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ANA MARIA CALIXTO PEREIRA

POPULATION GROWTH, PREY PREFERENCE AND PEST CONTROL POTENTIAL OF THREE PREDATORY BUGS SPECIES (Hem.: Miridae), NATURAL ENEMIES OF *Tuta absoluta* (Lep.: Gelechiidae)

LAVRAS - MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutor.

Orientadora

Dra. Vanda Helena Paes Bueno

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(CRESCIMENTO POPULACIONAL, PREFERÊNCIA POR PRESAS E POTENCIAL NO CONTROLE DE PRAGAS DE TRÊS ESPÉCIES DE MIRIDEOS PREDADORES (Hem.: Miridae), INIMIGOS NATURAIS DE Tuta absoluta (Lep.: Gelechiidae))

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutor.

APROVADA em 03 de agosto de 2015.

Dr. Luís Cláudio Paterno Silveira	UFLA
Dra. Lívia Mendes Carvalho	EPAMIG
Dra. Maria Fernanda Gomes Villalba Peñaflor	UFLA
Dr. Geraldo Andrade Carvalho	UFLA

Dra. Vanda Helena Paes Bueno
Orientadora

LAVRAS - MG

2015

A Deus. Que sempre me dá forças e saúde para enfrentar os desafios de cada dia.

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GENERAL ABSTRACT

Tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), tomato fruit borer *Neoleucinodes elegantalis* (Gueéne) (Lepidoptera: Crambidae) and *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) are important pests and can be responsible for major damages and economic losses in tomato crops if no control is used. The main control for these pests in Brazil is chemical, while in Europe, the mirid predators *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) are commercialized and used to control *T. absoluta*. Recently, in Brazil, three species of mirid predators [*Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal)] were found, having been evaluated regarding biological and behavioral traits, as well as potential use against *T. absoluta* and other tomato crop pests. In this study, we evaluated (1) the effect of different temperatures over the reproduction, longevity and population growth parameters (r_m, R_0, λ, T, DT) of *C. infumatus*, *E. varians* and *M. basicornis*;, (2) prey preferences for *T. absoluta*, *N. elegantalis* and *H. armigera,* in addition to predation behavior when having *T. absoluta* and *N. elegantalis* as prey in chance and no-choice tests, and; (3) evaluate population dynamics against *T. absoluta* in greenhouse experiment. The results showed that temperature affected the reproductive parameters of *C. infumatus*, *E. varians* and *M. basicornis*. The optimal temperature range for reproduction and longevity ranged from 24°C to 28°C. Prey preference tests indicated that the predators were able to prey on *T. absoluta*, *N. elegantalis* and *H. armigera* in no-choice tests. In choice tests, the predators had preference for *T. absoluta*. In the population dynamics tests against *T. absoluta*, *C. infumatus* and *M. basicornis* were able to establish and reproduce in tomato plants, decreasing the population of *T. absoluta*. *E. varians* was not able to establish in tomato plants within greenhouse conditions. The best release rate at the evaluated conditions was of two couples of mirid predators per plant. Predators *C. infumatus* and *M. basicornis* did not damage the tomato plants. Predators *C. infumatus* and *M. basicornis* have potential as good candidates for the biological control of *T. absoluta* and other tomato crop pests.

Keywords: *Campyloneuropsis infumatus*. *Engytatus varians*. *Macrolophus basicornis.* Mirid predators. Biological control. Fecundity. Increased intrinsic rate. Predation behavior. Release rates.

RESUMO GERAL

A traça-do-tomateiro *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a traça pequena do fruto *Neoleucinodes elegantalis* (Gueéne) (Lepidoptera: Crambidae) e *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) são pragas importantes que causam danos e perdas econômicas na cultura do tomate. O principal método de controle dessas pragas no Brasil é a aplicação de inseticidas, sendo que na Europa os mirídeos predadores *Macrolophus pygmaeus* (Rambur) e *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) são comercializados e usados para o controle de *T. absoluta*. Recentemente no Brasil, foram encontradas três espécies de mirídeos predadores [*Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) M*acrolophus basicornis* (Stal)], os quais estão sendo avaliados em suas diversas características biológicas e comportamentais assim como potencial para uso contra *T. absoluta* e outras pragas em cultivos de tomate. Neste estudo foram avaliados (1) o efeito de diferentes temperaturas na reprodução, longevidade e parâmetros de crescimento populacional (r_m, R_0, λ, T, DT) de *C. infumatus, E. varians* and *M. basicornis*; (2) suas preferencia pelas presas *T. absoluta*, *N. elegantalis* e *H. armigera* e o comportamento de predação quando predando *T. absoluta* e *N. elegantalis* em testes com chance e sem chance de escolha; (3) suas dinâmicas populacionais por meio de suas liberações em plantas de tomate com *T. absoluta* em casa de vegetação experimental. Os resultados demonstraram que a temperatura afetou os parâmetros reprodutivos de *C. infumatus*, *E. varians* e *M. basicornis*. O intervalo de temperatura ideal para a reprodução, longevidade e parâmetros de crescimento populacional foi de 24°C a 28°C. Nos testes de preferência, os predadores foram capazes de predar *T. absoluta*, *N. elegantalis* e *H. armigera* em testes sem chance de escolha. Já nos testes com chance de escolha, os predadores preferiram *T. absoluta.* Nos testes de liberação, as espécies *C. infumatus* e *M. basicornis* foram capazes de se estabelecer e reproduzir em plantas de tomate em casa de vegetação e diminuir a população de *T. absoluta*. Já *E. varians* não se estabeleceu dentro das mesmas condições. A melhor taxa de liberação foi de 2 casais de mirídeos predadores por planta. Em todos os testes realizados os predadores *C. infumatus* e *M. basicornis* não causaram danos nas plantas de tomate. A partir destes resultados pode-se concluir que *C. infumatus* e *M. basicornis* têm potencial como bons candidatos a agente de controle biológico de *T. absoluta* e de outras pragas presentes na cultura do tomate.

Palavras-chave: C*ampyloneuropsis infumatus*. *Engytatus varians*. *Macrolophus basicornis.* Mirídeos predadores. Controle biológico. Fecundidade. Taxa intrínseca de aumento. Comportamento de predação. Taxas de liberação.

SUMÁRIO

FIRST PART

FIRST PART

1 GENERAL INTRODUCTION

The tomato (*Solanum lycopersicum* L.) is a very popular product as salad vegetable. The estimative of its global production was approximately 160 million tones harvested from an area of about 5 million hectares in 2011, and it was the fourth most valuable crop after rice, wheat and soya bean, but followed closely by potato (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED STATES - FAO, 2014). In Brazil, the tomato crops are cultivated in area of 64.371 hectares and with a production of 4.987.102 ton (ANUÁRIO…, 2014).

