ANDRÉ COSTA CARDOSO

SEARCH FOR PREDATORY MITES TO CONTROL TOMATO PESTS

Dissertation presented at the Universidade Federal de Viçosa as part of the requirements of the Graduate Program in Entomology, to obtain the title of *Magister Scientiae.*

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A minha grande avó Dinah Alves Corgozinho

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SUMMARY

ABSTRACT

CARDOSO, André Costa, M.Sc., Universidade Federal de Viçosa, February, 2019. **Search for predatory mites to control tomato pests.** Advisor: Arnoldus Rudolf Maria Janssen.

With the excessive use of pesticides and the increasing impact on the environment and human health, safer pest control practices such as biological control have gained strength. It is applied in many agricultural areas and provides an alternative to chemical pest control. One of the most important crops in the world, the tomato (*Lycopersicon esculentum* Miller), is among the crops that require many sprays with agrochemicals. Biological control on tomato is often difficult because its glandular trichomes can release substances that are toxic to arthropods and may hinder the foraging of natural enemies of the pests. Adaptation of natural enemies to this crop is one of the selection criteria for potential biocontrol agents. Tomato originates from southern America, therefore, predators from wild plants from this continent may be adapted to tomato. Therefore, predatory mites were collected from wild and feral plants in the Minas Gerais state, Brazil, where the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) was found. Our aim here was to evaluate the potential of this predator as a biological control agent on tomato plants. In Chapter 1 and 2, I evaluated the potential of this predatory mite to control the whitefly, *Bemisia tabaci* and the tomato russet mite, *Aculops lycopersici. Amblyseius herbicolus* develops and reproduces when feeding on these two important tomato pests. Moreover, it was able to significantly reduce *B. tabaci* densities on tomato plants.

RESUMO

CARDOSO, André Costa, M.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Busca por ácaros predadores para controle de pragas em tomate**. Orientador: Arnoldus Rudolf Maria Janssen.

Com o uso excessivo de pesticidas e o crescente impacto sobre o meio ambiente e a saúde humana, práticas mais seguras de controle de pragas, como o controle biológico tem ganhado atenção. O controle biológico é aplicado em muitas áreas da agricultura e fornece uma alternativa ao controle químico de pragas. Uma das culturas mais importantes do mundo, o tomate (*Lycopersicon esculentum* Miller), está entre as culturas com maior quantitate de aplicações de agroquímicos. O controle biológico no tomate é muitas vezes difícil de ser realizado porque os seus tricomas glandulares podem liberar substâncias que são tóxicas para os artrópodes e podem dificultar o forrageamento dos inimigos naturais das pragas. Assim, a adaptação de inimigos naturais a esta planta é um dos critérios de seleção para potenciais agentes de biocontrole, e como o tomate é originário da América do Sul, os predadores de plantas silvestres deste continente podem ser adaptados ao tomate. Portanto, foram coletados ácaros predadores de plantas silvestres e selvagens no estado de Minas Gerais, Brasil, e o ácaro predador *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) foi o encontrado. Nosso objetivo aqui foi avaliar o potencial deste predador como um agente de controle biológico em plantas de tomate. Nos capítulos 1 e 2, foi avaliado o potencial deste ácaro predador para controlar duas importantes pragas do tomate: a mosca-branca *Bemisia tabaci* e o ácaro-do-bronzeamento-tomateiro, *Aculops lycopersici.* O mesmo foi capaz de se desenvolver e se reproduzir quando alimentado de ambas as pragas. Além disso, ele foi capaz de reduzir a densidade de *B. tabaci* em plantas de tomate.

General introduction

The excessive use of pesticides is inflicting damage on human health and ecosystems around the world (UN Human Rights Council, 2017; Hyland et al. 2019). The use of synthetic insecticides increases resistance of pests and leads to pest resurgence, which results in increased application frequencies and dose rates (Calvo et al., 2015). This vicious circle causes more pest problems, increasing residue levels and negative side effects on the environment. Therefore, a transition to more sustainable practices such as biological control is needed (UN Human Rights Council 2017).

One strategy of biological control is augmentative biological control, in which natural enemies (parasitoids, predators or micro-organisms) are mass-reared for release in large numbers to control pests in the crops (van Lenteren et al., 2018; van Lenteren and Bueno, 2003). This strategy is applied in many areas of agriculture and provides an alternative to chemical pest control (Cock et al., 2010; van Lenteren and Bueno, 2003).

Generalist predators were not considered for biological control for a long time. It was believed that biological control with specialist natural enemies was more successful (Murdoch et al. 1985; Symondson et al. 2002) because the dynamics of generalist natural enemies was not synchronized with the pest and because generalist predators usually do not have high growth rates (Murdoch et al. 1985). However, the lack of synchronization of generalist predators with their prey may actually be advantageous for biocontrol because pest outbreaks can be prevented by maintaining populations of generalist natural enemies by supplying them with alternative food sources, which is much more difficult with specialists (Ramakers 1990; Ramakers and Voet 1995; Nomikou et al. 2002).

The presence of alternative foods can indeed increase densities of natural enemies and subsequently cause a reduction of the pest populations, a phenomenon similar to apparent competition (Holt 1977; van Rijn et al. 1999; Nomikou et al. 2002; Grosman et al. 2014; Nomikou et al. 2010). Apparent competition refers to an indirect negative interaction between prey species that share a population of natural enemies (Holt 1977; Holt and Bonsall 2017). According to this theory, the presence of one prey will result in an increase of densities of the natural enemy, resulting in a decrease of the equilibrium density of the other prey. An example of this interaction is the biological control of thrips and whiteflies, where the control of whiteflies by the predatory mite *Amblyseius swirskii* was improved when thrips, another prey of this predator, was also present (Messelink et al. 2008). A similar increase in natural enemy densities and decrease of a pest can be obtained by supplying alternative food. In contrast to generalist natural enemies, the densities of specialist predators will decrease when pest densities are low, and this may facilitate new pest invasions (Janssen and Sabelis 2015). Therefore, the use of generalist natural enemies with alternative food has increased considerably.

Tomato (*Lycopersicon esculentum* Miller) is one of the most important crops in the world, and receives high agrochemical sprays to keep pests below the economic threshold levels (van Dam et al., 2006; Yardim and Edwards, 1998). Originating from the Andean region of South America, it was domesticated in Mexico (van Dam et al., 2006). Wild tomatoes are currently found in several parts of southern and central America and domesticated tomatoes can be found on all continents (van Dam et al., 2006). Like other plants of the *Lycopersicon* genus, tomato possesses glandular and non-glandular trichomes, which have generally been reported to provide protection against biotic and abiotic environmental factors (Farrar and Kennedy 1991; Glas et al. 2012). However, glandular trichomes can release substances that are toxic to arthropods and may hinder phytophagous arthropods as well as natural enemies of the pests (Van Haren et al. 1987; Kennedy 2003; Glas et al. 2012; van Houten et al. 2013).

Adaptation to a crop is one of the criteria in the search for natural enemies (van Lenteren et al., 2018) and because tomato originates from southern America, predators from wild or feral plants may be adapted to tomato. Brazil has a large biodiversity of predatory mites of the family Phytoseiidae (Demite et al., 2014) which are efficient biological control agents in several agricultural systems (van Lenteren et al., 2018). Therefore, predatory mites were collected from wild and feral plants in the Minas Gerais state, Brazil, and the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) was found.

Amblyseius herbicolus reproduces by thelytokous parthenogenesis, hence, the species consists of females only (de Moraes and Mesa, 1988). Thelytoky is sporadically present in mites (Oliver 1983), and this may be an advantage in this case because adaptive traits of this population will not be diluted because of outcrossing with other populations of this species in the field (Hoy 1985; Hoy and Cave 1986). *Amblyseius herbicolus* is a generalist predatory mite, abundant in several crops and able to feed on pollen and on prey such as immature whiteflies and broad mites (Rodríguez-Cruz et al. 2013; Cavalcante et al. 2015; Duarte et al. 2015). Therefore, our aim here was to evaluate the potential of this predator as a biological control agent of two important tomato pests: the whitefly *Bemisia tabaci* and the tomato russet mite *Aculops lycopersici.*

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Chapter 1:

Potential of the predatory mite Amblyseius herbicolus Chant (Acari: Phytoseiidae) to control Bemisia tabaci on tomato plants

Abstract

The whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) is an important pest of tomato and responsible for the transmission of more than 128 types of viruses. It is often controlled with pesticides, which have negative impact on the environment and to which pests have become resistant. Sustainable alternatives such as biological control are needed and several predators and parasitoids are efficient biological control agents of this pest. Although predatory bugs and beetles can be used to control whiteflies on tomato, predatory mites and parasitoids that are commercially available for control of *B. tabaci* on several crops are not recommended for this plant host. Predatory mites are often entrapped by the glandular trichomes of tomato and removal of leaves, which is a common management practice, removes parasitized whiteflies. Therefore, adaptation to this crop is one of the criteria in the search for natural enemies. Because tomato originates from southern America, predators collected from wild and feral tomato on this continent may be adapted to this plant. Predatory mites were collected from feral tomato plants and *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) was found. Laboratory experiments showed that this predator can develop and reproduce when feeding on whitefly eggs. It was also capable of feeding on the first instar (crawlers) of *B. tabaci* and of controlling populations of whitefly on tomato plants with pollen as alternative food.

