of diet on survival and developmental were analyzed with a Cox's proportional hazards model (Survival package in R, Therneau 2022). All statistical analyses were done using the software R 4.2.0 (R. Core Team 2021).

Results

Predation and oviposition of A. tamatavensis*,* A. herbicolus *and* P. ovatus *on first instar of* F. occidentalis

There was no statistically significant effect of time on predation rate of predatory mite species tested (d.f. = 5, Likehood ratio = 0.025, p = 0.61). However, there was a significant effect of predatory mite species on predation rate (d.f. = 3, Likehood ratio = 59.21, p < 0.0001). Predation rate of *P. ovatus* was lower than *A. tamatavensis* and *A. herbicolus* with about two larvae preyed per day (Fig. 1A), and predation rate of these others predators did not differ significantly with about 6 larvae preyed per day. At the same way, there was no significant effect of time on oviposition of tested predators (d.f. = 5, Likelihood ratio = 0.024, p = 0.98; d.f. = 4, Likelihood ratio = 2.34, p = 0.12).

Oviposition rate of *P. ovatus* was significantly lower than the other species (Fig. 1B), and oviposition of *A. tamatavensis* was significantly higher than that of *A. herbicolus* (Fig. 1B).

Predation and oviposition of* A. tamatavensis *and* A. herbicolus *on first and second instar* F. occidentalis *larvae

Predation rate of *A. herbicolus* and A*. tamatavensis* did not differ though time on first and second instar larvae of *F. occdentalis* (d.f. = 5, Likelihood ratio = 2.24, p = 0.1344 and d.f. = 5, Likelihood ratio = 0.047, p = 0.826 respectively). There was also no significant difference on predation rate of both predatory mite species on each thrips larvae stage (Fig. 2A; d.f. = 1, F-value = 2.65, p-value = 0.113 and d.f. = 1, F-value = 2.194, p-value = 0.149 respectively). However, predation rate of both predatory mite species was higher on first instar larvae than on second instar larvae (Fig. 2A). Oviposition rate of *A. herbicolus* and A*. tamatavensis* did also not differ over time (Fig. 2B; d.f. = 4, Likelihood ratio = 0.642, p = 0.422 and Likelihood ratio = 0.163, p=0.685, respectively). There was a significant difference on oviposition rate of *A. herbicolus* among treatments (Fig. 2B; d.f. = 2, F-value = 3.89, p=0.028 and F-value = 12.094, p=1e-04, respectively). Oviposition of *A. herbicolus* was higher on pollen than on second instar larvae of *F. occidentalis*, and oviposition on first instar larvae did not differ from the other diets (Fig. 2B). On the other hand, oviposition of *A. tamatavensis*, was significant higher on pollen and first instar larvae than on second instar larvae (Fig. 2B).

Survival and development of* A. tamatavensis *and* A. herbicolus *on first instar larvae of* F. occidentalis *and on* Typha *sp. pollen

Juvenile survival of *A. tamatavensis* was higher on pollen than on *F. occidentalis* first instar larvae (Fig. 3A; d.f. = 1, Likelihood ratio = 4.2, p = 0.04) and they also took more time to reach adulthood preying on *F. occidentalis* larvae than feeding on pollen (Fig. 3A, Likelihood ratio test= 9.94, d.f. = 1, p = 0.002). Survival of *A. herbicolus* did not differ on both diets (Fig. 3B; Likelihood ratio = 2.12, d.f. = 1, p = 0.1). However, development was faster on a diet of pollen than on a diet of *F. occidentalis* larvae (Fig. 3B; Likelihood ratio = 20.08, d.f. = 1, p < 0.001). *Amblyseius tamatavensis* sex ratio was about 86% female whereas *A. herbicolus* was a hundred percent female (Telitoky).

Discussion

We showed that predation and oviposition rates of the predatory mite *P. ovatus* on *F. occidentalis* first instar larvae were very low indicating that this predatory mite is not a promising candidate for biological control of this thrips (Fig. 1). *Amblyseius herbicolus* and *A. tamatavensis,* in turn, can prey and reproduce on first and second instar of *F. occidentalis* larvae and both also can develop into adults feeding on newly emerged larvae (Fig. 2 and 3).

Predation rates of both *Amblyseius* species reported here were slightly lower than those found from another commercial predatory mites such as *Amblydromalus limonicus*, *Amblyseius cucumeris* and *Amblyseius swirskii* (van Houten et al. 1995a, b, 2008; Vangansbeke et al. 2015). Both *Amblyseius* species tested were capable to prey on second instar larvae, which are much larger than the first instar. Larger thrips larvae can defend and counterattack or even use rectal fluids to deter their predators and, therefore, they are usually killed at a lower rate by predatory mites (Bakker and Sabelis 1989; Steiner et al. 2003). Although predation rate was much lower on second instar larvae than on first (Fig. 2A), oviposition of both treatments did not differ significantly (Fig. 2B). This may be due to the fact that first instar is smaller than the second, thus, the predators need to consume more of the smaller prey to obtain the same amount of food. It was also observed by Nomikou et al. (2004), where the predatory mite *Euseius scutalis* killed much more eggs and first instars of *Bemisia tabaci* than the larger second instar of this pest and the oviposition did not decrease proportionally.

These predators were also able to complete their developmental cycle feeding *on F. occidentalis* larvae. Although they took more time to develop into adults than those fed with *Typha* sp. pollen, their feeding on this diet indicate that they can increase its population preying only on this pest. This information is crucial because it signals that both predators can remain in the field even in the absence of others preys or alternative food sources. Both predators were also able to develop and reproduce feeding on pollen, indicating that it can be introduced in the field when prey is absent to maintain the predators in the field. The pre-establishment of natural enemies, before the occurrence of pests can help to increase the success of biological control programs (Pijnakker et al. 2020). Although pollen must be applied carefully because can also benefit *F. occidentalis* (van Rijn and Sabelis 1993; Vangansbeke et al. 2015; Leman and Messelink 2015), pollen can improve predator-prey ratios resulting in enhanced

thrips control (Van Rijn and Sabelis 1990; van Rijn et al. 1999, 2002; Sabelis and Van Rijn 2006). Astigmatid mites, in turn, can increases the density of the predatory mites, and does not affect the *F. occidentalis* population (Pirayeshfar et al. 2020, 2021). However, applying living atigmatid mites prey also needs precaution because they can cause crop damage (Czaikowska et al. 1988) or allergies in growers (Johansson et al. 1994; Arlian et al. 1997).

We observed that both predatory mites can successfully complete their development, prey and oviposit *on F. occidentalis*. Therefore, these mites have potential to be used in the management of this pest. However, it would be important to determine whether there are limiting environmental issues for the use of each one. For example, temperatures, humidity and host plant can have different effects on predatory mite species (Dinh and Sabelis 1988; van Houten et al. 1995a; Walter 1996; Schausberger 1998; Mcmurtry et al. 2013; Pereira de Sousa Neto et al. 2021). Furthermore, *A. herbicolus* reproduces by thelytoky parthenogenesis, while *A. tamatavensis* has males in its population and only generate offspring after mating (Moraes and Mesa 1988; Cavalcante et al. 2015b, 2017). This difference in their biology will probably have implications on management of these predators on field.

Additionally, further studies need to evaluate the compatibility of these predators with other biological control agents commercially available. The predatory bug *Orius insidiosus* and the entomopathogenic fungi *Beauveria bassiana* are the only biological control agents registered to control *F. occidentalis* in Brazil (MAPA 2021). For instance, simultaneous releases of *O. insidiosus* with *Amblyseius swirskii* did not appear to either interfere with or enhance suppression of *F. occidentalis* on cut roses (Chow et al. 2010). Also, pest control with *A. swirskii* and *B. bassiana* appeared not be additive or synergistic (Midthassel et al. 2016). However, the use of these biological control agents can be complementary and they can be used at different situations in integrated pest management programs. For example, *A. swirskii* could be used for preventive control and *B. bassiana* as a curative treatment (Midthassel et al. 2016). Nevertheless, studies also need to evaluate the interactions with others biological control agents used with different pests on the same crops. The use of several biological control agents in agricultural crops increases the complexity of the interactions among plants, herbivores and natural enemies (Ehler 1996; Janssen et al. 1999). All these interactions can directly or indirectly affect the density of target pest species and,

therefore, affect the success of biological control (Janssen et al. 1999; Venzon et al. 2001).

In conclusion, our results suggest that the predatory mites *A. tamatavensis* and *A. herbicolus* have good potential to be used in the biological control of the thrips *F. occidentalis.* Generalists phytoseiid mites can be cheap to be mass reared (Barbosa and de Moraes 2015; Massaro et al. 2016, 2021) and, therefore, can be an alternative to the predatory bug *Orius insidiosus* which mass production can be relatively expensive (Bonte and De Clercq 2010). Hence, although these predatory mites are commonly found in several Brazilian states, they also naturally occur in many others countries on tropical and subtropical zones (Demite et al. 2014, 2021). Thus, they could also be used in other places.

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Figure legends

Fig.1 Average daily predation rates (± s.e.) of *Amblyseius herbicolus*, *Amblyseius tamatavensis* and *Proprioseiopsis ovatus* on first instar larvae of *F. occidentalis* (A). Average daily oviposition rates (± s.e.) of *A. herbicolus*, *A. tamatavensis* and *P. ovatus* fed with *F. occidentalis* first instar larvae (B). Different lowercase letters show significant difference on predation and oviposition rates among predatory mite species ($p < 0.05$).

Fig.2 Average daily predation rates (± s.e.) of *Amblyseius herbicolus* and *Amblyseius tamatavensis* on first (L1) and second (L2) instar larvae of *F. occidentalis* (A). Average daily oviposition rates (± s.e.) of *A. herbicolus* and *A. tamatavensis* fed with *F. occidentalis* first (L1) and second (L2) instar larvae*,* and *Typha* sp. pollen (B). Different lowercase letters show significant difference on oviposition rates of *A. herbicolus* and different uppercase letters show significant difference among on oviposition rates of *A. tamatavensis* among treatments ($p < 0.05$).

Fig.3 Juvenile development and survival of *Amblyseius tamatavensis* (A) and *Amblyseius herbicolus* (B) fed with *F. occidentalis* larvae or *Typha sp.* pollen. Shown is the cumulative proportion of adults as a function of time. Total survival is the final cumulative proportion that reached adulthood. Different uppercase letters show significant difference on survival different lowercase letters show significant difference on developmental time ($p < 0.05$).