Tomato plants are attacked for many species of pests. The one of the most important pest is the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). This pest prefers tomato over other solanaceous plants (PICANÇO et al., 1995; SIQUEIRA; GUEDES; PICANÇO, 2000) with devastating effects on it global production. Europe was free of this pest until 2006 when it was reported its introduction in Spain (THE FOOD AND ENVIRONMENT RESEARCH AGENCY - FERA, 2009; URBANEJA; MONTON; MOLLA, 2009) and within 5 years it had become one of the most serious and widespread tomato pests (DESNEUX et al., 2010, 2011). Current is present in almost all continents (CENTRE FOR AGRICULTURE AND BIOSCIENCES INTERNATIONAL - CABI, 2015) and in quarantine conditions in some countries. Larvae damage leaves, stems and fruits whilst feeding (AZEVEDO et al., 2003; FERA, 2009) and also cause bud abscission and/or fruit malformations (AZEVEDO et al., 2003). Damaged fruits infected with secondary rots are unmarketable (FERA, 2009). The tomato fruit borer *Neoleucinodes elegantalis* (Guenée) (Lep.: Crambidae) is also important pest attacking tomato crops and other Solanaceae. The larvae of *N. elegantalis* develops inside of the fruits, feeding on the mesocarp and the endosperm, causing production losses from 50% to 90% (MIRANDA et al., 2005; NUNES; LEAL, 2001). The species *H. armigera* is an important polyphagous pest spreading worldwide and can attack approximately 200 plant species including tomato crops (CZEPAK et al., 2013; GUO, 1997). Although feeding larvae can sometimes be seen on the surfaces of plants, they are often hidden within plant organs (i.e. flowers or fruits), in which case bore holes may be visible (SMITH-PARDO, 2014). Since 2013, *H. armigera* was considered quarantine pest and was detected in tomato crops in Espirito Santo state, Brazil (PRATISSOLI et al., 2015).

The control of *T. absoluta*, *N. elegantalis* and *H. armigera* is done mainly using chemical control by successive applications of insecticides that lead to the selection of resistant populations to active ingredients and elimination of natural enemies (PICANÇO et al., 1995). Although some studies have shown the use of *Bacillus thuringiensis* (MOLLÁ; GONZÁLEZ-CABRERA; URBANEJA, 2011; NIEDMANN; MEZABASSO, 2006) and *Trichogramma pretiosum* (FARIA et al., 2008; PRATISSOLI et al., 2005) to control these pests, still there are little information about the biological aspects and behavior towards entomophagous insects associated with these lepidopteran pests. In case of *T. pretiosum*, the parasitoid does not walk well in tomato plants because of glandular trichomes (KENNEDY, 2003).

Mirid predators are been used in Europe to control pests in tomato crops. These predators are zoophytophagous and can be associated feeding plants and with several pests in various crops like vegetables, for example, whiteflies, thrips, aphids, mites and lepidopteran, and in this last case, mainly *T. absoluta* (ALOMAR; RIUDAVETS; CASTAÑE, 2006; AVILLA et al., 2004). These predators were able to reproduce in tomato plants and walk on glandular trichomes and shown be good natural enemies of *T. absoluta* and others tomato pests (CALVO et al., 2012). Several mirid species have been reported as having great potential for use in biological control programs as *Dicyphus tamaninii* (Wagner) (AGUSTÍ; GABARRA, 2008; CASTAÑÉ; RIUDAVETS; ALOMAR, 2009; GHABEISH; SALEH; DABABAT, 2010), *Dicyphus hesperus* (Knight) (MCGREGOR et al., 1999; SHIPP; WANG, 2006) *Dicyphus errans* (Wolff) (TAVELLA; GOULA, 2001) and *Macrolophus pygmaeus* (Rambur) (HOMMES; HORST, 2002; PERDIKIS; LYKOURESSIS, 2002). The species *M. pygmaeus* and *Nesidiocoris tenuis* (Reuter) are commercialized and used for controlling *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (ARNO et al., 2009; LENTEREN, 2012; MOLLÁ et al., 2009; URBANEJA; MONTON; MOLLA, 2009). Also they have been reported in the control of thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and *Tetranychus* spp. (CALVO; URBANEJA, 2004; SANCHEZ et al., 2006). The species *N. tenuis* also is associated with *Helicoverpa armigera* (Hübner) (DEVI; YADAV; ANAND, 2002). This predator has shown great efficiency in *T. absoluta* control, attacking the eggs and all larval stages of this pest; especially the 1st instar larvae (ARNO et al., 2009).

However, in Brazil, there are few reports of mirids predators. The species *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal) (Heteroptera: Miridae) were recently (2012) reported in the municipality of Ribeirao Vermelho and Lavras, Minas Gerais state, Brazil (BUENO et al., 2013). The species *C. infumatus* was reported in tobacco plants (*Nicotiana tabacum* L.) and *Petunia* sp, *E. varians* in tobacco plants and *M. basicornis* before was not associated with a specific host plant, but now was reported in tobacco plants (BUENO et al., 2013; FERREIRA; HENRY, 2011; FERREIRA; SILVA; COELHO, 2001). Regarding to distribution of these species, *C. infumatus* is probably a native of Brazil, while

E. varians (= *Cyrtopeltis varians*) is found in Cuba (CASTINEIRAS, 1995) and in North and South of Brazil (FERREIRA; SILVA; COELHO, 2001), and also in Mexico (MARTÍNEZ et al., 2014). According to Castineiras (1995), *E. varians* is an important natural enemy of *B. tabaci* in various crops such as eggplant, tomato and cucurbits. Luna et al. (2006) reported a natural control by this predator on *Heliothis virescens* in field conditions. The species *M. basicornis*, can be found in Argentina, Brazil, Cuba, Guatemala and Venezuela (MELO et al., 2004). This species was reported by Ohashi and Urdampilleta (2003) in the province of Misiones (Argentina), preying on aphids, *Epitrix* sp. and eggs and larvae of first instar of *Manduca sexta* in tobacco plants. However, to date, there are no studies that evaluate the potential of *C. infumatus*, *E. varians* and *M. basicornis* as biological control agents of pests, including studies on its biology, mass rearing and release in a greenhouse. According to Bueno et al. (2012, 2013) these predators have a great potential as natural enemies of *T. absoluta* and other pests, and since then they are being evaluated at their different biological and behavioral characteristics.

Faced with the problems with several pests as in tomato crops, and the decision to look for using biological control of these pests, an important process is search for a good natural enemy, preferentially native and that could attack more than one prey. In this sense, evaluations of its biological and behavioural characteristics are necessary steps that could help to establish criteria for the selection of a good natural enemy.

There are many factors that influence the natural enemies and their success of biological control like temperature, prey preference and population dynamics of predators and pests in greenhouse or in the field. The temperature has a fundamental role in the development, survival, population growth parameters and reproduction of insects. The temperature can influence the population dynamics of natural enemies and consequently its regulatory capacity and keeping pests below the economic injury level (DIXON et al., 2009; TRUDGILL et al., 2005). The insects can only reach adulthood and reproduce within a temperature range, an optimum temperature exists. However, negative effects are expected when insects are subjected to temperatures close to minimum and maximum limits that can withstand (HADDAD; PARRA; MORAES, 1999; TRUDGILL et al., 2005). The effect of different temperatures in reproductive and growth parameters of insects has been determined at laboratory conditions for some species of mirid predators (KIM; RIELD, 2005; PERDIKIS; LYKOURESSIS, 2002).