Introduction

The whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) is an important pest of tomato (van Dam et al. 2006). It is able to feed on more than 600 plant species (Brown 2009) and is responsible for the transmission of more than 128 types of plant viruses (Hogenhout et al. 2008). In addition to indirect damage through virus transmission, whiteflies can also cause direct damage such as leaf necrosis and irregular fruit ripening. When feeding, whiteflies release a sugary substance (honeydew), which serves as a substrate for the growth of saprophytic fungi, which can decrease the photosynthetic capacity of the plant (Brown et al. 1995).

The female deposits 90–95% of her eggs on the lower surface of young leaves and the older stages prevail on older leaves (Chandel et al., 2012). Whiteflies eggs are attached to plant leaf tissue by a pedicel (Rao and Reddy 1989; Byrne and Bellows 1991). The eggs turn brown before hatching. The first instar is mobile, and is commonly called “crawler”. Their movement is usually limited to the first few hours after hatching. The others three nymphal instars are scale-like and stationary (Byrne and Bellows 1991; Chandel et al., 2012). The fourth nymphal instar is commonly referred to as a pupa from which adults emerge (Byrne and Bellows 1991). The adult is white in color and measures 1.0–1.3mm in length (Chandel et al., 2012).

Besides having negative impact on the environment, chemical control of whiteflies is difficult because of the development of resistance to various active ingredients of pesticides (Omer et al. 1993; Dennehy and Williams 1997; Elbert and Nauen 2000; Ahmad et al. 2002). Therefore, sustainable practices such as biological control are needed (Calvo et al., 2015; UN Human Rights Council 2017). Several predators and parasitoids are efficient biological control agents of this pest (van Roermund et al. 1997; Gerling et al. 2001; Perdikis et al. 2008; Calvo et al. 2012). *Euseius scutalis* Athias-Henriot and *Amblyseius* (*Typhlodromips*) *swirskii* Athias-Henriot (Acari: Phytoseiidae) were found to suppress populations of *B. tabaci* on cucumber plants with alternative food (Nomikou et al. 2002, 2010). The presence of alternative foods can increase densities of natural enemies and subsequently cause a reduction of the pest populations, a phenomenon similar to apparent competition (Holt 1977; Nomikou et al. 2010; Holt and Bonsall 2017). Although predatory bugs and beetles can be used to control whiteflies on tomato, predatory mites and parasitoids that are commercially available for control of *B. tabaci* on several crops are not recommended for this host plant (Perdikis et al. 2008; Koppert Biological Systems, 2019). Predatory mites are often entrapped by glandular trichomes of this plant species (Van Haren et al. 1987; Gillespie and Quiring 1994; Cédola et al. 2001; Kennedy 2003) and the intensive practice of defoliation by the growers can result in the removal of immature parasitized whiteflies (Nannini et al. 2007; Bonato and Ridray 2007; Perdikis et al. 2008; Koppert Biological Systems, 2019).

Therefore, adaptation to the crop plant should be one of the criteria in the search for natural enemies (van Lenteren et al. 2018) and because tomato originates from southern America, predators from wild plants may be adapted to tomato. Despite the large biodiversity of phytoseiids (Demite et al. 2014), the use of predatory mites for the control of *B. tabaci* is still poorly studied in Brazil (Cavalcante et al. 2015b). Only five phytoseiid species were tested as possible control agents for whitefly in Brazil (Cavalcante et al. 2015b). Although they were all able to prey and reproduce feeding on whitefly eggs, only *Amblyseius tamatavensis* has been tested for its efficiency in controlling whitefly on bell pepper plants (Cavalcante et al. 2017). There are no studies of these predators on tomato plants and there are also no predators to control whiteflies available on the Brazilian market today. Thus, predatory mites were collected from wild and feral plants in the state of Minas Gerais, Brazil, and the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) was found. It is a generalist mite, abundant in several crops and able to feed on pollen and on prey such as whitefly immatures and broad mites (Rodríguez-Cruz et al. 2013; Cavalcante et al. 2015b; Duarte et al. 2015). Therefore, our aim here was to evaluate the potential of a strain of *A. herbicolus* that was collected from tomato plants in the field to control *B. tabaci* on such plants. Experiments were carried out in the laboratory to test the predator's ability to develop and reproduce when feeding on *B. tabaci* eggs. An experiment was also carried out to verify the ability of this predatory mite to control populations of whitefly on tomato plants.

Materials and methods

Predator collection

Predators were collected from tomato plants in gardens in the urban and rural areas of Viçosa (latitude: 20º 45 '14 "S; longitude: 42º 52' 55" W) and Prados (latitude: 21º 03 ' S; longitude: 44º 04' 47" W), both in the Minas Gerais state. Plants with chemical applications were avoided and leaves attacked by pest were removed and placed in paper bags and kept refrigerated. The leaves were inspected with a stereomicroscope and predatory mites were transferred to a plastic arena with a fine brush, and were reared as described below. The predatory mite *Amblyseius tamatavensis* was collect after it contaminated the *A. lycopersici* rearing. Owing to lack of time, it was not further studied.

Predator rearing

The predatory mites were reared on arenas consisting of a plastic sheet (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. In the beginning, several food types were supplied until we knew what would be the best to rear each species: *Typha sp.* pollen, bee pollen and tomato leaves infested with all stages of two spotted spider mite (*Tetranychus urticae*). It was noticed that *Typha sp.* pollen alone was sufficient to maintain laboratory rearings. This food was provided twice a week. After establishing the rearings, slides were made to send to a taxonomist to identify the species. Rearing arenas were maintained in a room with controlled conditions (25 ± 2° C, 70 ± 10% RH and 12 hours photophase).

Pollen

Pollen was collected from *Typha sp.* plants found in the rural area of Viçosa, and on the campus of the Federal University of Viçosa. It was dried in an oven at 60º C for 48 hours and placed in a sealed container in the freezer. Small quantities were periodically removed and stored in an Eppendorf tube in a refrigerator to use for rearings and experiments.

Cultivation of clean plants

Tomato plants used for breeding and experiments (var. Aguamiel) were cultivated in a greenhouse in 3-liter pots with substrate for plants based on pinus bark, peat and expanded vermiculite, enriched with macro- and micronutrients (Tropstrato HT). The plants were fertilized weekly with a mixture of NPK (20-5-20) and a phosphorus source (18% of P205), and supported with bamboo sticks tied with a string.

Tetranychus urticae rearing

Tetranychus urticae were collected from tomato plants from a greenhouse of the Laboratory of Acarology of the Federal University of Viçosa, Minas Gerais, Brazil. Spider mites were reared on detached tomato leaves kept in plastic trays containing water to maintain leaf turgor. The colonies were reared inside a room with controlled conditions (25 ± 2 ° C, 12 hours photophase).

Whitefly rearing

Whiteflies (*Bemisia tabaci* Gennadius biotype B) were collected from tomato plants cultivated on the campus of the Federal University of Viçosa, Minas Gerais, Brazil, with the aid of a sucker, and transferred to a cage (0.5 x 0.5 x 1.0 m) with clean tomato plants. A new tomato plant was added every week. The cages were placed on a table inside the laboratory of acarology.

Laboratory experiments

All the laboratory experiments were done in black plastic dishes (ø = 5.5 cm; 1.4 cm high) with a piece of wet filter paper inside and covered with a transparent lid. These experiments were performed at 25 ± 2 ° C, 70 ± 10% RH and 12 hours photophase.

Development and oviposition of *A. herbicolus* feeding on pollen and on *B. tabaci* eggs

Infested leaves were removed from the whitefly rearing and cut into pieces. Each piece was examined with a stereomicroscope to ensure the presence of at least 20 fresh *B. tabaci* eggs (based on their light green coloration) and subsequently added to half of the dishes. One mg of pollen with a clean piece of leaf was placed in the other dishes. A predator larva was placed in each dish and was checked once a day to verify the development until adulthood. After becoming adults, each dish was checked once a day to count the eggs produced by the predators. After each evaluation, the piece of leaf with the food was replaced and predator eggs were removed to prevent cannibalism. The oviposition of each predator was recorded for 5 days. Twenty replicates were done per treatment. The intrinsic rates of increase (r_m) of *A. herbicolus* were estimated for these two food sources using the developmental time, juvenile survival and average oviposition rate.

Oviposition and predation rate of *A. herbicolus* on crawlers and young and old eggs of *B. tabaci*

For treatments with *B. tabaci* eggs, a piece of leaf containing eggs of whiteflies was cut from one of the plants of the rearing unit. Depending on the treatment, new or old eggs were removed from the piece of leaf with a fine insect pin, based on the color of the eggs. Twenty new or old eggs were left per leaf piece. For the treatment with crawlers, twenty crawlers were transferred with a fine pin to a clean piece of leaf. One mg of *Typha sp.* pollen was put on a clean piece of leaf and offered as a control for oviposition. An egg wave was made to standardize the age of predators. When all the adult females of *A. herbicolus* were pregnant, in this case 9 ± 1 days old, one predator was transferred to each dish. Every 24 h, the piece of leaf was replaced with a new one with the same diet, and the numbers of prey attacked and eggs produced by the predators were recorded after 24, 48 and 72 h. Oviposition of the first day was excluded from analysis because of the possible effect of the previous diet (Sabelis, 1990). Eight replicates were done with exception of treatment with pollen, which was replicated six times.