Figures

Fig. 1

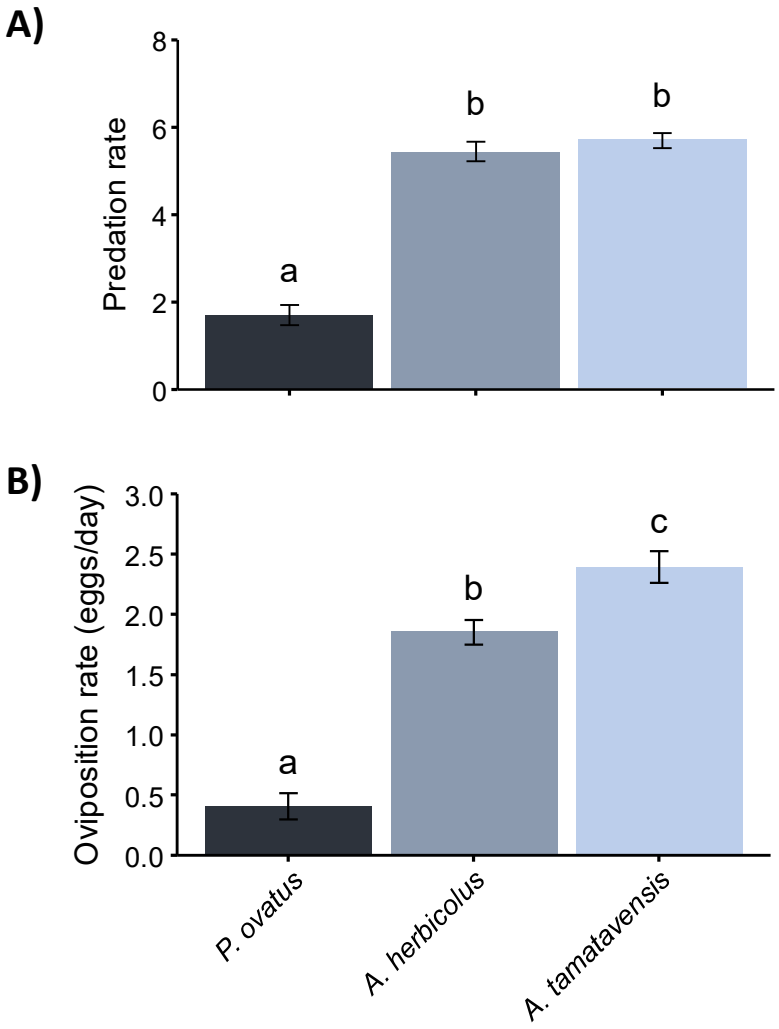


Fig. 2

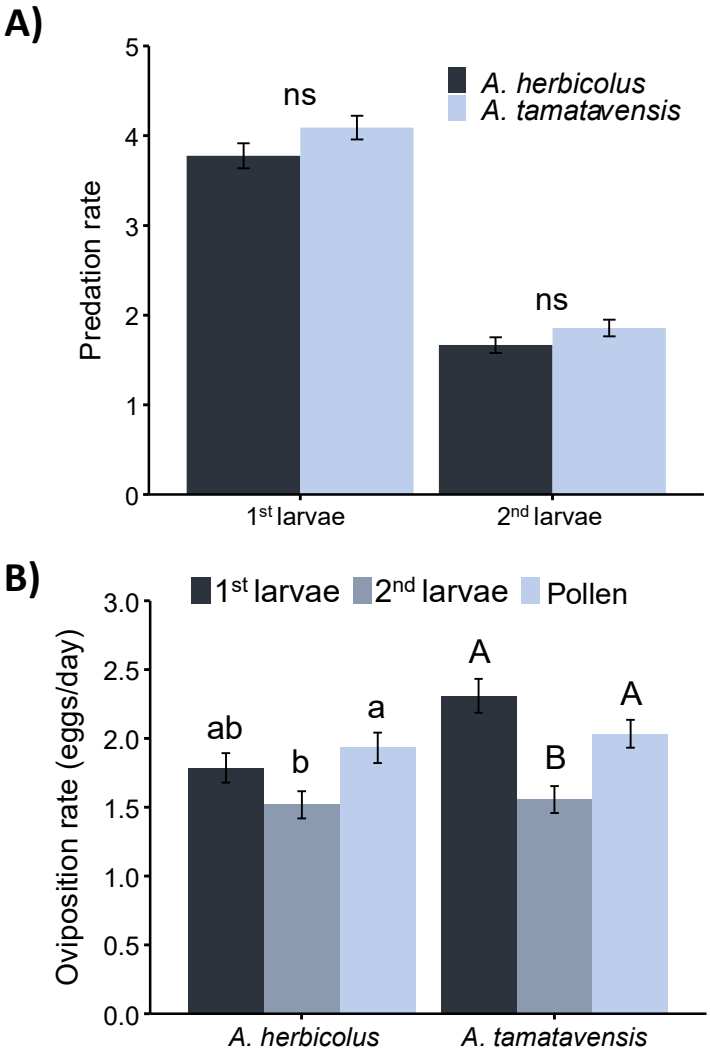
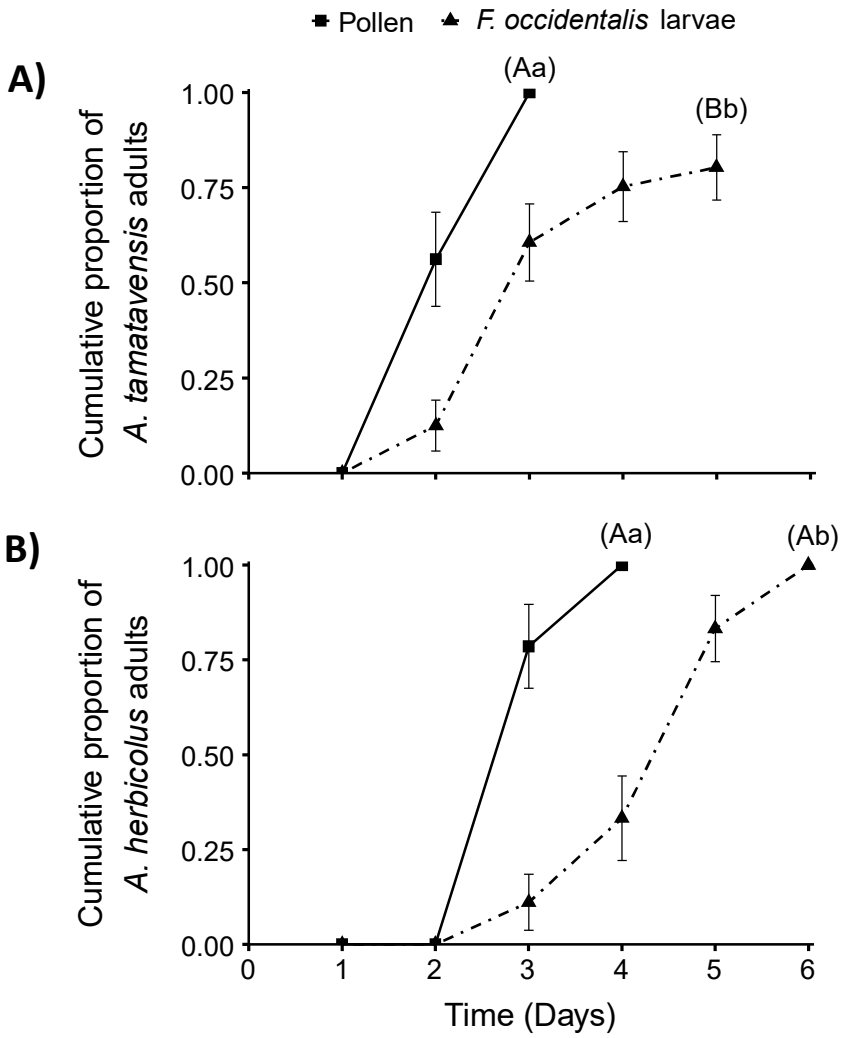

A)
Predation rate
5
4
3
2
1
0
ns
ns
A. herbicolus
A. tamatavensis
1st larvae
2nd larvae
B)
Oviposition rate (eggs/day)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1st larvae
2nd larvae
Pollen
ab
b
a
A
B
A
A. herbicolus
A. tamatavensis

Fig. 3


Pollen
F. occidentalis larvae
A)
(Aa)
(Bb)
Cumulative proportion of A. tamatavensis adults
1.00
0.75
0.50
0.25
0.00
B)
(Aa)
(Ab)
Cumulative proportion of A. herbicolus adults
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
5
6
Time (Days)

Chapter 2 - *Amblyseius tamatavensis* and *Amblyseius herbicolus* (Acari: Phytoseiidae) reduces *Frankliniella occidentalis* (Thysanoptera: Thripidae) population on bell pepper plants

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Abstract

Frankliniella occidentalis, a global invasive insect pest, can feed on a vast range of cultivated and non-cultivated plant species and is capable of transmitting numerous plant viruses. Generalist phytoseiid mites (Acari: Phytoseiidae) are commonly used as natural enemies in the biological control of this pest. Previously, we showed that Brazilian populations of the predatory mites *Amblyseius tamatavensis* Blommers and *Amblyseius herbicolus* Chant have potential to be used in applied biological control in the country since both species can reproduce and develop preying on this pest. However, its efficiency to control *F. occidentalis* on plants is still unknown. Therefore, the aim of this study to evaluate the potential of *A. tamatavensis* and A*. herbicolus* to control the western flower thrips on bell pepper plants. Hence, a population dynamics experiment was conducted on isolated plants inside cages to verify the capacity of these predators to reduce *F. occidentalis* population using pollen as alternative food. Predatory mites reduced *F. occidentalis* populations and, at the end, thrips populations were more than five times higher on control plants than on plants with predators. In addition, thrips damage on leaves was more accentuated on control plants than on plants with predators.

Keywords: Biological control. Thrips. Predatory mites. Phytoseiidae, Pollen.

Introduction

The western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a major cosmopolitan pest that can also act as vector of numerous plant pathogens (Gilbertson et al. 2015; Reitz et al. 2020). This pest is present on almost every continent in the world and is extremally polyphagous been capable to feed on many cultivated and uncultivated plant species (Lewis 1997; Nyasani et al. 2013).

Bell pepper (*Capsicum annuum* L.) is one of crops in which *F. occidentalis* is considered a key pest (Messelink et al. 2020). In this crop, feeding damage on the leaves reduces photosynthetic capacity of the plants and, damage on fruits, reduces their quality and commercial value (Shipp et al. 1998). In addition, *F. occidentalis* is vector of the Tomato spotted wilt virus (TSWV). This is one of the most economically important virus diseases of this crop (Messelink et al. 2020; He et al. 2020). TSWV causes huge stunting and yellow flecking of the leaves on young plants, and chlorotic line patterns with necrotic spots on mature plants and fruits (Turina et al. 2012). TSWV can also cause severe epidemics in bell pepper crops which can lead to production losses of up to 100 percent (Roselló et al. 1996).