Prey preference can have an important role to generalist insects. The generalist predators can forage a wide variety of prey, but often they can show distinct preferences for a particular prey (PROVOST et al., 2006). According Cock (1978), the preference of a predator is defined as the disproportionate attack rate to a kind of prey, when more than one kind of prey is available. Preference is measured in only one predator stage, mostly in adult stage (EUBANKS; DENNO, 2000), providing predator the choice for a prey in a relatively short time interval. Thus, voracity studies of a predator are usually preliminary investigations done to evaluate the potential of a new biological control agent. The knowledge of preference of a predator for a given prey is important both in assessing their potential for the control of particular pests and to obtain other important results for the implementation of a biological control program (MEYLING; ENKEGAARD; BRODSGAARD, 2003).

Further, in augmentative biological control where release of mass rearing natural enemies by inundative, inoculative and seasonal inoculative releases, the timing and release rate are tools important in the process of suppressing populations of insect pests (BUENO, 2009; LENTEREN, 2010). The increase of number of natural enemies released in greenhouses or in the field sometimes do not increase the level of pest control and increase the cost of implementation of biological control. Then is important the knowledge of ideal release rate, timing of release, climate conditions to better suppression pests species (COLLIER; STEENWYK, 2004; DRIESCHE et al., 2002).

In order to added more information on the predatory mirid bugs *C. infumatus*, *E. varians* and *M. basicornis* and its potential as biological control agents to control pests and their use in biological control programs, the aims of this study were (1) the effect of different temperatures on their reproduction, longevity and population growth parameters; (2) prey preference and predation behavior observations of these predators for 1st instar larvae of *T. absoluta*, *N. elegantalis* and *H. armigera* and (3) their population dynamics having *T. absoluta* as prey in cages in experimental greenhouse with tomato plants by testing two different release rates by five tests during a year period at different climate conditions.

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SECOND PARTS - ARTICLES

ARTICLE 1 Reproduction, longevity and population growth parameters of three predatory bugs (Hemiptera: Miridae) reared on *Anagasta kuehniella* eggs at five temperatures

Calixto, A.M.[a]
Bueno, V.H.P.[a,b]
Montes, F.C.[a]
van Lenteren, J.C.[c]

[a] Laboratory of Biological Control, Department of Entomology, Federal University of Lavras, P. O. Box 3037, 37.200-000 Lavras/MG, Brasil;
[b] Corresponding author. Department of Entomology, Federal University of Lavras, P.O.Box 3037, 37.200-000 Lavras/MG, Brasil (e-mail: vhpbueno@den.ufla.br);
[c] Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands.

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Abstract. Temperature is one of most important abiotic factors that affect reproduction and population growth of pests and their natural enemies. Mirid predatory bugs can be effective biological control agents. In Brazil, three mirid species *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal) are being evaluated as predators of important pests in tomato crops. Thus, this study aimed to evaluate the effect of five constant temperatures (16ºC, 20ºC, 24ºC, 28ºC and 32±1 ºC) on the reproductive and population growth parameters and longevity of these mirid predators. Newly-emerged adults, originating from nymphs reared at each temperature, were separated in pairs and kept in glass pots (1.7 L) containing tobacco plant seedlings (*Nicotiana tabacum* L., cv. TNN) as oviposition substrate and *ad libitum Anagasta kuehniella* eggs as food. The pre-oviposition period was shortest at 24ºC and 28ºC. The oviposition and post-oviposition periods were longer at 24ºC and 28ºC and shorter at 16ºC and 32ºC. The daily and total fecundity were highest for all three mirid species at 24°C. The intrinsic rate of increase (r_m) and the finite rate of increase (λ) were highest at 24ºC and 28ºC and the net reproductive rate (R_0) was highest at 24ºC for all three mirid species. The longevity of all three species was longest for both males and females at 24ºC and 28ºC. The size of tibia and weight of the three mirid predators was highest between 20°C and 28°C. The results indicate that temperatures between 24°C and 28°C are best for reproduction and population development of *C. infumatus, E. varians* and *M. basicornis.* This information can assist in development of a mass rearing method for the predators and also for timing of their release in the field.

Key words: *Campyloneuropsis infumatus*, *Engytatus varians*, *Macrolophus basicornis*, biological control, fecundity, longevity, intrinsic rate of increase.

Introduction

Predatory mirids bugs (Heteroptera: Miridae) are reported as biological control agents, and have been found associated with various prey as aphids, thrips, spider mites, and lepidopteran eggs and larvae (Devi, Yadav, & Anand, 2002; Perdikis & Lykouressis, 2002; Urbaneja et al., 2003; Blaeser, Sengonca, & Zegula, 2004). The interest in mirid predators is increasing due to the commercial success of the species *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Europe (van Lenteren, 2012). As a result, many species are currently being evaluated as potential biological control agents in various countries where *T. absoluta* is a very serious pest in tomato crops. The species *T. absoluta* is native from South America and was detected in Europe for the first time in Spain at the end of 2006, and currently is considered one of the most devastating pests of tomato crops worldwide, which has spread now to all continents (CABI, 2015).

The control of *T. absoluta* in Latin America still is mainly by the use of pesticides, although studies are being conducted to find an alternative control method. One of these alternatives might be biological control (Bueno et al., 2013). However, after finding natural enemies of a pest, the candidates have to be evaluated. This evaluation is based on ecological, biology (development and reproduction) and behavioral characteristics of both natural enemy and target pest, on the developmental synchronization with pest, climate adaptation and density dependent reaction to pest population fluctuations (van Lenteren, 2010).

Abiotic factors like temperature is an important environmental condition that influences insect's life history, and can affect the success of biological control. The insect body temperature is similar to the outside environment temperature, and an increase or decrease in environmental temperature results in an increased or decreased metabolic rate of the insect, which affects their activities and eventually its lifetime reproductive success (Huey & Berrigan, 2001). In addition, life table data help understanding changes in populations of the pest and its natural enemies, which is very helpful to get insight in synchronization of pest and natural enemy populations in greenhouses or in the field according to climate conditions (Kakde, Patel, & Tayade, 2014).

Three mirid predators, *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* are being evaluated in Brazil as candidates to control *T. absoluta* and other pests in tomato crops (Bueno et al. 2012, 2013). However, still little biological information is available about these three species and several characteristics that should be determined, as their reproduction and population growth, are important tools that could assist in the evaluation process and for the determination of their potential as biological control agents.