Population dynamics of *A. herbicolus* and *B. tabaci* on tomato plants

The population dynamics experiment was carried out between the months of July and August of 2016 in a small area, sheltered with plastic. The temperature was 13 - 26º C. A single tomato plant with four leaves was placed inside each of the six cages (0.5 x 0.5 x 1.0 m^3) used for the experiment. Three of these plants received twenty adult females of *A. herbicolus*. On the same day, three pairs of adult whiteflies (6 ± 1 days of age) were introduced in each cage. About 15 mg of *Typha sp*. pollen was added on all leaves of the six plants. The experiment was evaluated twice a week, whiteflies were counted with the aid of a sucker to avoid double counting, and mite stages visible to the naked eye were counted. After counting, the same amount pollen was added to the leaves, and the whiteflies were reintroduced. The plants were watered and fertilized regularly as above. The population growth rate of *A. herbicolus* was calculated based on the number of predators between the 4th and 18th day with the formula of exponential growth:

$$\mathbf{r} = \frac{\ln\left(\frac{N_t}{N_0}\right)}{t}$$

with r = growth rate, N_t = final number of predatory mites, N_0 = initial number of predatory mites, t = time interval (days). The time interval was chosen because of the characteristic curve of exponential growth in between these two time points (Figure 6).

Statistical analyses

The data of the oviposition and population dynamics experiments were analyzed with linear mixed effects models (LME) with replicate as a random factor. Contrasts between treatments were obtained with the function cld (package multcomp of R). Effects of diet on survival and developmental were analyzed with the survdiff function (package survival of R). All statistical analyses were done using the software R version 3.3.3 (R Project for Statistical Computing. http://www.r-project.org).

Results

Development and oviposition of *A. herbicolus* feeding on pollen and on *B. tabaci* eggs

The developmental time of *A. herbicolus* fed with *B. tabaci* eggs on pieces of tomato leaves differed significantly from that of pollen-fed predators (Chi² = 4.2, d.f. = 1, p = 0.04; Fig 1). Survival on these two diets did not differ significantly (Chi² = 0.9, d.f. = 1, p = 0.3; Fig. 1). There was a significant interaction between diet and time on the oviposition of *A. herbicolus* (lme: d.f. = 1, Likelihood ratio = 4.24, p = 0.0395). Oviposition was significantly higher on a diet of whiteflies on day 3, whereas there was no significant difference on the other days (Fig. 2). The intrinsic rate of increase obtained on the diet of *Typha sp.* pollen and *B. tabaci* eggs were respectively 0,205 and 0.186.

Oviposition and predation rate of *A. herbicolus* on crawlers and young and old eggs of *B. tabaci*

There was a significant effect of the interaction between day and diet on the oviposition rate (lme: Likelihood ratio = 11.78, d.f. = 3, p = 0.008). On the third day, the oviposition of *A. herbicolus* was lower when fed with old *B. tabaci* eggs than when fed young eggs, crawlers or pollen (Fig. 3). The predation rate was significantly lower on old whitefly eggs than on young eggs (Fig. 3), and there was no significant interaction between day and diet (lme: d.f. = 3, Likelihood ratio < 0.01, p = 1; Fig. 4).

Population dynamics of *A. herbicolus* and *B. tabaci* on tomato plants

In the population dynamics experiment, the effect of the interaction between treatment and time on whitefly densities was significant (lme: d.f. = 1, $Chi^2 = 18.5$, $p < 0.001$; Fig. 5). This was because the treatments started with the same number of whiteflies and finished with more whiteflies on plants without predators than on plants with predators. The difference in whitefly densities between plants with and without predators became significant on day 47 (Fig.5) and increased until the end of the experiment. At the end of the experiment, more than eleven times fewer whiteflies were found on average on plants with *A. herbicolus* than on plants without them. In the first month of the experiment, the predator densities increased until reaching an average of one hundred and twenty predators per plant around the thirty-seventh day, after which they began to decrease. At the end of the experiment there were still about forty predatory mites visible to the naked eye per plant (Fig. 6). The intrinsic rate of increase calculated through the number of predatory mites observed on the tomato plants among the 4th and 18th days was 0.137.

Discussion

Although it is known that the predatory mite *Amblyseius herbicolus* can feed and reproduce on *B. tabaci* eggs (Cavalcante et al. 2015b), it was not clear yet whether it can control this pest on tomato plants, a difficult host plant species for natural enemies. At the end of the population experiment, *A. herbicolus* reduced the numbers of whiteflies with more than 90% compared to plants without predators (Fig. 5). Similar results were obtained with *Euseius scutalis* Athias-Henriot and *Amblyseius* (*Typhlodromips*) *swirskii* Athias-Henriot, which were able to suppress populations of whiteflies on cucumber plants with alternative food (Nomikou et al. 2002). Cavalcante et al. (2017) showed a reduction of 69 to 87% of whitefly immatures on bell pepper plants with the predatory mite *A. tamatavensis*. Although a direct comparison of their results with ours is impossible because of the different host plants and methodologies utilized, *A. herbicolus* showed satisfactory results even more for having obtained such control on a hostile plant as tomato.

Amblyseius herbicolus as well as *A. swirskii* showed to be able to feed and reproduce when feeding on first instars of whitefly (crawlers) (Nomikou et al. 2004). Was observed that new whitefly eggs were more susceptible to predation than older eggs, and also resulted in a higher oviposition rate by *A. herbicolus*. Similar results were obtained with two populations of *A. swirskii* that also had greater difficulty in preying and reproducing on eggs of more than 48h old (Cavalcante et al. 2015a).

The rate of increase measured in the experiments was consistent with other published values for *A. herbicolus* (Reis et al. 2007; Rodríguez-Cruz et al. 2013), and did not differ much from that of *A. swirskii* (Nomikou et al. 2001; Nguyen et al. 2013), which is a much-used whitefly control agent (Janssen and Sabelis 2015; Calvo et al. 2015). The growth rate measured in the experiment on tomato plants was lower than that found in the laboratory experiments. This could be due to the greater effect of the host plant on the survival of this predator, or also due to climatic conditions, which could not be controlled on the experiment on plants.

While Cavalcante et al. (2017) reported that supplementary food apparently did not promote the persistence of *A. tamatavensis* on the plants when the densities of *B. tabaci* were low, the control with *A. swirskii* was improved by the presence of pollen, which increased the number of predators per plant and consequently decreased the density of whiteflies (Messelink et al. 2006; Nomikou et al. 2010). Although we did not have a control with predators without pollen, predators persisted when densities of whiteflies were low (Fig. 4). Thus, pollen can be used to maintain a population of this predator on plants and prevent further whitefly infestations.

It was long believed that biological control with specialist natural enemies was more successful than with generalists (Murdoch et al. 1985; Symondson et al. 2002) because the dynamics of generalist natural enemies was not synchronized with the pest and because generalist predators usually have longer generation times (Murdoch et al. 1985). However, the lack of synchronization of generalist predatory mites may actually be advantageous for biocontrol because pest outbreaks can be prevented by maintaining populations of generalist natural enemies by offering alternative food sources (Janssen and Sabelis 2015). The presence of alternative foods can increase densities of natural enemies and subsequently cause a reduction of the pest populations, a phenomenon similar to apparent competition (Holt 1977; Nomikou et al. 2010; van Rijn et al. 1999; Nomikou et al. 2002; Grosman et al. 2014;). In contrast, the densities of specialist predators will decrease when pest densities are low, and this may facilitate new pest invasions (Janssen and Sabelis 2015).

Nowadays, supplying food for natural enemies is a common practice in many countries and various biological control companies sell such alternative food. Future experiments with larger numbers of replicates should further confirm our results.

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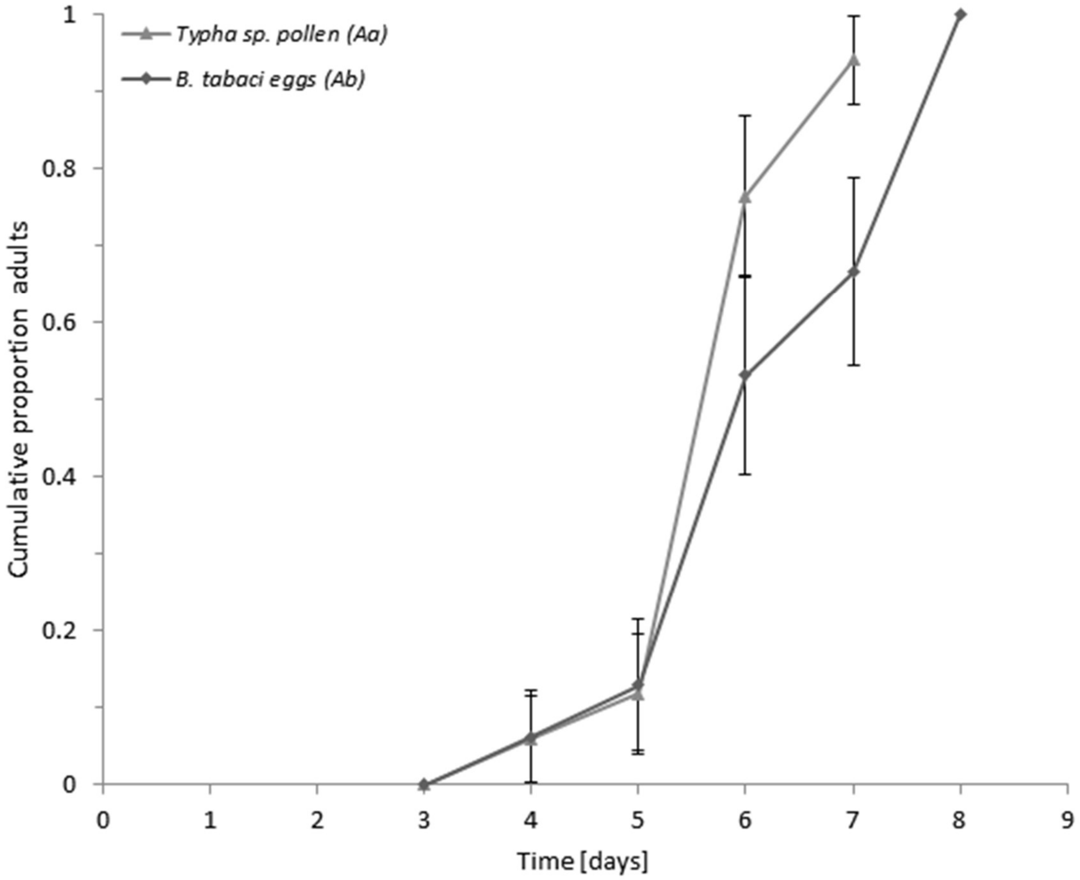