Despite insecticides still being the primary method employed to manage this pest (Bielza 2008), health and environmental problems associated with the use of chemical products and occurrence of resistant populations (Gao et al. 2012a) makes necessary the search for safer pest control methods (Bale et al. 2008). Several biological control agents have been shown to be effective in managing *F. occidentalis* (van Lenteren et al. 2018). Among these, phytoseiid mites (Acari: Mesostigmata) have been widely used in biological control programs for this pest globally (Mouden et al. 2017a; van Lenteren et al. 2018; He et al. 2020). However, currently there are no predatory mite species commercially available for controlling *F. occidentalis* in Brazil (MAPA 2021). Previously, we showed that Brazilian populations of predatory mites *Amblyseius tamatavensis* and *Amblyseius herbicolus* have potential to be used in applied biological control in the country since both species can reproduce and develop preying on this pest. However, its efficiency to control *F. occidentalis* on plants is still unknown. Therefore, the aim of this study was evaluating the potential of *A. tamatavensis* and A*. herbicolus* to control the western flower thrips on bell pepper plants. For this, a population dynamics experiment was conducted on isolated bell

pepper plants inside cages to verify the capacity of these predators to reduce *F. occidentalis* population using pollen as alternative food.

Materials and Methods

Cultivation of clean plants

Bell pepper plants used in the experiments were obtained by transplant commercial seedlings (average of 10 cm high) to 5-liter pots with moist substrate (Mecplant, MecPrec®). When plants reached 20 cm high, they were supported with bamboo sticks tied with a string and were grown for more two weeks until they were used in rearings and experiments (average of 15 leaves and 40 cm high). Bell pepper plants were fertilized three times a week with a solution of 0,060 g of calcium nitrate $Ca(NO_3)_2$; 0,060 g of potassium nitrate (KNO3) and 0,070 g of Monoammonium phosphate (MAP; 2% N, 61% P_2O_5). Plants were kept in a greenhouse with automatic drip irrigation and equipped with exhaust fans, which regulated temperature to remain below 27ºC.

Pollen

Pollen used in *F. occidentalis* rearing and in experiment was collected from *Typha sp.* plants (Viçosa, Minas Gerais, Brazil) and was dried in an oven at 40℃ for 12 h and later stored in a sealed 15-ml polypropylene Falcon tubes in the freezer (- 6℃). Small amounts were periodically placed in 1.5-ml Eppendorf tube and stored in the refrigerator (8℃) for use during few weeks.

Frankliniella occidentalis *rearing*

Frankliniella occidentalis individuals were collected from commercial rose greenhouses in Barbacena, Minas Gerais, Brazil and taken to Federal University of Viçosa, Viçosa, Minas Gerais, Brazil. The thrips rearing was adapted from the method described by (DeGraaf and Wood 2009). They were reared inside 6-L plastic food storage boxes with two rectangles (12 x 7 cm) cut out of 2 opposite walls in the box that were covered with a fine mesh (160 µm). The open top of the box was sealed with clingwrap pulled tight and secured with a rubber band. Each box contained two plastic dishes (ø = 5.5 cm; 1.4 cm high) with wet cotton inside, and one dish with 200 mg of *Typha* sp. pollen and a drop of honey solution (10% v/v). Two absorbent paper towels (19x21cm, Elegante®) were placed in the base of the box for thrips pupation. Adults

were allowed to oviposit in two Japanese cucumber fruits with the ends sealed with paraffin for 3-4 days and then, the fruits were removed and put into a new plastic box as described above with two new fruits. Pollen was provided twice a week until the pupae emerged into adults. Newly emerged adults were then aspirated into a pipet tip and returned to the oviposition box.

Predatory mites rearing

Amblyseius tamatavensis population was obtained from *F. occidentalis* and *Tetranychus urticae* rearings at the Acarology Laboratory of the Federal University of Viçosa. *Amblyseius herbicolus* was collected from orange jasmine plants*, Murraya paniculata,* (L.) Jack*,* Rutaceae, in the rural area of Viçosa, Minas Gerais, Brazil. These predators were reared on plastic arena (10 x 15 cm²) placed on foam, which was placed in a tray with about 2 cm of water to prevent the escape of the mites and to serve as a source of water. The method was adapted from that described by McMurtry and Scriven (1965). Predators were feed twice a week with 2 mg of *Typha sp*. pollen 200 mg of wheat germ containging *Thyreophagus cracentiseta* Barbosa, OConnor & Moraes (Acari: Acaridae). Wheat germ with prey mites was offered inside a cap of a 1.5ml Screw Cap Microtube. Rearing arenas were kept in a room at 25 ± 2° C with 70 ± 10% RH and 12 hours photophase.

Population dynamics on bell pepper plants

This experiment was design to evaluate the capacity of the predatory mites *A. tamatavensis* and *A. herbicolus* to reduce the population of *F. occidentalis* on bell pepper plants. The experiment was conducted in twelve BugDorm-4F insect cages (0.5 x 0.5 x 1.0 m). The bases of the cages were lined with a plastic bag and filled with a 3 cm layer of substrate to provide suitable pupation site for *F. occidentalis.* A 40 cm high bell pepper plant with one open flower was placed inside each cage. Each cage was placed on a platform above four 3-liter pots which were surrounded by a layer of two centimeters of a water and detergent solution, forming a barrier to prevent contamination. The cages were arranged inside a greenhouse and minimum and maximum temperature ranged between 11.19 ± 0.24 and 31.9 ± 0.59 °C respectively, while RH ranged between 20.5 ± 0.46 and 66.22 ± 0.77 %. Fifty *A. herbicolus* females were released on one-third of the plants, 50 *A. tamatavensis* females were released

on another one-third and the last one-third were left without predatory mites as a control. Seven days after the release of the predatory mites 25 adult *F. occidentalis* were collected randomly from the rearing cages and released inside each experimental cage. Two milligrams of *Typha sp.* pollen were add to all plants three times a week. Because the sprouts of the plants grew to the point of touching the top of the cages, the apexes of these sprouts were pruned to stimulates side sprouts that had more space for their development inside the cages. In addition, fruits were removed 2 weeks after pollination to stimulate more flower bud production. The experiment was evaluated weekly by carefully handling 6 leaves selected randomly on all open flowers and recording all *F. occidentalis* adults and larvae, and predatory mites with the aid of a hand magnifying glass (magnification 45x). These evaluations were carried out during seven weeks and in the eighth week all plants were fully sampled detaching all leaves and flowers. On this last evaluation, all leaves and flowers were evaluated using a stereomicroscope (50 x magnification; Zeiss Stemi 508, Germany) to count the adult thrips and larvae and the predatory mites as well. After that, all leaves were photographed and latter rated on an arbitrarily damage scale from 0 to 4: 0 = no visible feeding damage; 1 = clearly visible damage with less than 25 percent of the leaf area; 2 = 25-50 percent of leaf area damaged; 3 = 50-75 percent of leaf area damaged and; 4 = 75-100 percent of the leaf area damaged (see Fig. 5 with examples of damage scales)

Statistical analyses

The numbers of thrips adults and larvae on sampled leaves and flowers over the first seven weeks were analyzed with a linear mixed effect model (LME of the package nlme, (Pinheiro et al., 2017) with replicate as random factor and treatment and time as fixed factors. Models were Log (x+1) transformed to normalize error distribution. Because densities of larvae were not linear over the time, a polynomial function was fit to the data with adding a quadratic and cubic term of time and its interactions with treatment. Models were checked with normal error plots and plots of residuals against fitted values. Significance of factors and interactions were determined with likelihood ratio (L.R.) tests. The presence and absence of damage on leaves over the first seven weeks was analyzed using a glmer with binomial error distribution and replicate as random factor and treatment and time as fixed factors with the package MASS (Ripley et al. 2013). The final densities of adults and larvae of thrips, and predatory mites were

analyzed using a generalized linear model with negative binomial distribution (NB-GLM) with the package MASS (Ripley et al. 2013) due the over dispersed count data (Crawley 2013). The final leaf damage was analyzed using a generalized linear model with quasi-binomial with the package MASS (Ripley et al. 2013) due the over dispersed binomial data (Crawley 2013). We first compare if the presence or absence of damage was different among treatments (scale 0 vs scale 1,2,3 and 4) and, after that, if the damaged leaves differ in damage scale (scale 1 vs scale 2, 3, 4). When possible, models were simplified by stepwise deletion aggregating non-significant factor levels in a stepwise a posteriori procedure (Crawley 2013). Contrasts among treatments over the time were obtained with the package emmeans with a Tukey correction for multiple comparisons (Lenth and Lenth 2018).

Results

There was a significant interaction of treatment and time on adults of *F. occidentalis* densities on sampled leaves over the first seven weeks (lme, Likelihood ratio = 8.38, d.f. = 6, $p < 0.05$; Fig. 1A). The difference in densities of *F. occidentalis* adults among plants with and without both predatory mite species tested were significant from the fourth until the seventh week (Fig. 1 A). Effect of the factors time squared and cubed as well as its interaction with time were not significant ($Time^3$: L.R. = 2.54, d.f. = 10, $p < 0.28$; $Time^2$: L.R. = 0.19, d.f. = 12, $p = 0.091$; Treatment:$Time^3$: L.R. = 1.16, d.f. = 7, $p = 0.28$; Treat:$Time^2$: L.R. = 0.77, d.f. = 6, $p = 0.038$), and were removed from the model of thrips larvae on sampled leaves. Interaction of time and treatment was also not significant (L.R. = 5.58, d.f. = 8, $p = 0.06$). However, time and treatment were significant (Time: L.R. = 21.37, d.f. = 5, $p < 0.0001$; Treatment: L.R. = 21.03, d.f. = 3, $p < 0.0001$). Because parameter estimates of *A. herbicolus* and *A. tamatavensis* treatments did not differ so much in , model was simplified by aggregating these factor levels in a stepwise a posteriori procedure (Crawley 2013).Number of thrips larvae on sampled leaves were significantly different on plants with both predatory mite species from control plants from the second until seventh week (Fig. 1B). Both species of predatory mites were found on sampled leaves over all evaluations, and almost all control plants stayed without predatory mites over the experiment. One plant of control treatment was contaminated with predatory mites on fifth week. However number of predators was low compared with plants that received these mites at the beginning of the experiment (Fig. 1C). Interaction of treatment and time was not significant when