The objectives of this study were evaluate the effects of five constant temperatures on the growth populations parameters and longevity of *C. infumatus*, *E. varians* and *M. basicornis* when having *Anagasta kuehniella* (Zeller) eggs as food. Our hypothesis concerning reproduction and life table parameters were: Null hypothesis (H_0): there are differences in reproductive and life table parameters among *C. infumatus*, *E. varians* and *M. basicornis* for each temperature to which they are exposed, and there are effects at the highest and lowest temperatures. Alternative hypothesis (H_1): there are no differences in reproductive and life table parameters among *C. infumatus*, *E. varians* and *M. basicornis* for each temperature to which they are exposed, and there are no effects at the highest and lowest temperatures. With the results of this study we can determine the best temperatures for mass rearing of the predators in the laboratory. In addition, knowing the population growth parameters of a natural enemy can assist determining the possibilities for establishment, the synchronization of predator and prey, and the predation rate of the prey.

Material and Methods

Collecting and rearing of mirid predators

Adults of mirids predators were collected in tobacco *Nicotiana tabacum* L. located in the district of Santos Dias, in Ribeirão Vermelho, Minas Gerais, Brazil. The individuals were examined in the laboratory using a key proposed by Ferreira and Henry (2011), and in addition, adults (males and females) were sent for identification to a taxonomist of Family Miridae, Paulo S. F. Ferreira at the Department of Animal

Biology, Federal University of Viçosa (UFV). Three mirid species was identified: *Campyloneuropsis infumatus*, *Engytatus varians* and *Macrolophus basicornis*. Mass rearing of the three species was performed according to methodology proposed by Bueno et al. (2013). Adults were released inside acrylic cages (30x30x60 cm) containing a tobacco plant (*N. tabacum* cv. TNN) as oviposition substrate and *ad libitum Anagasta kuehniella* (Zeller) eggs as food offered three times a week. Every seven days, a new tobacco plant was placed inside the cages. The old tobacco plant containing the eggs was individually placed in a new acrylic cage (30x30x60 cm) to allow hatching and development of the nymphs. This rearing was maintained at 25 ± 2ºC, RH 70 ± 10% and 12h photophase.

Obtaining tobacco seedlings

Seeds of tobacco (*N. tabacum* L., cv. TNN) were sowed in plastic trays containing organic substrate Plantmax® (Pinus bark, peat, expanded vermiculite, enriched with macronutrients and micronutrients). Later, the tobacco seedlings were transplanted into plastic cups (200 mL) with soil + organic substrate (Plantmax®) and kept in a greenhouse. Seedlings with 3 leaflets were used as oviposition substrate for the mirid females during the experiments.

Reproduction, longevity and population growth

Newly-emerged adults, originating from 100 nymphs of each mirid predator which developed at the same temperatures (Montes, 2013), were kept in glass pots (1.7 L) (one couple/glass pot) containing a tobacco seedling in a plastic cup + soil (200 mL) with up to three leaflets as oviposition substrate and *ad libitum A. kuehniella* eggs as food. Daily, the old seedling was replaced by a new one into the glass pot. The old seedlings were observed under a stereoscopic microscope (Leica EZ4) (40X) and the number of predator eggs and nymphs was recorded daily. Later, the stem was wrapped with a peace of moistened cotton and placed in Petri dishes (15 cm diameter) for the total development of the predator. The experiment was done in climate chambers at 16ºC, 20ºC, 24ºC, 28ºC and 32 ±1ºC, RH 70 ± 10% and 12h photophase. The mortality of adults was recorded daily, and longevity was determined for males and females. The data on daily and total fecundities were based on counting of emerged nymphs due the difficulty of observing predator eggs inside the plant tissue (Agustí & Gabarra, 2008, 2009; Sanchez et al., 2009). The pre-oviposition, oviposition and post-oviposition periods, fecundity (daily and total) of *C. infumatus*, *E. varians* and *M. basicornis* at the different temperatures were determined. The numbers of replicates for the different tests are given in Table 1.

Weight and size

Weight and size of newly-emerged females and males of each mirid predator species was determined at the five temperatures. To weigh the predators, 20 males and 20 females from each temperature were placed individually in a glass tube (2.5 x 8.0 cm) covered with plastic film, and weighed using a precision balance (Shimadzu, model AW-220, 0.0001g of precision). Next, the right hind tibiae of the weighed insects were measured by placing it on a slide in a drop of Hoyer solution under an optical microscope (100 x magnifications) with an ocular micrometer.

Data analysis

The experiment was conducted in factorial design 5 x 3, being five temperatures (16ºC, 20ºC, 24ºC, 28ºC and 32ºC) and three mirid predators species (*C. infumatus*, *E. varians* and *M. basicornis*). All data were checked for normality ($P \geq 0.05$) by using a Shapiro-Wilk test. Data from pre-oviposition, oviposition and post-oviposition periods, daily and total fecundity and longevity showed normal distributions and, thus, they were exposed to an analysis of variance (Two-way ANOVA) (factors: mirid species and temperatures), using the Tukey's test ($P \leq 0.05$). To compare the weight, size of tibia and longevities of females and males for the same temperature the *t* test was used ($P \leq 0.05$). Data of weight and size of females and males for all temperatures were not normally distributed, and were, thus, transformed by square root (x^0.5) and then exposed to an analysis of variance (Two-way ANOVA) (factors: mirid species and temperatures) using the Tukey's test ($P \leq 0.05$). All data were analyzed by software R Development Core Team (2014).

To calculate the life table parameters of the three mirid predators, the data of survival and fecundity of each female was used. Age interval (x), fecundity (m_x), probability of survival (l_x) data were used to calculate the net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r_m), finite rate of increase (λ) and double time (DT), by the

Jackknife method (Andrewartha & Birch, 1954). The age (in days) at which the insects began adulthood, immature survival and sex ratio of offspring of the juveniles exposed to the same temperature (Montes 2013) were used in the calculation of the population growth parameters. The life table parameters and their standard errors were estimated by the Lifetable Sas Method (Maia, Luiz, & Campanhola, 2000, Maia et al., 2014). Means of the life table parameters were compared in pairs by applying a unilaterally Student *t* test for independent samples ($P \leq 0.05$) using the statistical program SAS (SAS Institute, 2000).

Results

Pre-oviposition, oviposition and pos-oviposition periods.

The pre-oviposition periods for *E. varians, C. infumatus* and *M. basicornis* were on average 4-5 days at 24ºC and 28ºC and significantly shorter than at the higher and lower temperatures ($F_{4,145} = 4.315$, $p<0.0001$). At 16ºC the pre-oviposition period for all three species was longer than those at the other four temperatures ($F_{4,145} = 5.896$, $p<0.001$) (Figure 1).

The oviposition periods of all mirid species were longer at 24ºC and 28ºC ($F_{4,145} = 7.458$, $p = 0.002$) than at the other temperatures. At 24°C the oviposition periods of *M. basicornis* (26.6 days) and *E. varians* (25.9 days) were longer than that of *C. infumatus* (23.9 days) ($F_{4,145} = 2.367$, $p<0.001$). The oviposition period of *M. basicornis* (26.8 days) was longer than that of the other two species at 28ºC (*C. infumatus* – 23.8 days and

E. varians – 23.7 days) ($F_{4,145}$ = 10.325, p = 0.001). There were no differences between oviposition periods for all three species at 16ºC and 32ºC (Figure 2).