Figure 1: Development and survival of *A. herbicolus* fed *B. tabaci* eggs (dark line) or *Typha sp*. pollen (light line). Shown is the mean cumulative proportion of adults as a function of time. Total survival is the final cumulative proportion that reached adulthood. Treatments with same capital letters did not differ significantly in survival and with different small letters were different in development.

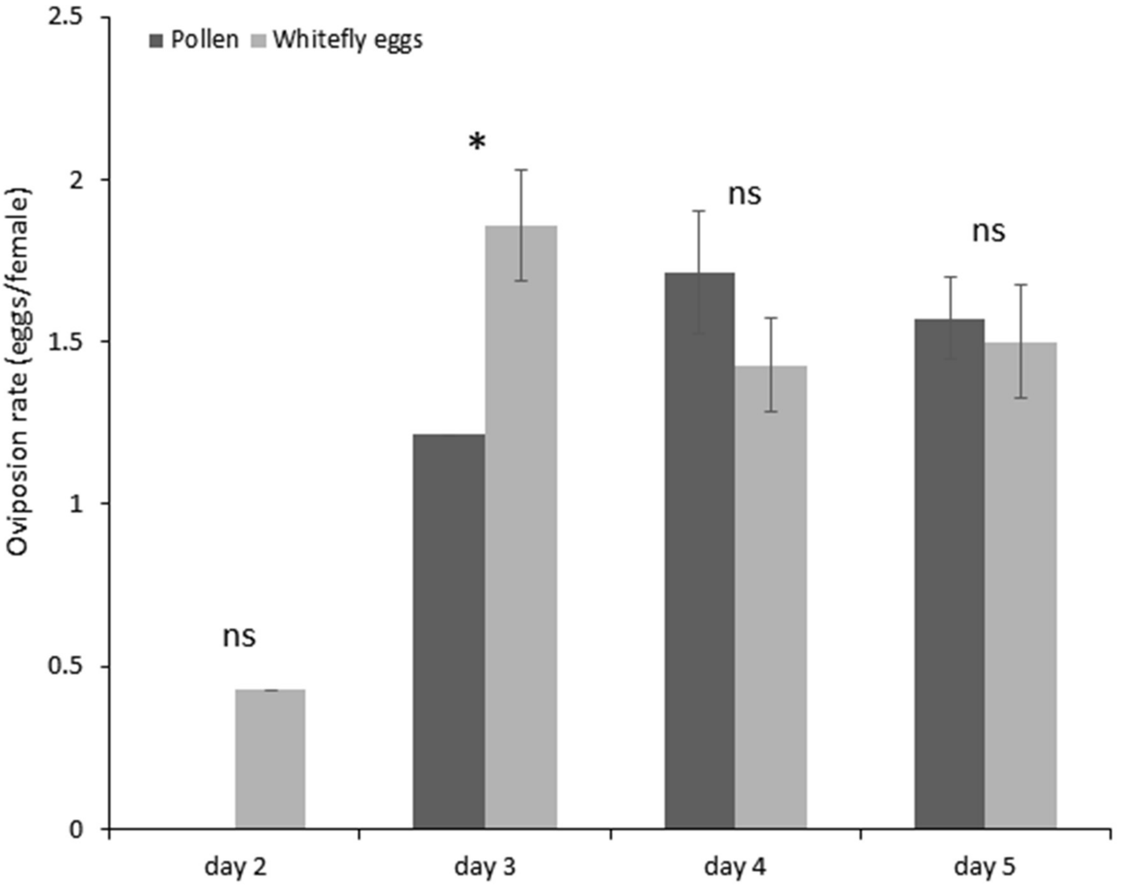

Figure 2: Average oviposition rate of female predators fed with *B. tabaci* (light bars) or *Typha sp*. pollen (dark bars). (ns) indicates that the treatments did differ significantly, asterisks (*) show significant difference among treatments ($p < 0.05$).

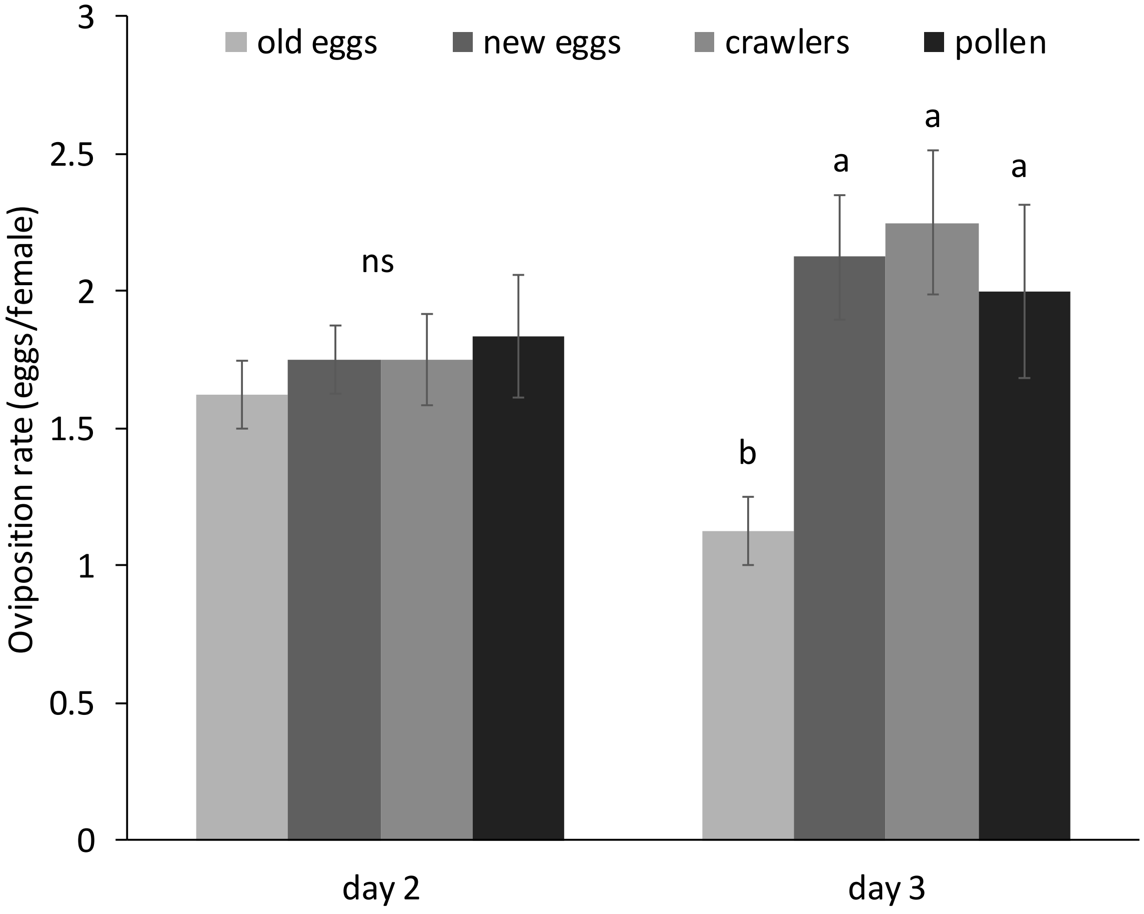

Figure 3: Average oviposition rates of female predators fed with *B. tabaci* or *Typha sp*. pollen. ns: treatments did not differ significantly between days; different letters show significant difference among treatments per day ($p < 0.05$).