we analyzed the number of flowers produced by plants of each treatment over the experiment (Fig. 2A; L.R. = 4.28, d.f. = 6, p = 0.12). Treatment was also not significant (L.R. = 0.37, d.f. = 5, p = 0.83), however, the factor time was significant because the numbers of flowers decrease over the experiment (Fig. 2A; L.R. = 20.28, d.f. = 5, p < 0.0001). Interaction of treatment and time and the factor time were not significant when we analyzed the number of thrips per flower over the experiment (Fig. 2B; L.R. = 1.84, d.f. = 6, p = 0.4 and; L.R. = 0.28, d.f. = 5, p = 0.59), however, there were significantly more thrips on control plants than on plants with predatory mites (Fig. 2B; L.R. = 6.68, d.f. = 3, p = 0.01). At the end of the experiment, when all plants were destroyed and evaluated on stereomicroscope, there were a significant effect of treatment on the number of thrips adults and larvae (Fig. 3, A and B; L.R. = 9.75, d.f. = 2; p = 0.008 and; L.R. = 13.8, d.f. = 2, p = 0.001). There were significantly more thrips adults and larvae on control plants than on plants with predatory mites (Fig. 3, A and B). Numbers of predatory mites also differ among treatments (Fig 3C; L.R. = 101.76, d.f. = 2, p < 0.0001). Only few predatory mites were found contaminating one control plant (Fig 3C). Interaction of treatment and time was also not significant when we analyzed the presence and absence of thrips damage on sampled leaves (L.R. = 5.05, d.f. = 7, p = 0,08), but the factors treatment and time were significant (L.R. = 127.11, d.f. = 5, p < 0.0001 and; L.R. = 10.07, d.f. = 5, p = 0.006 respectively). Plants that received the predatory mite *A. tamatavensis* had less leaves with thrips damage than control plants from the fourth to the seventh week and plants with *A. herbicolus* did not differ from the others treatments (Fig. 4A). At the end, when all leaves of the plants were classified at four damaged scales, there was no significant difference in proportion of leaves without thrips damage (scale 0) when compared with damaged leaves (scale 1, 2, 3 and 4) among treatments (Fig. 4B; d.f. = 11, F = 0.78, p = 0.48). In addition, there were also no significant difference in proportion of leaves with damage scale 2, 3 and 4 than leaves with few thrips damage (scale 1) among treatments (Fig. 4B; d.f. = 11, F = 3.2 p = 0.08).

Discussion

Previously, was showed that *A. herbicolus* and *A. tamatavensis* can develop and reproduce when feeding on *F. occidentalis larvae*. Here we demonstrated that the presence of these predators reduced populations of this pest on bell pepper plants over eight weeks of experiment. Bolckmans et al. (2005) and Calvo et al. (2012)

evaluated other species of predatory mites to control *F. occidentalis* on bell pepper and *A. swirskii* was the most promising species tested. Comparison with our results is difficult due the different methodologies used such as plant size, sample methodology, and number of predators and thrips released. In our experiment, populations of *F. occidentalis* considering larvae and adults were significantly lower on plants with predators from the second and fourth sampling, respectively (Fig. 1 A and B). At the end of the assessed period, there were more than five times thrips on control plants than on the treatment that received predatory mites (Fig. 3, A and B). Experiments that evaluate population dynamics and show pest control on plants over multiple pest generations, are crucial when evaluating the potential of a biological control agent (Huffaker et al. 1976; Murdoch et al. 1985). They not only show if pest numbers decrease in the presence of the tested control agent, but also if the decrease is sustained over pest generations (Gerson and Weintraub 2007). Furthermore, they verify the ability of natural enemies to establish on the host plant, which plays a crucial role in the pest-predator dynamics (Walter 1996; Cedola et al. 2001; Schmitz 2007; Mcmurtry et al. 2013). Based on life table studies of *F. occidentalis* at different temperatures and considering the average temperature and the duration of our experiment, this pest probably would go over three generations (Mcdonald et al. 1998; Zhang et al. 2007). Given the low numbers of thrips found on plants with predators at the last destructive sampling and the fact that predatory mites were found on plants over all experiment, it suggests that the two tested predatory mite species have the potential to effectively control *F. occidentalis* on bell pepper plants.

Although the damage to the control plants appears to be more severe when compared to the plants with predators, there was no statistically difference among treatments (Fig. 4B). Despite that, results obtained can be improved by adapting the methodology, such as the use of slow-release sachets (Baxter et al. 2011; Bolckmans 2013; Calvo et al. 2015) or by doing multiple releases of predators. Slow-release sachets could contain both predatory mites and an alternative prey, providing a sustainable food source for the predators to feed, reproduce and disperse into the crops for several weeks as was done by Midthassel et al. (2014). in similar system. Periodic releases, in turn, can increase the density of predatory mites on crops, especially when alternative food sources are scarce (Ridgway and Vinson 1977). Because periodic releases are not always economically feasible for growers, supplemental food sources may be an alternative due to having better cost-benefit

(Vangansbeke et al. 2016). Here, we used *Typha* sp. pollen and although predatory mites were introduced one week before thrips infestation, they were found on sampled leaves of treated plants throughout all the experiment (Fig. 1 C and 3C). Even though many studies had demonstrated positives results in applying pollen to *F. occidentalis* control, it must be applied with careful due to the potential benefits to this pest (van Rijn and Sabelis 1993; Vangansbeke et al. 2015; Leman and Messelink 2015). Research has been carried out to minimize the risks by providing alternative food sources, such as the use of astigmatid mites and decapsulated brine shrimp cysts (*Artemia* sp.)(Vangansbeke et al. 2015; Pirayeshfar et al. 2021). These food items can increase and maintain predators on plant and do not sustain the *F. occidentalis* population (Vangansbeke et al. 2015; Pirayeshfar et al. 2021).

Additionally, it is important to use predatory mites within an integrated pest management. They can be combined with other strategies of thrips control such as cultural and chemical control or even with other biological control agents (Reitz et al. 2003). Numerous biological control agents are well known to be efficient in reducing *F. occidentalis* population, such as predatory bugs, edaphic predatory mites, and entomopathogens (Van Lenteren 1995; van Lenteren et al. 2018). The use of different agents can be particularly important because they can act in different pest stages and could reduce pest population more quickly (Ridgway and Vinson 1977; Hokkanen 1995; Van Lenteren 1995). However, prior research is necessary before introducing multiple natural enemies, as it could lead to negative impacts on pest populations due to competition or intraguild predation (Rosenheim et al. 1995; Rosenheim and Harmon 2006). Because *F. occidentalis* is vector of the Tomato Spotted Wilt Virus, only few pest individuals could cause extensive economic damage. Therefore, depending on the thrips infestation level, the use of more than one biological control agent must be carefully evaluated (Van Lenteren 1995). Hence, although there are cases where the use of multiple natural enemies cannot be additive or synergistic they can be complementary and used in different situations (Midthassel et al. 2016).

Concluding, two tested predatory mite species have the potential to effectively control *F. occidentalis* on bell pepper plants. The use of these predators can be a valuable tool in pest management in Brazil because most of the predatory mites used to control *F. occidentalis* do not occur naturally in the country (van Lenteren et al. 2018; Demite et al. 2021). Importation of exotic natural enemies must pass through a rigid risk assessment protocol to evaluate potential environmental impacts (Bigler et al.

2006). In addition, it is expected that native species will be more adapted to environmental conditions. Future studies must evaluate the efficiency of these predators in the field and assess the performance of these predatory mite species on other crops where *F. occidentalis* is considered a key pest.

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Figure legends

Fig. 1 Mean number (± SE) of *Frankliniella occidentalis* adults (A)*, F. occidentalis* larvae (B) and predatory mites (C) on sampled leaves of control plants (solid line with black squares), plants with *Amblyseius tamatavensis* (dot dashed line with black circles) and *Amblyseius herbicolus* (dotted line with white squares) over the first seven weeks. Contrasts among treatments were obtained after a linear mixed effects model. Asterisks indicate significant differences among treatments per day of evaluation ($p<0.05$).

Fig. 2 Mean number (± SE) of flowers (A) and *F. occidentalis* adults per flower (B) on control plants (black bars and solid line with black squares), plants with *Amblyseius tamatavensis* (dark grey bars and dot dashed line with black circles) and *Amblyseius herbicolus* (light grey bars and dotted line with white squares) over the first seven weeks. Contrasts among treatments were obtained after a linear mixed effects model. Different uppercase letters on legend indicate significant differences among treatments per day of evaluation ($p<0.05$).

Fig. 3 Final densities of *F. occidentalis* adults (A), F. occidentalis larvae (B) and predatory mites on control plants (black bars), plants with *A. tamatavensis* (light grey bars) and plants with *A. hebicolus* (dark grey bars) after destructive sampling on eighth week. Contrasts among treatments were obtained after a generalized linear mixed model and generalized linear model with binomial and quasi binomial error distribution respectively. Different lowercase letters indicate significant differences among treatments ($p<0.05$).

Fig. 4 Proportion (± CI) of sampled leaves with *F. occidentalis* damage over the first seven weeks (A) and proportion (± CI) of leaves per damage scale at destructive sampling on the eighth week (B) on control plants (black bars and solid line with black squares), plants with *Amblyseius tamatavensis* (dark grey bars and dot dashed line with black circles) and *Amblyseius herbicolus* (light grey bars). Contrasts among treatments of the first figure were obtained after a linear mixed effects model and after generalized linear model with quasi-binomial to the second figure. Different uppercase letters on legend indicate significant differences among treatments per day of evaluation ($p<0.05$).

Fig. 5 Example of leaves of different damage scales. (A) damage scale 0 = no visible feeding damage; (B) damage scale 1 = visible damage on less than 25 percent of the

leaf area; (C) damage scale 2: 25-50 percent of leaf area damaged; (d) damage scale 3 = 50-75 percent of leaf area damaged and; (E) damage scale 4 = 75-100 percent of the leaf area damaged.