The pos-oviposition period were longer at 24ºC and 28ºC and shorter at 16ºC and 32ºC ($F_{4,145}$ = 3.345, $p<0.001$) (Figure 3) for all three mirid species.

Daily and total fecundity.

The daily fecundity was higher at 24ºC for all three species (*C. infumatus* – 10.4, *E. varians* – 10.3 and *M. basicornis* – 11.7 eggs/female/day) than at the other temperatures ($F_{4,145}$ = 12.752, $p<0.001$). The daily fecundity of *M. basicornis* was higher than that of the other two species at 16°C ($F_{2,73}$ = 4.233, p = 0.014), 28°C ($F_{2,99}$ = 2.684, $p<0.001$) and 32°C ($F_{2,69}$= 5.212, p = 0.004) (Table 1). At 28°C the daily fecundities were 6.7 eggs/female/day (*C. infumatus*), 6.4 eggs/female/day (*E. varians*) and 8.3 eggs/female/day for *M. basicornis*.

The total fecundity was higher at 24ºC (*C. infumatus* – 248.5, *E. varians* – 266.7 and *M. basicornis* – 311.2 eggs/female) than at the other temperatures ($F_{4,145}$ = 6.158, p = 0.003) (Table 1). The total fecundity of *M. basicornis* was higher than that of the other two species at 16°C ($F_{2,73}$ = 3.664, $p<0.001$), 24°C ($F_{2,93}$ = 4.551, p = 0.0013) and 28°C ($F_{2,99}$ = 6.127, p = 0.006). The total fecundity of *C. infumatus* was lower than that of *E. varians* and *M. basicornis* at 20°C ($F_{2,90}$ = 7.354, $p<0.001$) and at 32°C ($F_{2,69}$ = 2.443, $p<0.001$) (Table 1).

Average fecundity (mx), survival (lx) and growth population parameters.

Average fecundity (m_x) reached a maximum of 4 eggs/female at 16°C and 32°C (Figs 4A and 8A). At 20°C, 24°C and 28°C, the average fecundity (m_x) reached a maximum of 7, 14 and 13 eggs/female, respectively (Figs 5A, 6A and 7A). The number of eggs/female/day increased with female age at 16°C, 20°C, 24°C, 28°C and 32°C (Figs 4B to 8B). The number of eggs/female/day start to increase after the 4th day of their adult life (pre-oviposition period) and decreased after the 25th day at 24°C and 28°C (Figs 6B and 7B). At 24°C and 28°C the females of *C. infumatus*, *E. varians* and *M. basicornis* laid more eggs than at the other three temperatures (Figure 6A, B and 7A, B). The survival rates (l_x) decreased linearly with the increase of female age in all temperatures (Figs 4A, 5A, 6A, 7A and 8A). The longest survival (l_x) of females was observed at 24°C and 28°C (Figs 6A and 7A) for all three mirid species.

There were no significant differences among the species *C. infumatus*, *E. varians* and *M. basicornis* for the population growth parameters at each temperature. However, at the different temperatures, the intrinsic rate of increase (r_m) was significantly higher at 24ºC and 28ºC for all three species ($t = 2.314$, $df = 4$, $p<0.001$) than at the other temperatures. The net reproductive rate (R_0) was higher at 24ºC ($t = 3.987$, $df = 4$, $P<0.001$) than at the other temperatures. The mean generation time (T) was higher at 28ºC and 32ºC (~40 days) ($t = 7.698$, $df = 4$, $p = 0.002$) than at the other temperatures, the double time (DT) was higher at 24ºC, 28ºC and 32ºC than at the other temperatures, and the finite rate of increase (λ) was higher at 24ºC and 28ºC than at the other temperatures (Table 2).

Longevity of females and males.

The longevity were longer for males ($F_{4,145}$ = 6.987, p<0.001) and females ($F_{4,145}$ = 5.324, p<0.001) at 24ºC and 28ºC than at the other temperatures for all three mirid species. At all temperatures (16°C, 20°C, 24°C, 28°C and 32°C) the longevity of *C. infumatus* and *E. varians* females was longer than that of males (Table 3). At 20°C, 24°C, 28°C and 32°C the longevity of females and males of *M. basicornis* did not differ significantly (Table 3).

Weight and size of tibia.

The size of right hind tibia of males and females of all three species did not differ significantly at any of the temperatures (t=1.115, df=2, p=0.233). However, the size of the right hind tibia of *E. varians* females were larger than that of the other two species at 20ºC, 24ºC and 28ºC (1.97, 2.29 and 2.05 mm) (Table 4). Females and males of *E. varians* were heavier than those of the other two species at all temperatures.

Discussion

The results obtained in this study provide the first data on reproduction and growth population parameters of *C. infumatus*, *E.*

varians and *M. basicornis.* Temperature can affect mirid life histories, as those of all other insects, reflecting genotypic constraints that are modifiable through an individual's interaction with its environment (Wheeler, 2001). The shortest pre-oviposition and longest oviposition periods for all three mirid species at the temperature interval 24 and 28°C were similar to results reported for other mirid species as *M. pygmaeus* (Perdikis & Lykouressis 2002) and *Deraeocoris brevis* (Uhler) (Kim & Riedl, 2005). A short pre-oviposition period generally is associated with development at optimal temperature and with reproduction earlier in life (Wheeler, 2001).

The number of eggs/female laid was highest in higher temperatures. According to Wheeler (2001), oviposition in heteropteran can be limited by above - and below -optimum temperatures. Low temperatures can adversely affect fecundity because the feeding period is extended at the expense of time devoted to oviposition. In this study, the daily and total fecundity of the three mirid species was lower at lowest and highest temperature (16ºC and 32ºC) than at the other temperatures. The values of daily fecundity of all three mirid species at 24ºC were higher than values obtained for *Deraeocoris brevis* (9.5 eggs/female/day) (green beans as oviposition substrate) (Kim & Riedl 2005), *N. tenuis* (4.3 eggs/female/day) and *M. pygmaeus* (3.1 eggs/female/day) (Mollá et al. (2014) (tomato plants), all having *A. kuehniella* eggs as food at 25ºC. This shows that not only the temperature can affect the reproduction of zoophytophagous predators, but also the quality of the prey offered as food and the source of water obtained from the plant that is used as oviposition substrate. Lepidopteran eggs as food increase the longevity and fecundity of heteropteran predators, as they have a high nitrogen content and consequently high quality protein (Ferkovich et al., 2007, Calixto et al 2013, Mollá et al., 2014). Eggs of *A. kuehniella* are often used as factitious prey to rear heteropteran predators in the laboratory (Bueno et al., 2006; Lundgren, 2011, Mollá et al, 2014). In this study, eggs of *A. kuehniella* (food) and tobacco seedlings (substrate for oviposition and source of water) were adequate for reproduction of three mirid species and maybe contributed to enhance the fecundity of the three mirid species at 24°C when compared to others studies. Lower fecundity values were reported by Sanchez et al. (2009) for *N. tenuis* (60 nymphs/female) and by Mollá et al. (2014) for *N. tenuis* (83.7 eggs/female) and *M. pygmaeus* (48.1 eggs/female) when they were fed on *A. kuehniella* eggs at 25ºC and using a tomato plant as source of water and oviposition substrate. Mollá et al. (2014) stressed also that *M. pygmaeus* was not able to reproduce when fed on *T. absoluta* eggs on tomato leaf discs in the laboratory, and thus, lepidopteran eggs of some species probably do not have the same nutritional quality as *A. kuehniella* eggs for mirid predators.