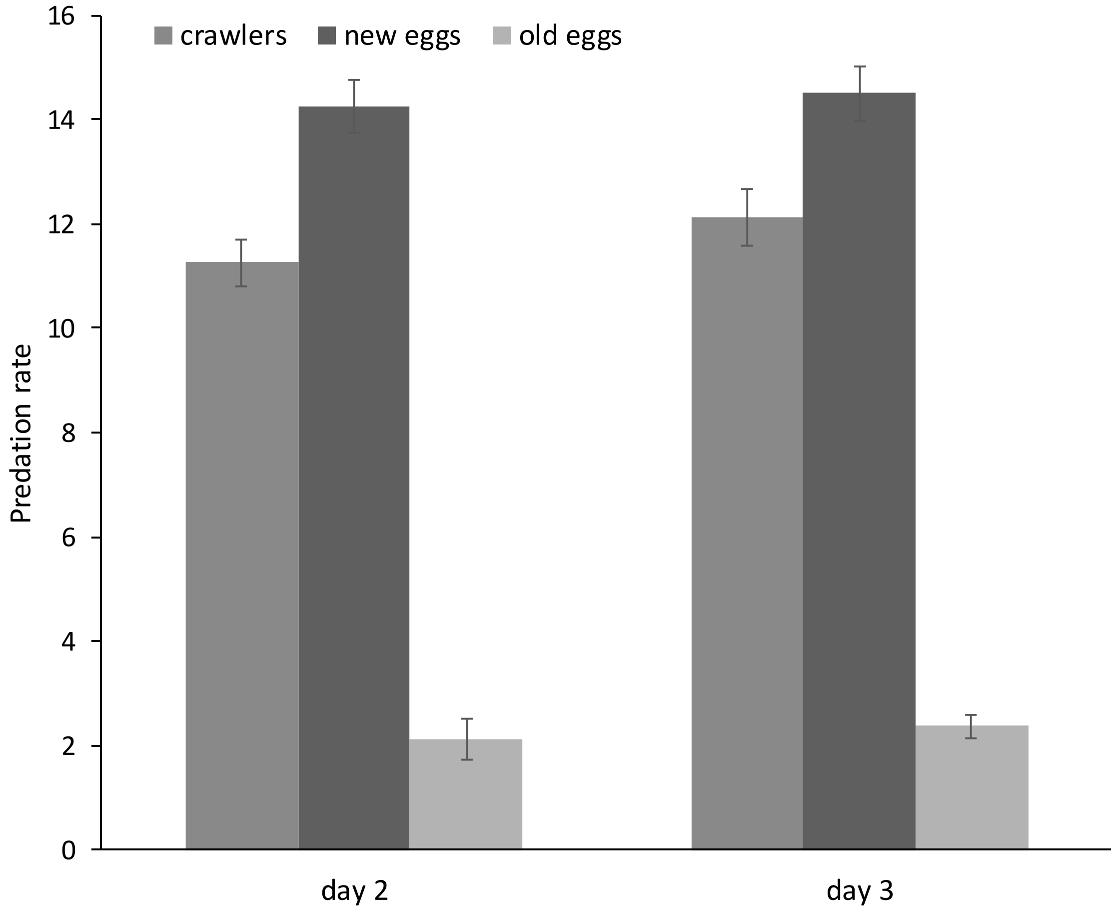

Figure 4: Predation rate of *A. herbicolus* on crawlers, young or old eggs of *B. tabaci*.

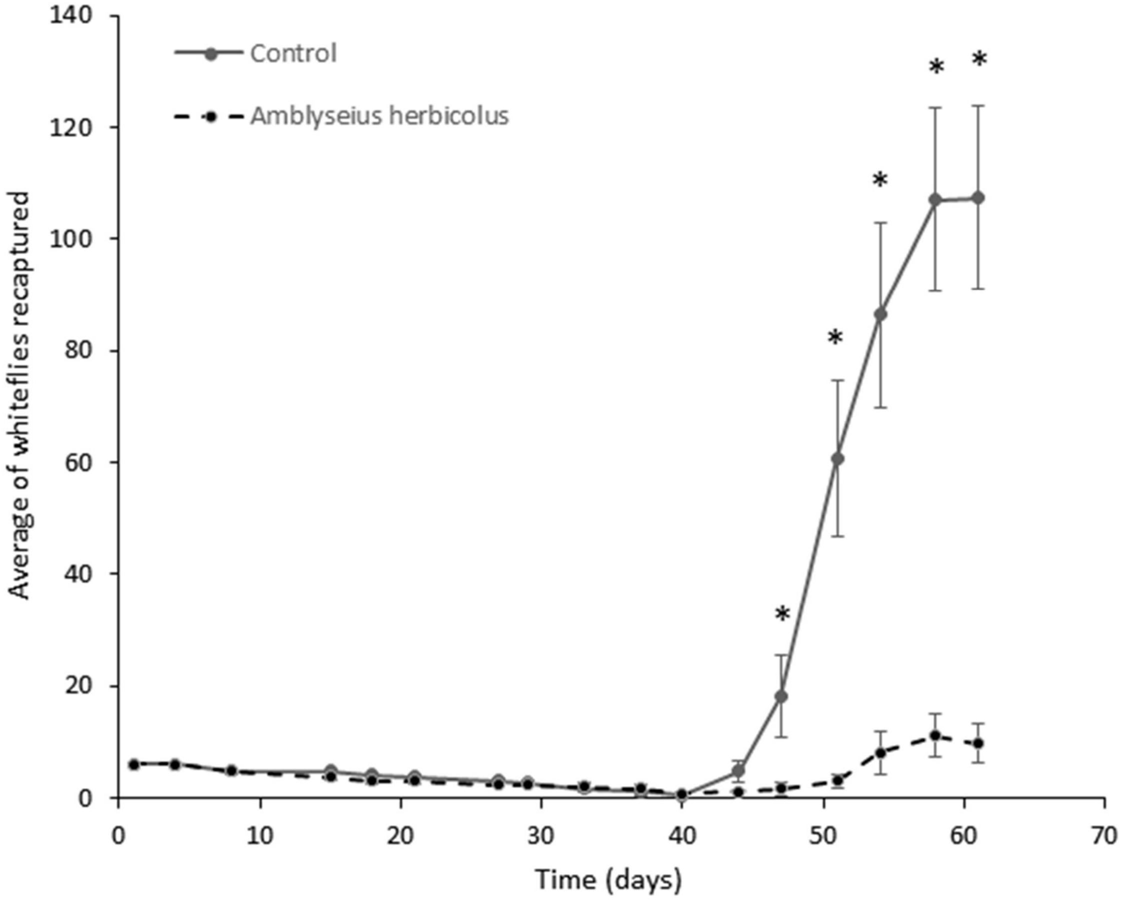

Figure 5: Average numbers of whiteflies on tomato plants with (dashed line) and without (solid line) predators during two months. Asterisks (*) indicate significant differences between treatments per day ($p < 0.001$).

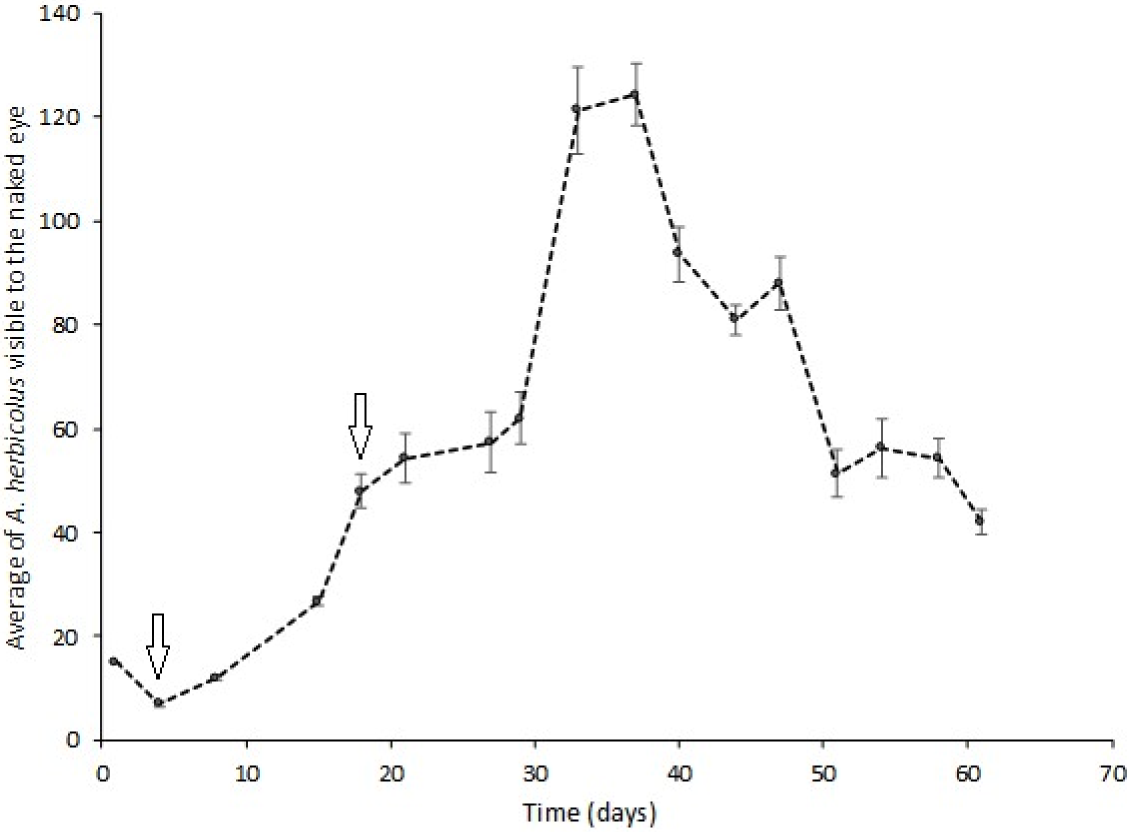

Figure 6: Average *A. herbicolus* visible to the naked eye in treatment with this predator (dashed line). The arrows shown the interval used to calculate the population growth rate.