Figures

Fig.1:

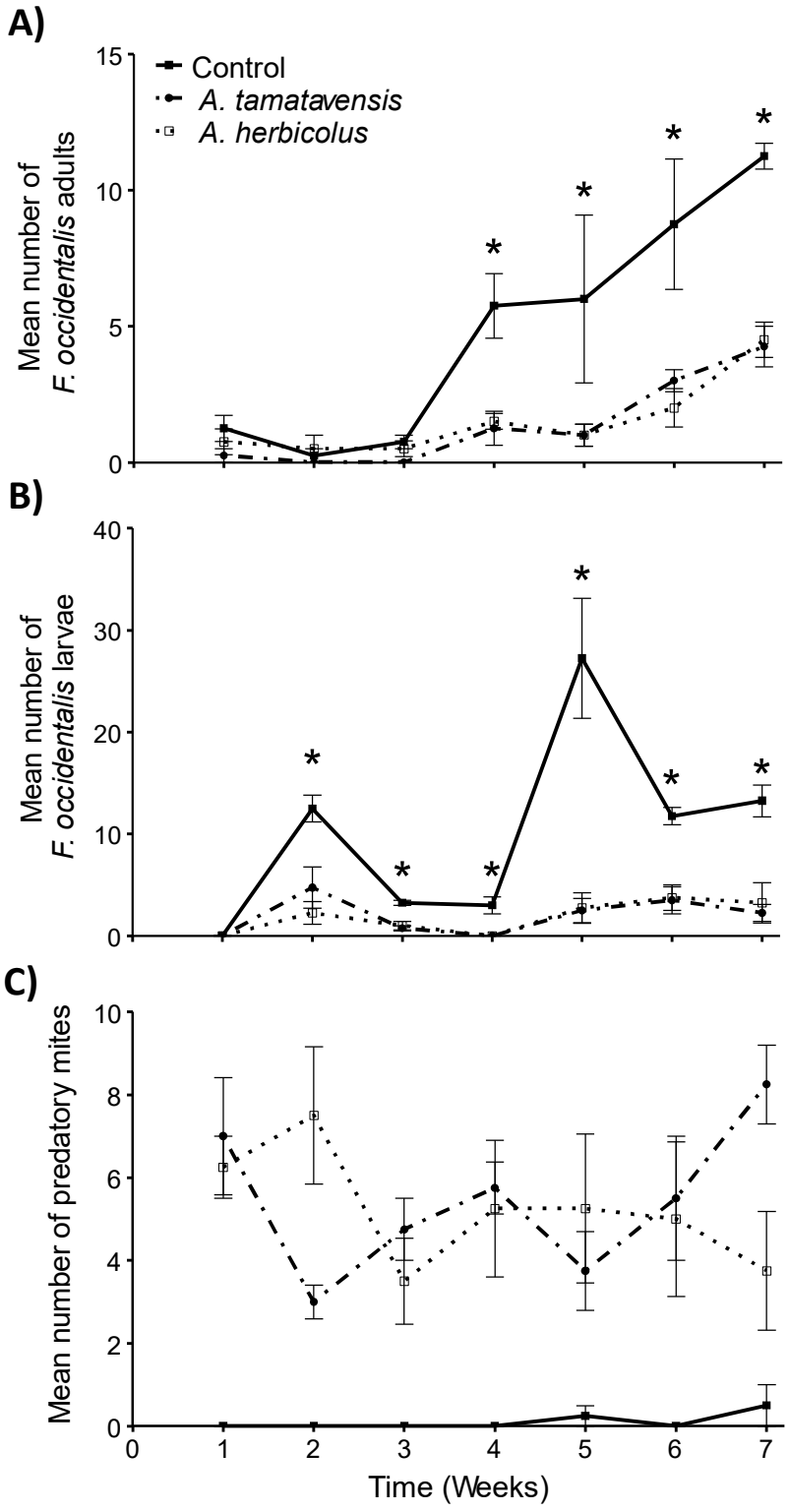

Fig. 2

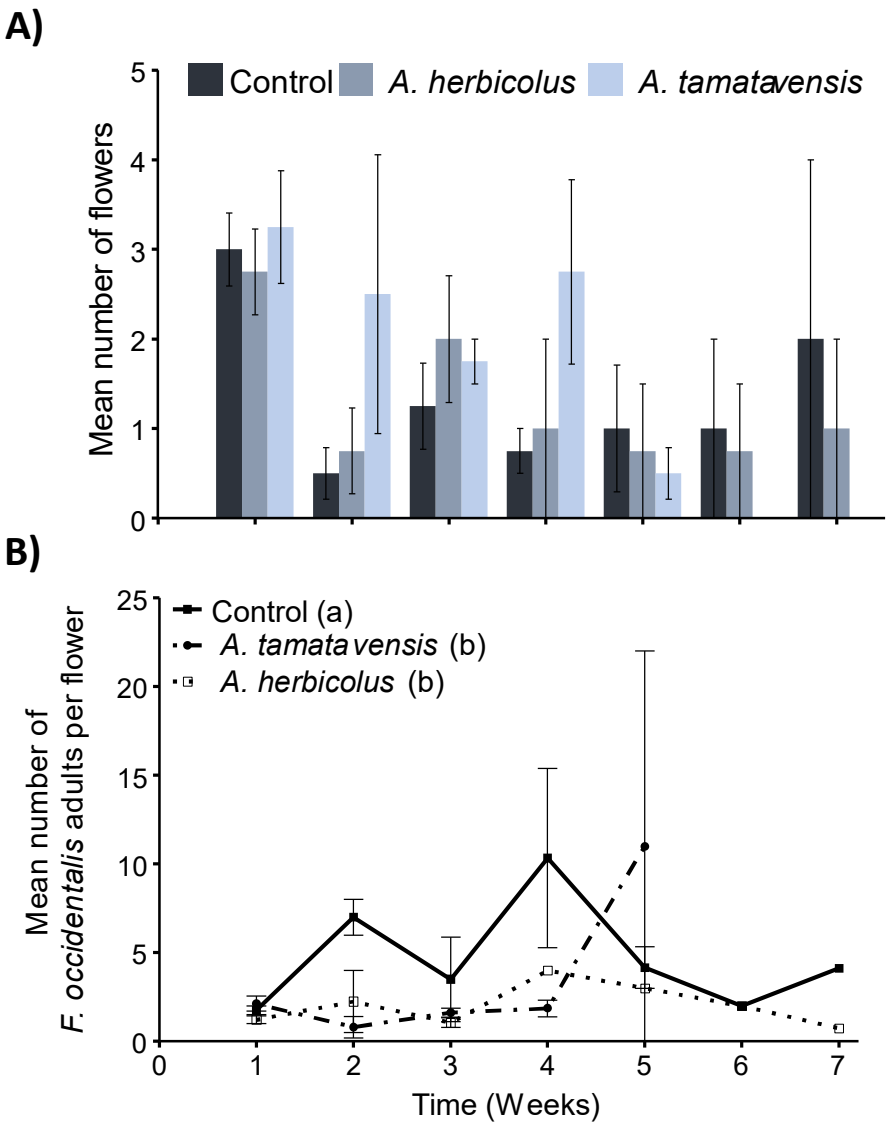
A)
Control
A. herbicolus
A. tamatavensis
Mean number of flowers
0
1
2
3
4
5
B)
Control (a)
A. tamatavensis (b)
A. herbicolus (b)
Mean number of F. occidentalis adults per flower
0
5
10
15
20
25
0
1
2
3
4
5
6
7
Time (Weeks)

Fig. 3

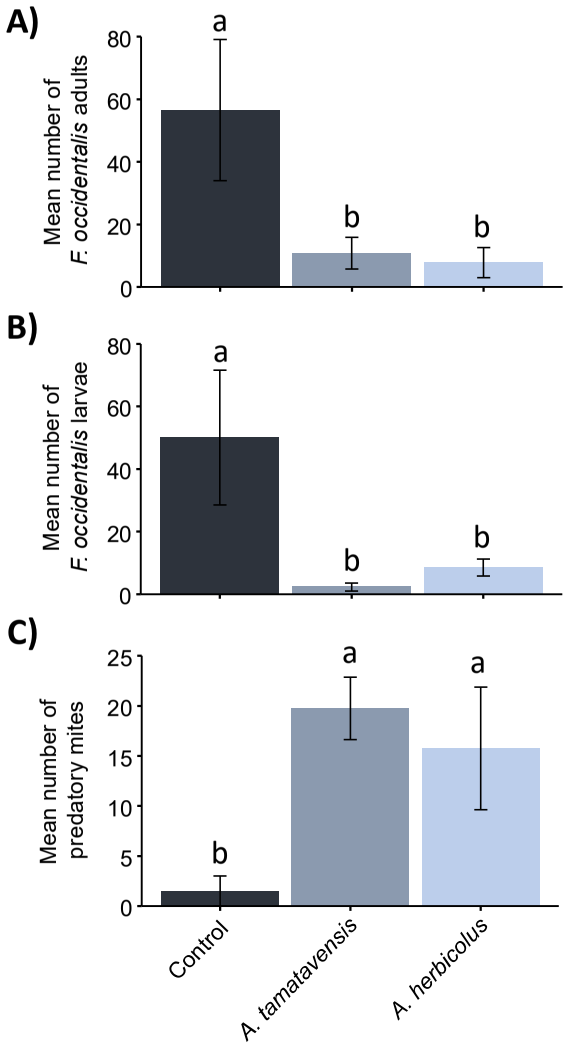
A)
Mean number of F. occidentalis adults
80
60
40
20
0
a
b
b
B)
Mean number of F. occidentalis larvae
80
60
40
20
0
a
b
b
C)
Mean number of predatory mites
25
20
15
10
5
0
b
a
a
Control
A. tamatavensis
A. herbicolus

Fig. 4

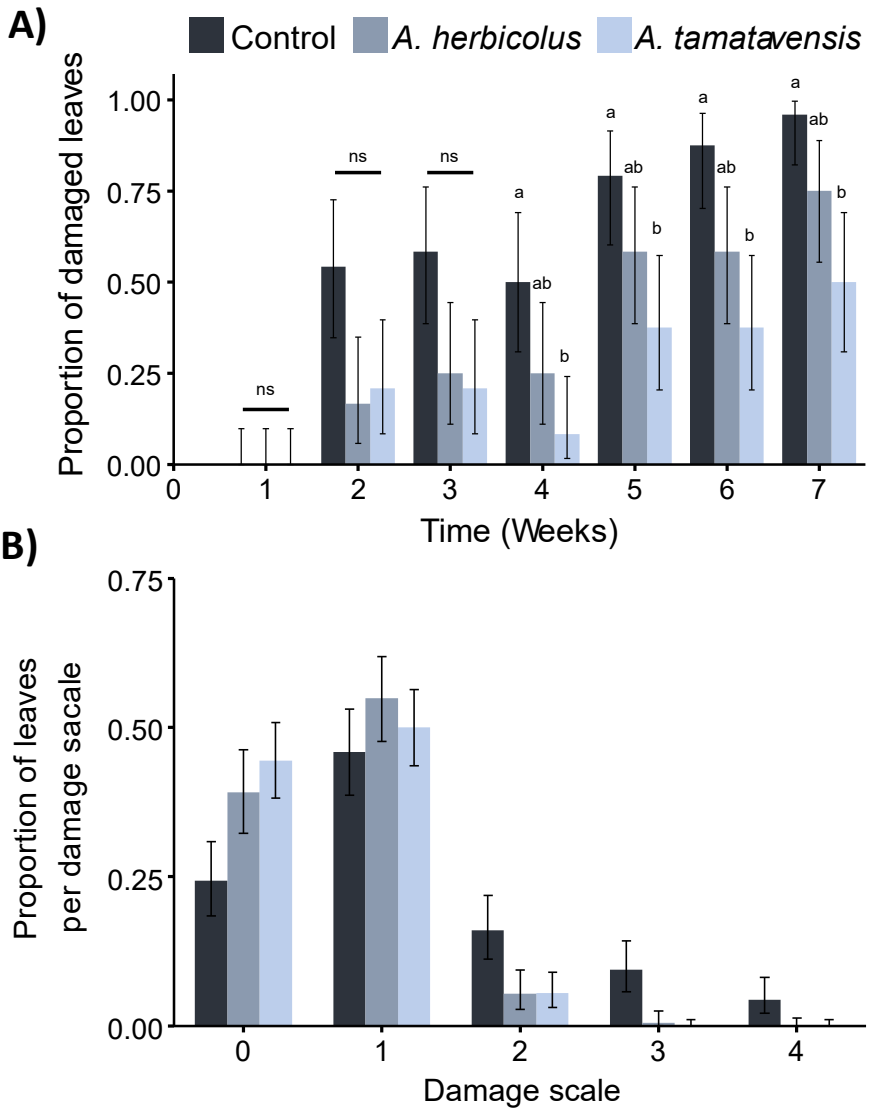
A)
Control
A. herbicolus
A. tamatavensis
Proportion of damaged leaves
1.00
0.75
0.50
0.25
0.00
ns
ns
ns
a
ab
b
a
ab
b
a
ab
b
0
1
2
3
4
5
6
7
Time (Weeks)
B)
Proportion of leaves per damage sacale
0.75
0.50
0.25
0.00
0
1
2
3
4
Damage scale