A suitable temperature and high nutritional food also increase the longevity of females, and consequently the females have a higher fecundity. Longevity in insects generally depends on the oviposition rate, that is, the number of eggs laid per day (Wheeler, 2001). This relationship was also demonstrated in this study, where temperatures of 24°C and 28°C resulted in longer longevity, high fecundity and longer oviposition period for all three mirid predators species. Female longevity values obtained in this study were similar to those found for *M. caliginosus* (=

M. pygmaeus) females (28.68 days at 22ºC) (Hansen et al. (1999), and lower than those of *M. pygmaeus* females (49.25 days) at 25ºC) (Perdikis & Lykouressis, 2002).

The effect of temperature experienced during the period of growth (e.g. nymphal development) appears to be particularly important in explaining the weight and size of newly emerged adults (Atkinson 1994). In this study, males and females of *C. infumatus* and *M. basicornis* showed similar weights and sizes, but *E. varians* was heavier and longer than these two other mirid species. Sanchez et al. (2009) recorded lighter females and males of *N. tenuis* at 25ºC.

Temperature affected the survival rate (l_x), average fecundity and population growth parameters of *C. infumatus*, *E. varians* and *M. basicornis*. The survival rate (l_x) in all temperatures showed a curve type II pattern (Towsend, Begon, & Harper, 2006), indicating that the mortality rate of these predators was constant from birth until older age.

Life table data provide understanding of the changes in population size of insects and having such data for both the pest and its natural enemies is very important to understand the role which natural enemies can play in pest management (Kakde, Patel, & Tayade, 2014). The intrinsic rate of increase (r_m) is a key demographic parameter used to show the population growth potential of insects under given environmental and food conditions (Andrewartha & Birch, 1954; Watson, 1964). For arthropods, especially for insects, temperature is main determinant of r_m since developmental period, longevity, fecundity and other life history parameters are highly correlated with temperature (Birch, 1948). The net reproductive rate (R_0) is the average number of female born to a female over her lifetime (Huey & Berrigan, 2001). Southwood and Henderson, (2000) reported that values of the net reproductive rate (R_0) excessing 1, and values of intrinsic rate of increase (r_m) higher than 0 indicate the population is increasing in size. In this study, the values of intrinsic rate of increase (r_m) were above 0 and the net reproductive rate (R_0) values were above 1. In this study at 24°C and 28°C for the three mirid predators *C. infumatus*, *E. varians* and *M. basicornis*, we found that the intrinsic rate of increase (r_m) values (varied from 0.0899 to 0.1003) were slightly lower, but, the net reproductive rate (R_0) values (varied from 79.88 to 91.08) were higher than ones of *T. absoluta.* The values obtained in this study for all life table parameters (mainly r_m and R_0) were similar to values found by Perdikis and Lykouressis (2002) and Mollá et al. (2014) to *M. pygmaeus.* However, the statement that an efficient natural enemy should have a potential maximum rate of population increase (r_m) equal to or larger than that of its host, can not simply be used for predators (van Lenteren, 2010). According to van Lenteren (2010), for predators, reproduction and killing are not strictly linked like in parasitoids and, in addition, predators usually kill prey during both immature and adult stages, and each of these stages has a different duration and predation efficiency. Therefore, studies on the determination of predators kill rate are necessary to be done in order to assist in the determining the efficiency of three mirid predators as biological control agents.

Conclusion

Our experimental results support our null hypothesis, i.e. temperature influences reproduction, longevity and life table parameters of *C. infumatus*, *E. varians* and *M. basicornis* and the lowest and highest temperatures (16°C and 32°) negatively affect these parameters. Further, the results show that temperatures in the range from 24°C to 28°C result in the best reproductive, population growth and longevity of *C. infumatus*, *E. varians* and *M. basicornis*. These results are very important to improve the mass rearing of mirid predators and can assist in the releases of these predators in the field or greenhouse conditions.

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Tables

Table 1: Daily (eggs/female/day) and Total fecundities (eggs/female) of *C. infumatus*, *E. varians* and *M. basicornis* at constant temperatures (± 1°C). 70±10% RH and 12h photophase.

T °C	Species	n	Daily Fecundity	Total Fecundity
16	*C. infumatus*	20	3.0±1.17 B*d**	17.4±1.13Bd
	E. varians	25	3.1±0.97Bd	18.9±0.87Bd
	M. basicornis	33	4.6±2.01Ad	32.6±1.21Ad
20	*C. infumatus*	12	5.4±1.23Ac	69.1±1.16Bc
	E. varians	37	5.8±1.12Ac	81.2±1.02Ac
	M. basicornis	44	5.6±0.89Ac	77.2±0.77Ac
24	*C. infumatus*	19	10.4±0.22Aa	248.5±3.23Ba
	E. varians	39	10.3±0.16Aa	266.7±4.16Ba
	M. basicornis	38	11.7±0.31Aa	311.2±4.31Aa
28	*C. infumatus*	15	6.7±0.24Bb	160.1±3.17Bb
	E. varians	45	6.4±0.31Bb	151.6±2.41Bb
	M. basicornis	42	8.3±0.88Ab	222.4±2.68Ab
32	*C. infumatus*	11	3.2±0.13Bd	19.2±1.33Bd
	E. varians	24	3.5±0.21Bd	25.5±0.88Ad
	M. basicornis	36	4.1±0.11Ad	25.8±0.94Ad

*Means (± SE) followed by same capital letter in columns indicate non-significant differences for species at the same temperature (Tukey's test ($P \leq 0.05$). **Means followed by same lower case letter in the columns indicate non-significant differences between the species at different temperatures (Tukey's test ($P \leq 0.05$). n= number of replicates (females).

Table 2: Growth parameters of *C. infumatus*, *E. varians* and *M. basicornis* at constant temperatures (± 1°C). 70±10% RH and 12h photophase.