Chapter 2:

Potential of the predatory mite Amblyseius herbicolus Chant (Acari: Phytoseiidae) to control the tomato russet mite, Aculops lycopersici Tryon (Acari: Eriophyidae)

Abstract

An important pest of tomato is the tomato russet mite *Aculops lycopersici* Tryon (Acari: Eriophyidae). Populations of this mite develop quickly under favorable conditions and attack stems, leaves, flowers, and fruits. It is mainly controlled with acaricides. Control of this pest is mainly done with pesticides because sustainable alternatives, such as biological control, are often difficult on tomato because the dense trichomes often hinder natural enemies. Although several families of predatory mites such as Phytoseiidae, Stigmaeidae and Tydeidae have been tested as biocontrol agents for the tomato russet mite, there are still no commercial biocontrol products available. Thus, this study confirms that the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) is capable of developing and reproducing when feeding on the tomato russet mite. It is important to try to maintain predator populations on plants before pest invasion. The addition of pollen or other alternative food could maintain densities of natural enemies to prevent invasion by *A. lycopersici.*

Introduction

An important pest of tomato is the tomato russet mite (TRM) *Aculops lycopersici* Tryon (Acari: Eriophyidae), which has a world-wide distribution (Perring and Farrar 1986). Populations of this mite develop quickly under favorable conditions (Rice and Strong 1962; Haque and Kawai 2002) and attack stems, leaves, flowers, and fruits. A heavy infestation of *A. lycopersici* affects plant height, the number of leaves and the diameter of the stem (Haque and Kawai 2002). Severe infestations cause damage such as yellowish-brown-colored stems and branches and dried leaflets (Haque and Kawai 2002). Initially, the damage is found on the lower parts of the plants, and subsequently on the upper parts as the mites gradually move upward (Haque and Kawai 2002). Feeding on young tomato plants by TRM reduces yield because it decreases photosynthetic processes (Royalty and Perring 1989). The pest damages the upper and lower epidermal cell layer of leaflets and it can kill the host-plant, which is uncommon among Eriophyoidea (Royalty and Per Ring 1988).

Tomato possesses glandular and non-glandular trichomes, which have generally been reported to provide protection against biotic and abiotic environmental factors (Farrar and Kennedy 1991; van Houten et al. 2013). However, glandular trichomes can release substances that are toxic to arthropods and may hinder phytophagous arthropods as well as natural enemies of the pests (Van Haren et al. 1987; Kennedy 2003; Glas et al. 2012; van Houten et al. 2013).

TRM is mainly controlled with acaricides (Duso et al. 2010). Nevertheless, several families of predatory mites, such as Phytoseiidae, Stigmaeidae and Tydeidae have been tested as biocontrol agents of this pest (Osman and Zaki 1986; Hessein and Perring 1986; Brodeur et al. 1997; Haque and Kawai 2002; Trottin-Caudal 2005; Momen and Abdel-Khalek 2008; Park et al. 2010, 2011; van Houten et al. 2013; Lopes 2015). Life-history studies of several phytoseiids such as *Amblydromalus limonicus Amblyseius swirskii, Typhlodromus athiasae, Paraseiulus talbii* and *Euseius concordis* were performed with TRM (Momen and Abdel-Khalek 2008; Park et al. 2010, 2011; van Houten et al. 2013; Lopes 2015). Although *A. limonicus* can feed and reproduce on a diet of TRM, it was ineffective to control this pest. This predatory mite was able to establish on tomato plants only after heavy infestation of TRM when the glandular trichomes collapsed as a result of TRM feeding (van Houten et al. 2013). Moreover, the TRM populations escaped by moving up in the plant where the trichomes were still intact (van Houten et al. 2013). Trichomes create competitor-free and enemy-free space for TRM because it can seek refuge and feed between the glandular trichomes (van Houten et al. 2013).

Amblyseius swirskii was also able to reproduce when feeding on TRM, however apparently it has the same problems of establishment in the crop (Momen and Abdel-Khalek 2008; Park et al. 2010, 2011; van Houten et al. 2013). A study conducted with different populations of *Euseius concordis* showed a decrease in russet mite density on plants with predators, but this did not result in effective control (Lopes 2015). Moreover, mass rearing of *E. concordis* is still problematic: Astigmatina mites may not be suitable for rearing this species (Silva 2004; Barbosa and de Moraes 2015). Therefore, there are still no commercial biocontrol products for the control of TRM available.

Thus, after observing that the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae), found on tomato plants and therefore possibly adapted to this host, can feed on this pest, the present study was performed to evaluate its ability to develop and reproduce when fed with the tomato russet mite.

Materials and methods

Cultivation of clean plants

Tomato plants used for breeding and experiments (var. Aguamiel) were cultivated in a greenhouse in 3-liter pots with substrate for plants based on pinus bark, peat and expanded vermiculite, enriched with macro- and micronutrients (Tropstrato HT). The plants were fertilized weekly with a mixture of NPK (20-5-20) and a phosphorus source (18% of P205), and stems were tied to bamboo sticks for support.

Pollen

Pollen was collected from *Typha sp.* plants found in the rural area of Viçosa and on the campus of the Federal University of Viçosa. It was dried in an oven at 60º C for 48 hours and placed in a sealed container in the freezer. Small portions were periodically removed and stored in an Eppendorf tube in a refrigerator and were used for experiments and cultures.

Tomato russet mite rearing

Tomato russet mites were obtained from the Rural University of Pernambuco and reared on tomato plants surrounded by water to prevent contamination with other species. A new tomato plant was added every week. It was reared inside a room with controlled conditions (25 ± 2 ° C, 12 hours photophase), which was kept closed to prevent contamination with flying insects.

Predator rearing

Amblyseius herbicolus was reared on arenas consisting of a plastic sheet (10 x 15 cm^2) 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. *Typha sp.* pollen was supplied twice per week to maintain laboratory rearings. Rearings were maintained in a room with controlled conditions (25 ± 2° C, 70 ± 10% RH and 12 hours photophase).

Experiments

All experiments were done in the laboratory in black plastic dishes (ø = 5.5 cm; 1.4 cm high) with a piece of wet filter paper inside and covered with a transparent lid. All experiments were performed at 25 ± 2° C, 70 ± 10% RH and 12 hours photophase.

Development of *A. herbicolus* on *A. lycopercisi* on tomato petioles

On the first day, leaves infested with *A. lycopersici* were removed from the rearing and the leaflets removed. Then, pieces of petiole were cut and examined under a stereomicroscope to ensure the presence of at least 10 *A. lycopersici* adults with all immature stages. The amount of TRM was based on the studies conducted with *A. swirkii* feeding on this pest (Park et al. 2010). Each piece was added to half the dishes and 1 mg of pollen with an uninfested petiole was added to the other dishes. Predator larvae were collected from the rearing and one larva was added to each dish. The dishes were covered with a transparent lid and were opened every day to check whether the individuals had turned into adults and to add food. After the first adult was observed, observations were done every twelve hours. Each treatment had 22 replicates.

Oviposition of *A. herbicolus* feeding on *A. lycopersici* on tomato petioles

Dishes with clean petioles with pollen or with petioles with 10 *A. lycopersici* were prepared as above. An egg wave was made to standardize the age of predators.

When all adult females of *A. herbicolus* were seen to carry an egg (10 ± 1 days old), one predator was added to each dish. The dishes were covered with a lid and were opened every day to check the number of eggs. Subsequently, the eggs were removed to prevent cannibalism and food was added. Oviposition was measured during 5 days and the first day was excluded from the analysis to avoid the influence of the previous diet (Sabelis 1990). Sixteen and 15 replicates of treatments with *A. lycopersici* and *Typha sp.* pollen were done respectively.

Development and oviposition of *A. herbicolus* feeding on pollen and on *A. lycopercisi* on a piece of tomato leaf

On the first day, a piece of infested leaf with at least 10 *A. lycopercisi* adults from the rearing was added to half of the dishes and 1 mg of pollen with a clean leaf piece was added to the other dishes. A predator larva was added to each dish, which was then covered with a transparent lid and opened once a day to verify the development of the predator and to count the eggs produced by the predators. After each evaluation, the leaf piece with the food was replaced and the predator eggs were removed as above. The oviposition of each predator was recorded for 5 days. The first day of oviposition was included in the analysis because the predators had received the same diet during their development (20 replicates per treatment).

Statistical analyses

The oviposition data were analyzed with linear mixed effects models with replicate as a random factor. Contrasts between treatments through time were analyzed with the function cld (package multcomp of R). Survival and developmental analyses were done with the survdiff function (package survival of R). All statistical analyses were done with the software R, version 3.3.3 (R Project for Statistical Computing. http://www.r-project.org).

Results

Development of *A. herbicolus* on *A. lycopercisi* on tomato petioles

A. herbicolus developed successfully when feeding on *A. lycopersici* on tomato leaf petioles, but took significantly longer to reach adulthood than when they fed on

Typha sp. pollen (Chi² = 10.4, d.f.= 1, p = 0.001; Fig. 1). Survival did not significantly differ with predator diet (Chi² = 1.8, d.f. = 1, p = 0.2; Fig. 1).