Fig. 5

Chapter 3 – Efficiency of *Amblyseius tamatavensis* to reduce simultaneously two key pests on bell pepper plants

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Abstract

Frankliniella occidentalis and *Bemisia tabaci* are two major invasive pests worldwide. They are capable to feed on hundreds of cultivated and noncultivated plants species and are vectors of many plant viruses. Besides hazardous to the human health and environment, insecticides remain the main strategy used to control these pests. Biological control is an environmentally safer and healthier method, and are helping to reduce the actual excessive use of pesticides. Generalist phytoseiid mites (Acari: Mesostigmata) are among the most used natural enemies applied in biological control programs of these pests. In the second chapter we showed that the predatory mite A*mblyseius tamatavensis* Blommers is able to reduce *F. occidentalis* population on bell pepper plants and this predator species is also being used to control the whitefly *B. tabaci* in Brazil. Therefore, the aim of this study was evaluating the population dynamics of *F. occidentalis* and *B. tabaci* simultaneously in the presence and absence of the predatory mite *A. tamatavensis* Therefore, populations of these pests were evaluated in isolated bell pepper plants over 50 days. Thrips and whitefly populations were significantly higher on plants without predatory mites from the day 36 and 26 respectively and, at the end, there were more than nine time more adults of both pests on control plants than on plants with *A. tamatavensis* as bodyguards. The damage of thrips on fruits and leaves were also significantly higher on plants without *A. tamatavensis* and these plants also produced lighter fruits than plants with predators. So, our results indicates that *A. tamatavensis* could be used to control these two pests simultaneously on bell pepper plants

Key-word: Biological control. Whitefly. Thrips. Predatory mite. Phytoseiidae.

Introduction

Western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) are two major invasive pests worldwide (Basu 2019a; Reitz et al. 2020). In few decades, these pests spread from its origins and today they are distributed in almost every continent of the world (De Barro et al. 2011; He et al. 2020). So-called supervectors, they are capable of transmitting numerous plant viruses, therefore, their damage to farmers goes far beyond the direct damage caused by its feeding on cultivated plants (Gilbertson et al. 2015). This two insect species share many features that make their management difficult to the growers. In some crops, the viruses transmitted by these insects can be really devastating, leading to huge economic losses (Varma and Malathi 2003; Kirk and Terry 2003; Gilbertson et al. 2015). Their small size and high levels of aggregation made its detection difficult when densities are low in the crop and uncontrolled populations can increase quickly at optimum conditions due to their high intrinsic rates of population increase (Reitz 2009; Sutherland and Parrella 2011; Basu 2019b; Ribeiro et al. 2021). In addition, they are extremely polyphagous being capable to survive on native and spontaneous vegetation as well as neighbor crops which makes even more difficult their management (Wan and Yang 2016; Basu 2019a; Reitz et al. 2020).

Bell pepper (*Capsicum annuum* L.) is one of the most consumed vegetables in the world and one of crops in which both *F. occidentalis* and *B. tabaci* are considered key pests (Messelink et al. 2020). *Frankliniella occidentalis* drain the epidermal cells content leading to its characteristic silvered damage (Kirk 1995; Reitz et al. 2020). Feeding damage on the leaves can reduces the photosynthetic capacity of the plant and when the attack occurs in the fruits it can cause cosmetic damages, diminishing the quality and the commercial value of the fruits. (Shipp et al. 1998). In addition, *F. occidentalis* is vector of the Tomato spotted wilt virus (TSWV), which can cause huge economic losses to bell pepper crops (Messelink et al. 2020; He et al. 2020). TSWV causes huge stunting and yellow flecking of the leaves on young plants, and chlorotic line patterns with necrotic spots on mature plants (Turina et al. 2012). *Bemisia tabaci*, in turn, feed on plant sap and can cause yellowing, mottling and fall of leaves. In addition, development of sooty mould fungi on its excretion (honeydew) may reduce photosynthetic capacity the plant (Byrne and Bellows 1991). Although *B. tabaci* is vector of devastating viruses to others crops such as tomato, cucumber and melon

plants, most of viruses are not known to infect peppers (Messelink et al. 2020). However, some cultivars of pepper are symptomless hosts of Tomato yellow leaf curl virus (TYLCV), one of the most devastating pathogens affecting tomato crop, and can serve as virus reservoir for the acquisition and transmission of TYLCV by *B. tabaci* (Polston and Lapidot 2007).

Insecticides remain the main strategy used to control these pests in most of the agricultural areas (Bielza 2008; Basit 2019). *Because F. occidentalis* and *B. tabaci* are pest of many crops, populations of these pests are often under constant insecticide pressure which resulted in several relates of resistant populations (Gao et al. 2012b; Horowitz et al. 2020). Biological control is environmentally safer pest control method and has been applied with success in several cropping systems, playing an important role to achieve sustainability in crop protection (Bale et al. 2008). Several biological control agents are efficient controlling *F. occidentalis* and *B. tabaci* being many of them already commercialized by biological control companies around the world (van Lenteren et al. 2018). Generalist phytoseiid mites (Acari: Mesostigmata) are among the most used natural enemies applied in biological control programs of these pests and some species can feed on both (Mouden et al. 2017b; van Lenteren et al. 2018; He et al. 2020; Kheirodin et al. 2020). The fact that these natural enemies can feed on more than one pest species help to reduce the number of natural enemies to be introduced in the crop (Messelink et al. 2008). Moreover, many generalist phytoseiid mites can feed on pollen of several plant species (McMurtry and Scriven 1966; Leman and Messelink 2015) and therefore it is possible to establish populations of these predators in a crop before pest invasions (Janssen and Sabelis 2015).

Brazil is one of the main food producers in the world and despite the richness of Brazilian phytoseiid fauna, few species are been used in applied biological control in the country (Cavalcante et al. 2015b; Demite et al. 2021). The generalist predatory mite *Amblyseius tamatavensis* was recently registered to control the whitefly *B. tabaci* and there are still no predatory mites being marketed to control *F. occidentalis* in Brazil (MAPA 2021). *O*ther species of its genus are also used in the control of whiteflies and other pests such as thrips and mites in other countries (van Lenteren et al. 2018). Therefore, the aim of this study was evaluating the potential of this natural enemy to control simultaneously the western flower thrips and the whitefly *B. tabaci*.

Materials and methods

Cultivation of clean plants

Bean plants used in the thrips rearing were obtained by sowing weekly seven Carioca Bean seeds (*Phaseolus vulgaris* L.) one centimeter deep and equidistantly directly on the moist substrate (Mecplant, MecPrec®) inside 3-liter pots. Emerged plants were grown for two weeks until they were taken to the rearing cages. To obtain the Bell pepper plants used in the experiment, seeds cv. Cascadura Ikeda (*Capsicum annuum* L.) were sowed on 128-cell expanded polystyrene seedling tray (6cm high, 34 cm width, 67cm length) filled with substrate. The seedlings (average of 10 cm high) were transplanted to 3-liter pots two weeks after emergency. When the plants reached 20 cm high, they were supported with bamboo sticks tied with a string and were grown for more two weeks until they were used in the experiment (average of 15 leaves and 40 cm high). Bell pepper plants were fertilized every two weeks with 5g of NPK (20-5-20) and 10g of superphosphate (18% of P205). The plants were cultivated inside a greenhouse with controlled temperature (26±1 °C) and with automatic drip irrigation.

Pollen

Cattail pollen was used for the *F. occidentalis* rearing and for the experiment because it was a good food source for thrips and for *A. tamatavensis* (Hulshof et al. 2003; Cavalcante et al. 2015a). The pollen was collected from *Typha sp.* plants (Viçosa, Minas Gerais, Brazil) and was dried in an oven at 40℃ for 12 h and later stored in a sealed 15-ml polypropylene Falcon tubes in the freezer (- 6℃). Small amounts were periodically placed in 1.5-ml Eppendorf tube and stored in the refrigerator (8℃) for use during few weeks.

Frankliniella occidentalis *rearing*

Frankliniella occidentalis were collected from greenhouses with commercial rose in Barbacena, Minas Gerais, Brazil and taken to Federal University of Viçosa, Viçosa, Minas Gerais, Brazil. The thrips were reared inside a BugDorm-4F insect cages (0.5 x 0.5 x 1.0 m) with bean plants and with *Typha sp.* pollen supply. Pots with clean bean plants were added to the cages every week and were placed above the old pots (with dead plants) which were maintained inside the cages due to the presence of *F.*

occidentalis pupae on the soil. Two milligrams of *Typha sp.* pollen was supplied twice a week on the leaves with the aid of fine brush. When there were a large population of *F. occidentalis* 100 adults were transferred to a clean cage with clean bean plants inside.

Predatory mite rearing

Amblyseius tamatavensis population was obtained from *F. occidentalis* and *Tetranychus urticae* rearings at the Acarology Laboratory of Federal University of Viçosa. This predator was reared on plant-pot dishes (15 cm diameter, 2 cm high) placed on a foam (15cm diameter, 3 cm high), which was placed in a tray (7,5cm high, 22,1cm width, 30,3cm length) with 2 cm of water layer and covered with another tray to keep the humidity. This tray was placed in a bigger tray (7,5cm high, 28,9cm width, 34,9cm length) filled with a solution of water and detergent to prevent scape or contamination. The predators were fed weekly with one g of wheat germ with *Thyreophagus cracentiseta* Barbosa, OConnor & Moraes (Acari: Acaridae). *Thyreophagus cracentiseta* rearing was adapted from (Freire and Moraes 2007) reared inside transparent plastic pots (15 cm diameter and 8 cm high) placed inside trays as explained above for the *A. tamatavensis* and was fed with wheat germ previously sterilized in autoclave (120 °C under 1.4 atm for 20 minutes).