T °C	Species	r_m	R_0	T	DT	λ
16	*C. infumatus*	0.0331 ± 0.004 A[*]c[**]	33.56 ± 0.21 Ac	81.03 ± 0.22 Ac	19.20 ± 0.12 Ac	1.0388 ± 0.003 Ac
	E. varians	0.0343 ± 0.003 Ac	35.21 ± 0.29 Ac	81.77 ± 0.26 Ac	20.03 ± 0.10 Ac	1.0355 ± 0.004 Ac
	M. basicornis	0.0379 ± 0.005 Ac	38.03 ± 0.28 Ac	80.89 ± 0.34 Ac	19.55 ± 0.09 Ac	1.0374 ± 0.005 Ac
20	*C. infumatus*	0.0651 ± 0.002 Ab	77.04 ± 0.42 Ab	78.03 ± 0.33 Ac	14.08 ± 0.08 Ab	1.0726 ± 0.002 Ab
	E. varians	0.0614 ± 0.006 Ab	78.66 ± 0.44 Ab	80.44 ± 0.34 Ac	14.15 ± 0.09 Ab	1.0831 ± 0.005 Ab
	M. basicornis	0.0678 ± 0.004 Ab	79.13 ± 0.40 Ab	77.22 ± 0.29 Ac	13.99 ± 0.07 Ab	1.0809 ± 0.003 Ab
24	*C. infumatus*	0.0899 ± 0.005 Aa	89.33 ± 0.39 Aa	56.04 ± 0.18 Ab	11.44 ± 0.06 Aa	1.1003 ± 0.010 Aa
	E. varians	0.0972 ± 0.007 Aa	88.45 ± 0.38 Aa	55.86 ± 0.21 Ab	10.89 ± 0.04 Aa	1.1025 ± 0.008 Aa
	M. basicornis	0.0988 ± 0.006 Aa	91.08 ± 0.41 Aa	57.63 ± 0.22 Ab	10.65 ± 0.08 Aa	1.1028 ± 0.009 Aa
28	*C. infumatus*	0.0956 ± 0.008 Aa	80.07 ± 0.55 Ab	41.23 ± 0.11 Aa	10.62 ± 0.11 Aa	1.1053 ± 0.008 Aa
	E. varians	0.0991 ± 0.009 Aa	79.88 ± 0.49 Ab	41.89 ± 0.14 Aa	11.46 ± 0.09 Aa	1.1042 ± 0.009 Aa
	M. basicornis	0.1003 ± 0.010 Aa	81.44 ± 0.51 Ab	42.14 ± 0.18 Aa	10.23 ± 0.12 Aa	1.1098 ± 0.008 Aa
32	*C. infumatus*	0.0645 ± 0.006 Ab	32.34 ± 0.24 Ac	40.02 ± 0.20 Aa	10.45 ± 0.10 Aa	1.0788 ± 0.007 Ab
	E. varians	0.0612 ± 0.007 Ab	31.72 ± 0.19 Ac	39.78 ± 0.23 Aa	11.07 ± 0.08 Aa	1.0767 ± 0.002 Ab
	M. basicornis	0.0701 ± 0.004 Ab	33.57 ± 0.28 Ac	37.04 ± 0.19 Aa	9.18 ± 0.11 Aa	1.0801 ± 0.008 Ab

[*] Means (± SE) followed by same capital letter in columns (difference between species in same temperature) and same [**] lower case letter (difference between species at different temperatures) in columns do not differ by Unilaterally *T* test at $P \leq 0.05$. r_m = Intrinsic rate of increase; R_0 = Net reproductive rate; T = Mean generation time; DT = Double time; λ = Finite rate of increase.

Table 3: Longevity (days) of *C. infumatus*, *E. varians* and *M. basicornis* at constant temperatures (± 1°C). 70±10% RH and 12h photophase.

		Longevity					
T °C	Species	Females	n	Males	n	*t*	*P* values
	C. infumatus	14.7±1.61A*c**	20	12.3±2.71Bc	20	0.704	0.012
16	*E. varians*	15.2±1.58Ac	25	11.7±1.56Bc	25	1.116	0.003
	M. basicornis	15.6±2.04Ac	33	12.1±1.12Bc	33	0.145	0.024
	C. infumatus	21.4±1.52Ab	12	18.2±1.05Bc	12	1.234	< 0.001
20	*E. varians*	22.6±1.13Ab	37	19.1±1.46Bc	37	0.983	0.004
	M. basicornis	22.5±0.99Ab	44	21.7±1.11Ac	44	1.119	0.014
	C. infumatus	31.4±2.15Aa	19	28.4±2.24Ba	19	2.136	0.026
24	*E. varians*	33.7±1.44Aa	39	29.3±1.88Ba	39	0.865	0.035
	M. basicornis	34.6±1.79Aa	38	30.2±1.96Aa	38	1.457	0.123
	C. infumatus	32.4±2.55Aa	15	27.6±1.89Ba	15	2.132	< 0.001
28	*E. varians*	31.5±1.77Aa	45	28.9±2.14Ba	45	1.479	0.002
	M. basicornis	35.1±1.19Aa	42	33.7±2.52Aa	42	0.788	0.214
	C. infumatus	13.7±1.86Ac	11	11.9±1.06Bc	11	0.955	0.006
32	*E. varians*	14.2±3.75Ac	24	12.8±0.98Bc	24	1.038	0.041
	M. basicornis	13.9±1.15Ac	36	12.5±1.65Ac	36	0.899	0.075

*Means (± SE) followed by same capital letter in columns indicate non-significant differences for species at the same temperature (Tukey's test (P≤0.05). **Means followed by same lower case letter in the columns indicate non-significant differences between the species at different temperatures (Tukey's test (P≤0.05). n= number of replicates (females or males).

Table 4: Size of tibia (mm) of *C. infumatus, E. varians* and *M. basicornis* at constant temperatures (± 1°C). 70±10% RH and 12h photophase.

T °C	Species	n	Female	Male	*t*	*P* values
	C. infumatus	20	1.68±0.85A*d**	1.65±0.72Ad	1.115	0.233
16	*E. varians*	20	1.82±1.54Ac	1.80±1.06Ac	0.968	0.314
	M. basicornis	20	1.71±0.47Ad	1.69±0.78Ad	1.010	0.064
	C. infumatus	20	1.83±1.25Ab	1.80±1.24Ab	1.066	0.078
20	*E. varians*	20	1.97±1.06Aa	1.94±0.78Aa	2.013	0.145
	M. basicornis	20	1.88±0.47Ab	1.86±1.15Ab	1.782	0.347
	C. infumatus	20	1.84±1.03Ab	1.82±0.22Ab	0.974	0.081
24	*E. varians*	20	2.29±1.14Aa	2.24±0.98Aa	1.257	0.129
	M. basicornis	20	1.98±0.46Ab	1.93±1.05Ab	2.335	0.206
	C. infumatus	20	1.87±1.37Ab	1.85±0.82Ab	2.153	0.312
28	*E. varians*	20	2.05±1.33Aa	2.03±0.75Aa	1.673	0.211
	M. basicornis	20	1.91±0.87Ab	1.92±1.12Ab	0.931	0.097
	C. infumatus	20	1.65±0.62Ad	1.63±0.34Ad	1.336	0.161
32	*E. varians*	20	1.81±0.98Ac	1.79±0.41Ac	1.099	0.082
	M. basicornis	20	1.72±1.04Ad	1.70±0.58Ad	1.121	0.215

*Means (± SE) followed by same capital letter in columns indicate non-significant differences for species at the same temperature (Tukey's test (P≤0.05). **Means followed by same lower case letter in the columns indicate non-significant differences between the species at different temperatures (Tukey's test (P≤0.05). n= number of replicates (females or males).