Oviposition of *A. herbicolus* feeding on *A. lycopersici* on tomato petioles

Oviposition was not significantly affected by the interaction between day and treatment (lme: Likelihood Ratio = 0.02, d.f.= 5, p = 0.88; Fig. 2). *A. herbicolus* produced fewer eggs when fed with *A. lycopersici* on tomato leaf petioles than when fed with *Typha sp.* pollen (lme: Likelihood Ratio = 27.65, d.f.= 3, p < 0001; Fig. 2) and there was no significant difference in oviposition among days (Likelihood ratio = 0.857, d.f. = 1, p = 0.355).

Development and oviposition of *A. herbicolus* feeding on pollen and on *A. lycopercisi* on a piece of tomato leaf

When *A. herbicolus* fed on *A. lycopersici* on infested pieces of leaves, its developmental rate and juvenile survival did not differ significantly from that on a diet of pollen (Chi² = 1.5, d.f. = 1, p = 0.2 and Chi² = 1, d.f. = 1, p = 0.3, respectively, Fig. 3). There was no significant difference in the oviposition of *A. herbicolus* when fed with *A. lycopersici* and *Typha sp.* pollen offered on pieces of leaves. (lme: Likelihood Ratio = 1.7, d.f. = 30, p < 0.41; Fig. 4). Oviposition differed significantly among days (lme, Likelihood ratio = 64.3, d.f. = 1, p < 0.0001). This was because the second day differed from the other days (Fig. 4) probably because many adult females had not reached the reproductive stage yet (Fig. 2).

Discussion

The predatory mite *A. herbicolus* was able to develop and reproduce on a diet of *A. lycopersici* (Fig. 1 to 4). In the experiment where pieces of leaves infested with tomato russet mite were offered, *A. herbicolus* was able to develop and reproduce as good as when it fed on pollen and there was no difference in juvenile survival between groups of predators fed with these two food sources (Fig. 3). Similar results were obtained by Park et al. (2011, 2010), who evaluated the life history parameters of the predatory mite *A. swirskii* feeding on *A. lycopersici* and on cattail pollen in Korea. The only work that evaluated the efficacy of the predators for control of the tomato russet mite on tomato plants was the study conducted by Lopes, (2015), which showed a decrease of the density of *A. lycopersici* by the predator *Euseius concordis*. However, this control was not sufficient. Moreover, mass rearing of *E. concordis* is still problematic because the Astigmatina may not be suitable for rearing this species (Barbosa and de Moraes 2015).

In experiments with leaf petioles of tomato plants infested with *A. lycopersici*, the oviposition and developmental rate of *A. herbicolus* was lower than on a diet of pollen (Figure 1 and 2). However, when *A. lycopersici* was offered on pieces of leaves, the differences in oviposition and development were not significant (Figure 3 and 4). Further studies should to be done to verify this difference in results on petioles and leaves. It is possible that is more difficult to attack the prey on petioles because the density of trichomes is higher than on leaves (Van Haren et al. 1987) and *A. lycopersici* can hide between these trichomes (Sabelis and Bruin 1996; van Houten et al. 2013). In addition, it was observed that the greater part of the population of *A. lycopersici* infested the leaves (Haque and Kawai 2002) and the difference in densities of *A. lycopersici* on leaves vs petioles may have caused this difference in development and oviposition.

Collapse of the glandular trichomes after heavy infestation of TRM showed to help predatory mites to establish on tomato plants (van Houten et al. 2013), however, they were ineffective in controlling this pest. Moreover, the TRM populations escaped by moving up in the plant where the trichomes were still intact (van Houten et al. 2013). Therefore, it is important to try to maintain predator populations on plants before TRM invasion using alternative food to maintain densities of natural enemies and prevent invasion by *A. lycopersici.*

In Chapter 1, I showed that the predatory mite *A. herbicolus* was able to reach considerable densities on tomato plants when pollen was added as alternative food. Moreover, unlike TRM, *B. tabaci* does not cause the collapse of glandular trichomes. Thus, if a population of predators is established on plants, as in the experiment with whiteflies, this might prevent the establishment of TRM.

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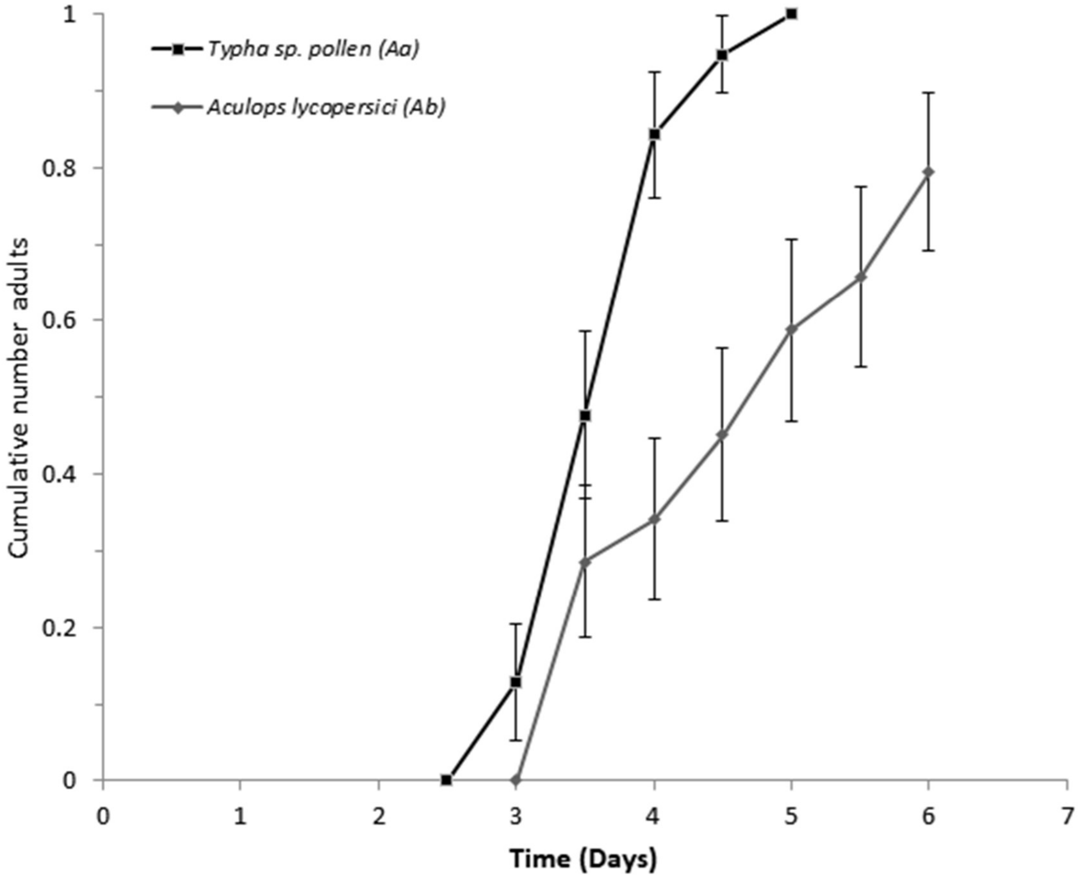

Figure 1: Development and survival of *A. herbicolus* fed *A. lycopersici* (diamonds and grey line) and *Typha sp*. pollen (squares and black line). Shown are the mean cumulative proportions of adults as a function of time. Total survival is the final cumulative proportion that reached adulthood (i.e., 79% on a diet of *A. lycopersici* and 100% on pollen). Treatments with the same capital letters did not differ significantly in survival and with different small letters differed in developmental rate.