Population dynamics of* Frankliniella occidentalis*,* Bemisia tabaci *and the predator* Amblyseius tamatavensis *on Bell pepper plants with alternative food supply

This experiment was firstly designed to evaluate the capacity of the predatory mite *A. tamatavensis* to reduce the population of *F. occidentalis* on bell pepper plants. However, on the first day of evaluation, we observed the presence of adult whiteflies in all cages (1-4 adults per plant) and therefore, its populations were also evaluated throughout the experiment. The experiment started with eight bell pepper plants as described above which were transplanted to a 7 litters pots. To isolate the plants, cages similar of those described by Nilakhe (1988) were used. The cages were made of a 2.2 meters fence wire (5.2 mm diameter), which was bent to form a circle of 44 cm of diameter at one side and at the opposite side a straight end (50 cm) was bent from the center of the circle. The straight end of this fence wire was pushed into 95 cm PVC

pipe (1/2") and inserting it into the soil at the center of the pot. This structure was covered with fine mesh (size 70 µm) that was closed by tying a rubber band around the mesh and fixing it to the pots. Each cage was placed on a tray (7.5 cm high, 29.6 cm width and 43.5 cm length) turned upside down inside another tray (8.60 cm high, 37. 20 cm width and 53,20 cm length). The larger tray was filled with a layer of two centimeters of a water and detergent solution, forming a barrier to prevent contamination. The cages were arranged in a completely randomized design inside a greenhouse (34.5 ±14.5˚C and 70±10 RH). At the first day of the experiment, 15 adult *F. occidentalis* were collected randomly from the rearing cages and released inside each of the eight experimental cages. Half of the plants also received 10 gravid females of *A. tamatavensis* collected randomly from the rearings. Three milligrams of *Typha sp.* pollen were add to all plants twice a week. The experiment was evaluated weekly by handling carefully all leaves of each plant and recording *F. occidentalis* and *B. tabaci* adults. Adults of the pests were sucked into a pipette tip (1000 µl; Nichiryo, Japan) connected to a transparent hose (ø = 1 cm) with a mesh (size 170 µm) between avoiding count the same insect more than once. Afterwards, we also assessed the presence or absence of predatory mites and then the adults of thrips and whiteflies were reintroduced to the cages by inserting the pointed part of the pipette tip closed with Parafilm “M” (Bemis Flexible Packaging, Neenah, WI 54956) into the soil. Plants without predatory mites were always evaluated first to avoid contamination. Because the sprouts of the plants grew to the point of touching the top of the cages, the apexes of these sprouts were pruned to prevent the curling of young leaves which would make sampling difficult and hinder plant growth. This pruning stimulates side sprouts that had more space for development inside the cage. These evaluations were carried out for six weeks and in the seventh week (50th day), all leaves, flowers and fruits were carefully cut and the adults of thrips and whiteflies were counted by sucking it into a pipette tip. Then, these plant parts were evaluated using a stereomicroscope (40 x magnification; Zeiss Stemi 508, Germany) to count the adult thrips that were not seen at naked eye as well as the predatory mites and immature thrips. We also evaluated the number of leaves and fruits per plant and the presence or absence symptoms of thrips damage. At the end, each fruit was weighed on a semi-analytical scale (Marte, AS2000, ±0.01 g). The immature whiteflies were not counted because its densities were extremely high in the control plants.

Statistical analysis

The numbers of *F. occidentalis* and *B. tabaci* adults on plants with and without predators over the first six weeks (day 8 to 43) were analyzed with a linear mixed effect model (LME) (Bates et al. 2015) with replicate as random factor and treatment and time as fixed factors. Models were log-transformed to normalize error distribution. Contrasts among treatments over the time were obtained with the package emmeans with a Tukey correction for multiple comparisons (Lenth 2018). The final densities of adults of thrips and whiteflies, thrips larvae and predatory mites, and the number of leaves, leaves dropped on the substrate, fruits, damaged leaves, and damaged fruits at 50th day were analyzed using a generalized linear model with negative binomial distribution (NB-GLM) with the package MASS (Ripley et al. 2013) due the over dispersed count data (Crawley 2013). The weight of the fruits was analyzed with a generalized linear model with Gaussian distribution. All statistical analyses were done with R version 4.0.2 (R. Core Team 2021) and the graphs were made using the ggplot2 package (Wickham 2016).

Results

There was a significant interaction of treatment and time on both *F. occidentalis* (lme, Likelihood ratio = 15.27, d.f. = 5, $p < 0.0001$; Fig. 1A) and *B. tabaci* densities (lme, Likelihood ratio = 19.5, d.f. = 5, $p < 0.0001$; Fig. 1B). The difference in densities of *F. occidentlais* and *B. tabaci* adults among plants with and without *A. tamatavensis* was significant from the 36th and 29th day respectively until the end of the experiment on day 50 (Fig. 1 A and B). At the end of the experiment, adults of both pests were more than nine times higher on plants without *A. tamatavensis* than on plants with these predators (nb.glm, z-value = -6.002, d.f. = 1, $p < 0.001$ and z-value = -2.614, d.f. = 1, $p < 0.01$; Fig. 1 A and B). We did not see any predatory mite throughout the experiment on the control plants. In the last evaluation, we found few predatory mites on two control plants, however, the densities of these predators were significantly higher on plants that received predatory mites at the beginning of the experiment (nb.glm, z-value = -7.00, d.f. = 1, $p < 0.001$; Fig. 2 A). Probably, the predatory mites invaded these plants close to the last evaluation, thus, we consider these plants as control plants for the analysis. At the end, there were significantly more immature thrips on control plants than on plants where predatory mites were released (nb.glm, z-value = -8.76, d.f. = 1,

$p < 0.001$; Fig. 2 B). Plants with predators had significantly more leaves (nb.glm, z-value = -3.06, d.f. = 1, $p < 0.001$; Fig. 2 C) and less *F. occidentalis* damage than control plants (nb.glm, z-value = -3.87, d.f. = 1; Fig. 2 D). There were significantly more leaves dropped in the soil on plants without predators (nb.glm, z-value = -5.14, d.f. = 1, $p < 0.001$; Fig. 2 E). The number of fruits did not differ between treatments (nb.glm, z-value = 0.351, d.f. = 1, $p = 0.72$; Fig. 2 F), however, the fruits of the plants with predatory mites had less *F. occidentalis* damage (nb.glm, $p < 0.001$ and z-value = -3.06, d.f. = 1, $p < 0.001$; Fig. 2 G) and the were significantly heavier than those of control plants (glm, t-value = -2.49, d.f. = 1, $p < 0.05$; Fig. 2 H).

Discussion

We showed that the presence of the predatory mite *A. tamatavensis* on bell pepper plants reduced both *B. tabaci* and *F. occidentalis* populations. Whitefly and thrips populations increased on plants without predatory mites and, at the end, populations on these plants were more than nine times higher on control plants than on plants with *A. tamatavensis* as bodyguards (Fig. 1, A and B). Pest suppression also result in plants with more leaves and fruits without *F. occidentalis* damage and fruits were also heavier on plants with predatory mites.

At the end of the experiment, there was a high predatory mite density on the plants with about 500 predatory mite per plant (Fig 2 A). This high number of predators may be due the presence of both pests simultaneously and due the pollen supplied. When two pests share the same natural enemy, it can result in better control of the pests species through an increase in predator densities through apparent competition (Holt and Bonsall 2017). Messelink et al. (2008) showed that the control of the whitefly *Trialeurodes vaporariorum* by the predatory mite *Amblyseius swirskii*, was improved when *F. occidentalis* were also present on cucumber crop.

Although *F. occidentalis* can also benefit from pollen supplied, populations of this pest were suppressed in the presence of *A. tamatavensis*. It is known that pollen can improve predator-prey ratios resulting in enhanced thrips control (Van Rijn and Sabelis 1990; van Rijn et al. 1999, 2002; Sabelis and Van Rijn 2006), however, due to the potential benefits of pollen to *F. occidentalis* and others herbivorous it must be applied with careful (van Rijn and Sabelis 1993; Vangansbeke et al. 2015; Leman and Messelink 2015). Provision of others kind of food sources such as astigmatid mites and decapsulated brine shrimp cysts (Artemia sp.) have been studied to minimize

these risks since it does not support *F. occidentalis* population (Vangansbeke et al. 2015; Pirayeshfar et al. 2021). Future studies must evaluate the potential use of *T. crasentiseta* used in predator rearing to establish or boost *A. tamatavensis* population on plants.

The presence of this predatory mite on plants also decreases the damage of the *F. occidentalis* on leaves and fruits (Fig 2 D and F). We also observed several leaves dropped on the substrate in control cages (Fig. 2 E) probably due to the high whitefly population (Fontes et al. 2012). At the end, plants with *A. tamatavensis* also had higher average fruit weight (Fig. 2 H). Besides the directly reduction of the pest densities, the large difference in *F. occidentalis* damage on plants can also be explained by change in the pest behaviors mediated by the presence of the predatory mites. For example, Jandricic et al. (2016) showed that the presence of the predatory mite *Neoseiulus cucumeris* changed *F. occidentalis* feeding behavior and consequently reduced their damage on the plants.

It was known that *A. tamatavensis* can prey, and reproduce on *B. tabaci* eggs (Cavalcante et al. 2015b) and reduced and *B. tabaci* immatures in bell pepper plants (Cavalcante et al. 2017). However, there were no results until now showing the control of this pest for a longer period of time with more than one generation of the pest. Therefore, our results suggests that the reduction of the *B. tabaci* population by the predatory mite *A. tamatavensis* may be persistent.

The only macro-biological agent registrated in Brazil to control *F. occidentalis* is the predatory bug *Orius insidiosus* (MAPA 2021). Although this predator is efficient in *F. occidentalis* control (Carvalho et al. 2008), mass production of this predator can be relatively expensive (Bonte and De Clercq 2010). Because generalists phytoseiid mites mass rearing may be cheaper because they can feed on astigmatid mites which can be easily produced on cheap substrates (Barbosa and de Moraes 2015; Massaro et al. 2016, 2021), *A. tamatavensis* would be alternative to use in management of this pest in the future. In addition, many crops are attacked by *B. tabaci* and *F. occidentalis* and therefore would be possible to use only one biological agent to control both, which would be less costly, and would avoid problems such as intraguild predation. Although Brazil gives priority to the registration of phytosanitary products to be used in organic agriculture (Togni et al. 2019), registration requirements can be a challenge for small- and medium-sized biological control companies (Mascarin et al. 2019) because is not only costly but also time consuming (Bale et al. 2008). The possibility to extend

registration of an biological agent already registered for another pest species may be faster because most of the information needed in this process will be the same (MAPA et al. 2006).