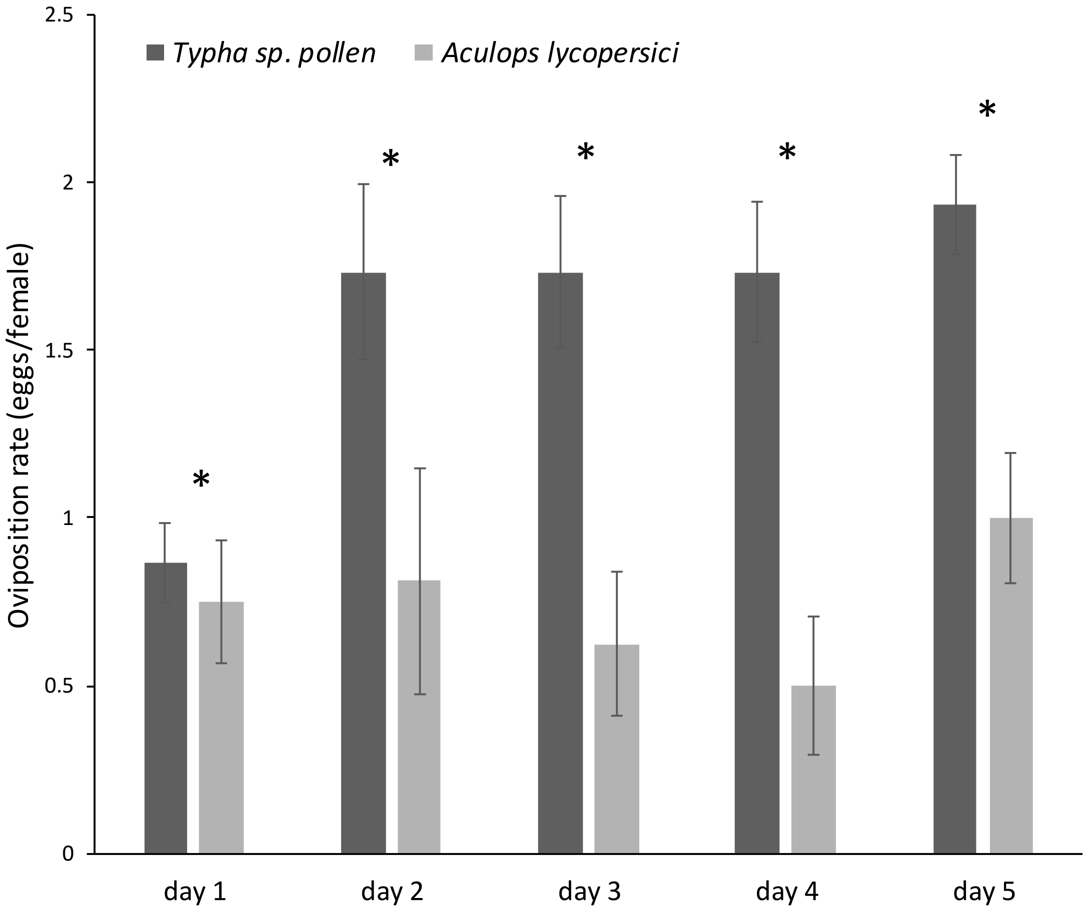

Figure 2: Average oviposition rate of female predators fed *A. lycopersici* (light bars) and *Typha sp*. pollen (dark bars). Asterisks (*) indicate significant differences in oviposition between treatments per day (p < 0.05).

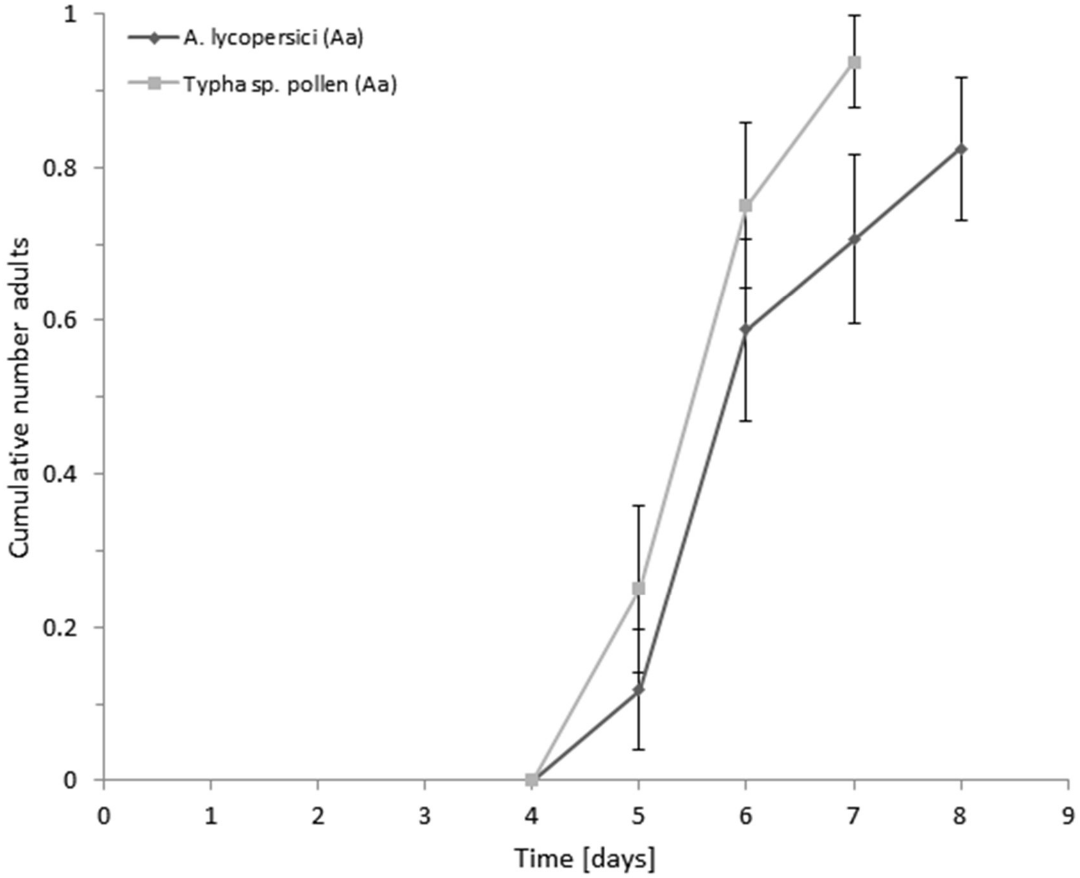

Figure 3: Development and survival of *A. herbicolus* fed *A. lycopersici* (dark line) and *Typha sp*. pollen (dark line). See legend to Fig. 1 for further explanation. Treatments with same capital letters did not differ significantly in survival and with different small letters were different in development.

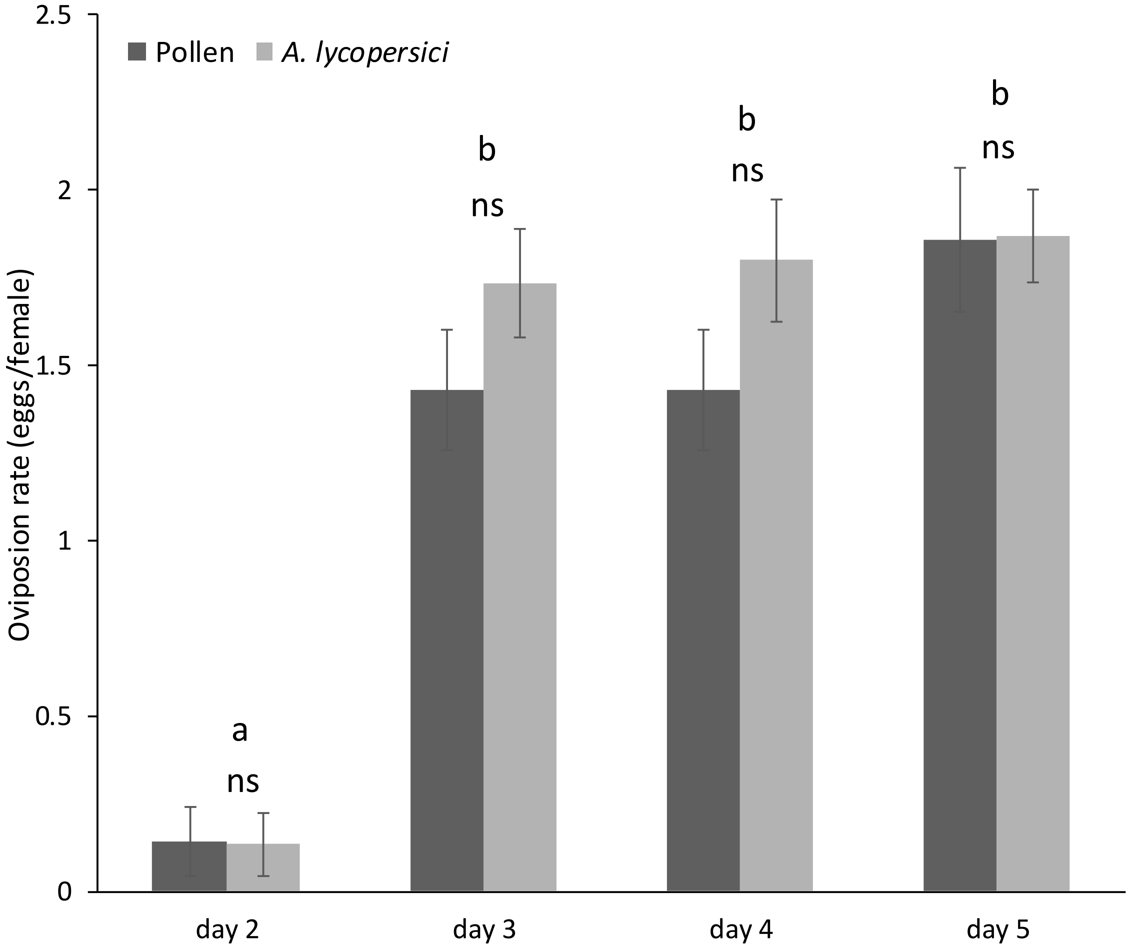

Figure 4: Average oviposition rate of female predators fed *A. lycopersici* (light bars) and *Typha sp*. pollen (dark bars). (ns): oviposition did not differ significantly between the two treatments per day ($p > 0.05$, Tukey test) and different letters show significant differences among days ($p < 0.05$).

General conclusions

From the work presented in this thesis, it can be concluded that the predatory mite *Amblyseius herbicolus* has potential for the control of tomato pests. Although further experiments on plants are needed, the current thesis shows that this predatory mite is able to develop and reproduce by feeding both on *B. tabaci* and *A. lycopersici*, which are important tomato pests. The population experiment on tomato plants with pollen as an alternative food showed that this mite is able to establish on tomato plants and control whiteflies. If this predator is able to reach similar high densities as observed in this population experiment, it may also significantly reduce the densities of A. *lycopersici.* Future experiments should focus on the dynamics of this predator and pests on tomato plants.