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Figure legends

Fig. 1 Mean number (± SE) of *Frankliniella occidentalis* (A) and *Bemisia tabaci* (B) adults on sweet pepper plants with (dot dashed line with circles) and without (solid line with squares) *Amblyseius tamatavensis* during 50 days. Final densities on plants of control (white squares) and with predators (white circles) were obtained by destructive sampling on day 50. Contrasts between treatments during the first six weeks were obtained after a linear mixed effects model and the last day after a generalized linear model with a negative binomial error distribution. Asterisks indicate significant differences between treatments per day of evaluation (* $p<0.05$, ** $p<0.001$, *** $p<0.0001$).

Fig. 2 Mean number (± SE) of predatory mites (A); *F. occidentalis* larvae (B); leaves (C); leaves with *F. occidentalis* damage (D), leaves dropped on the substrate (E); fruits (F); fruits with *F. occidentalis* damage (G); and mean (± SE) fruit weight (H). All data were obtained by destructive sampling on day 50. Contrasts between treatments were obtained with a generalized linear model with negative binomial distribution for figures A to G and with gaussian distribution for figure H. Asterisks indicate significant differences between treatments (* $p<0.05$, ** $p<0.001$, *** $p<0.0001$).

Figures

Fig. 1

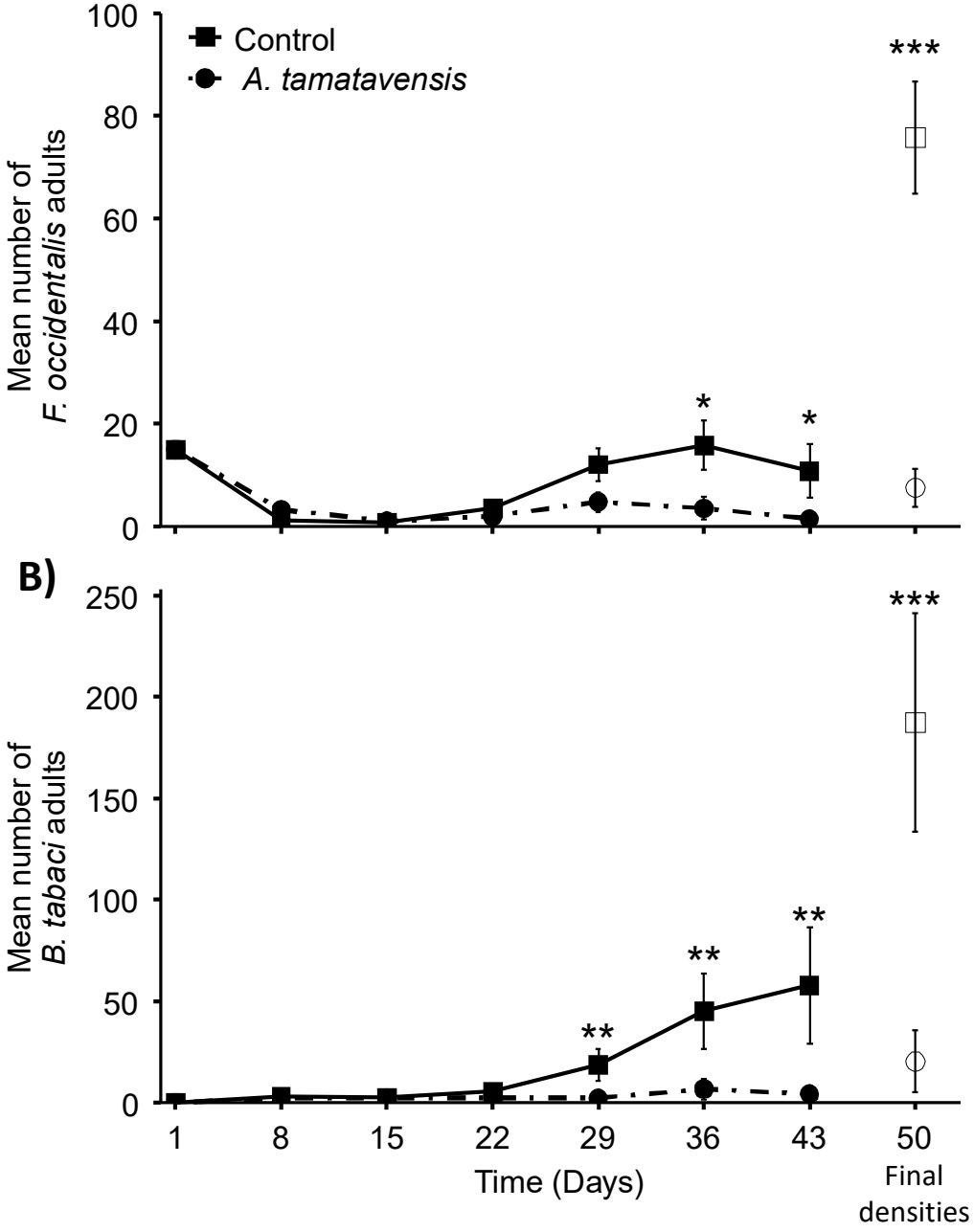
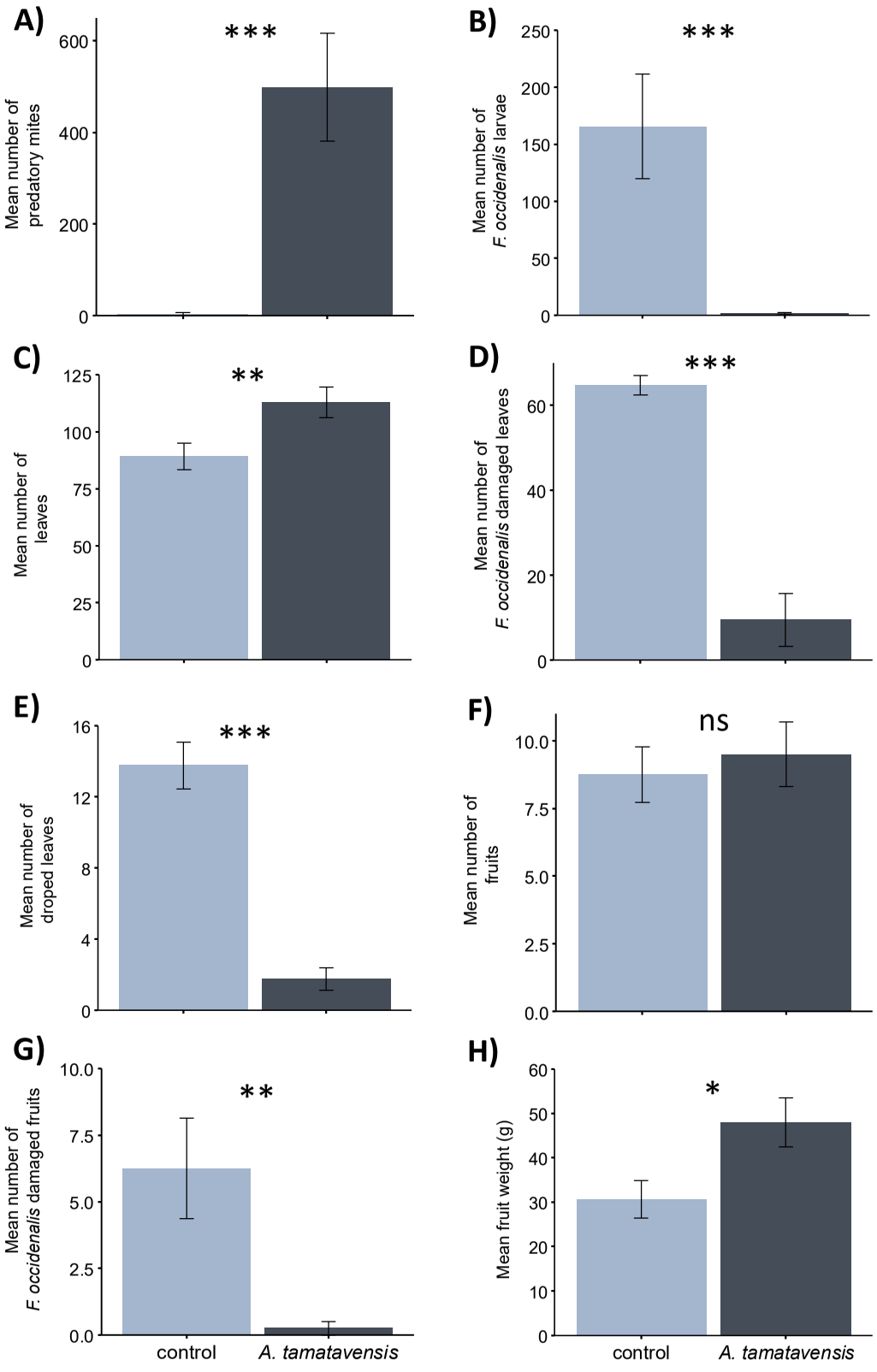


Fig. 2


A)
***
Mean number of predatory mites
600
400
200
0
B)
***
Mean number of F. occidenalis larvae
250
200
150
100
50
0
C)
**
Mean number of leaves
125
100
75
50
25
0
D)
***
Mean number of F. occidenalis damaged leaves
60
40
20
0
E)
***
Mean number of droped leaves
16
12
8
4
0
F)
ns
Mean number of fruits
10.0
7.5
5.0
2.5
0.0
G)
**
Mean number of F. occidenalis damaged fruits
10.0
7.5
5.0
2.5
0.0
control
A. tamatavensis
H)
*
Mean fruit weight (g)
60
50
40
30
20
10
0
control
A. tamatavensis

General Conclusions

- The assessed predation rate of *Proprioseiopsis ovatus* on *F. occidentalis* was very low as well as their oviposition, therefore this species is not a good candidate to be used in biological control of this thrips species.

- *Amblyseius herbicolus* and *Amblyseius tamatavensis* are able to prey on first and second instar larvae of *F. occidentalis.* They also have a good oviposition rate on both thrips stage. In addition, both predators can develop into adults feeding on first instar larvae of *F. occidentalis*.

- *Amblyseius herbicolus* and *Amblyseius tamatavensis* are able to reduce *F. occidentalis* population on bell pepper plants with *Typha* sp. pollen as supplementary food.

- Reduction of *F. occidentalis* population by *Amblyseius herbicolus* and *Amblyseius tamatavensis* also result in plants with less severe damaged caused by this pest.

- *Amblyseius tamatavensis* is able to control *F. occidentalis* and *Bemisia tabaci* populations simultaneously on bell pepper plants.

- Reduction of *F. occidentalis* and *B. tabaci* populations by *A. tamatavensis* increased plant yield.

- *Amblyseius herbicolus* and *Amblyseius tamatavensis* have potential to be used in biological control programs of *F. occidentalis* in Brazil.