Table 5: Weight (mg) of *C. infumatus*, *E. varians* and *M. basicornis* at constant temperatures (± 1°C). 70±10% RH and 12h photophase.

T °C	Species	n	Female	Male	*t*	*P* values
16	*C. infumatus*	20	1.60±0.75A*d**	1.58±0.22Ad	1.013	0.321
	E. varians	20	1.74±1.31Ac	1.70±1.18Ac	0.987	0.071
	M. basicornis	20	1.64±0.58Ad	1.59±0.83Ad	1.314	0.062
20	*C. infumatus*	20	1.88±1.57Ab	1.86±1.65Ab	0.968	0.115
	E. varians	20	1.98±1.24Aa	1.97±1.18Aa	1.231	0.214
	M. basicornis	20	1.93±1.13Ab	1.90±1.36Ab	0.996	0.147
24	*C. infumatus*	20	1.91±1.21Ab	1.89±0.16Ab	1.321	0.083
	E. varians	20	2.45±1.11Aa	2.39±0.87Aa	2.012	0.106
	M. basicornis	20	2.03±0.24Ab	2.00±1.42Ab	1.459	0.064
28	*C. infumatus*	20	1.92±1.62Ab	1.90±1.44Ab	0.995	0.102
	E. varians	20	2.39±1.26Aa	2.37±0.85Aa	1.132	0.111
	M. basicornis	20	1.92±0.77Ab	1.91±0.89Ab	1.087	0.088
32	*C. infumatus*	20	1.64±0.23Ad	1.61±1.09Ad	0.879	0.109
	E. varians	20	1.79±0.39Ac	1.75±1.04Ac	1.054	0.071
	M. basicornis	20	1.67±1.43Ad	1.66±0.94Ad	2.013	0.061

*Means (± SE) followed by same capital letter in columns indicate non-significant differences for species at the same temperature (Tukey's test (P≤0.05). **Means followed by same lower case letter in the columns indicate non-significant differences between the species at different temperatures (Tukey's test (P≤0.05). n= number of replicates (females or males).

Figures

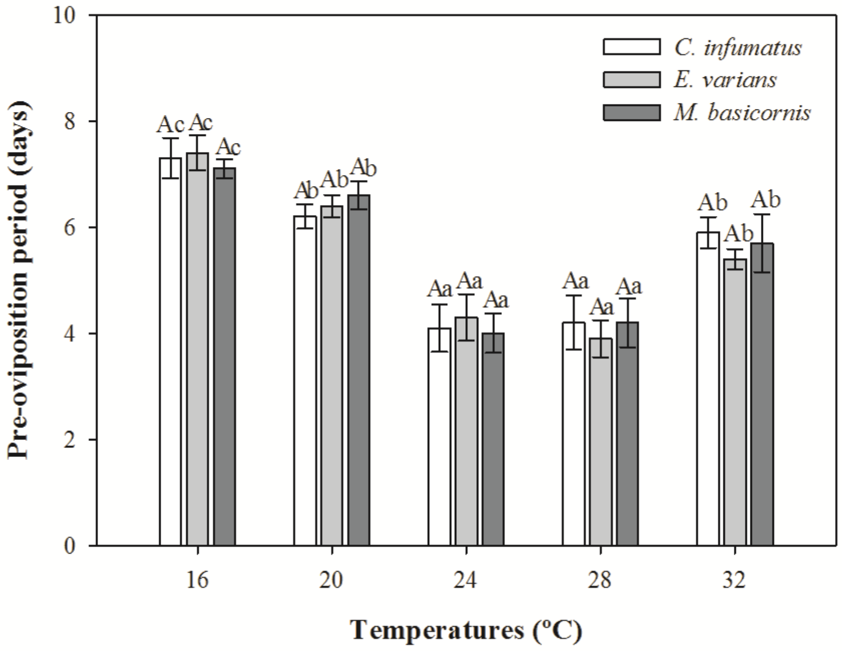

Figure 1: Pre-oviposition period in days (± SE) of *C. infumatus*, *E. varians* and *M. basicornis* at different temperatures, 70±10% RH and 12h photophase.

Means (± SE) followed by same capital letter indicate non-significant differences for the species in same temperature (One-way ANOVA) and same lower case letter (Two-way ANOVA) indicate non-significant differences between the species at different temperatures (Tukey's test (P≤0.05). n = number of replicates (females) of daily and total fecundity (Table 1).

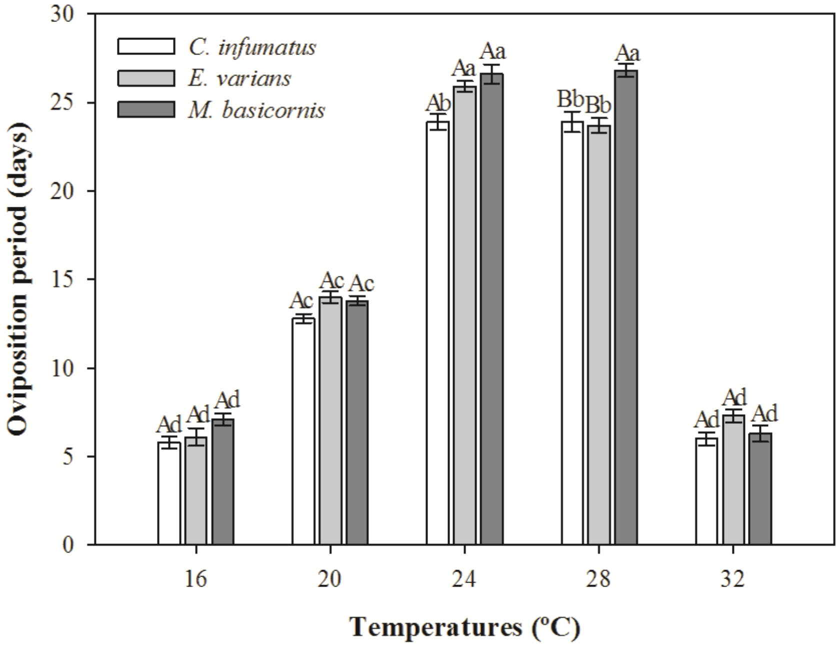

Figure 2: Oviposition period in days (± SE) of *C. infumatus*, *E. varians* and *M. basicornis* at different temperatures, 70±10% RH and 12h photophase.

Means (± SE) followed by same capital letter indicate non-significant differences for the species in same temperature (One-way ANOVA) and same lower case letter (Two-way ANOVA) indicate non-significant differences between the species at different temperatures (Tukey's test (P≤0.05). n = number of replicates (females) of daily and total fecundity (Table 1).

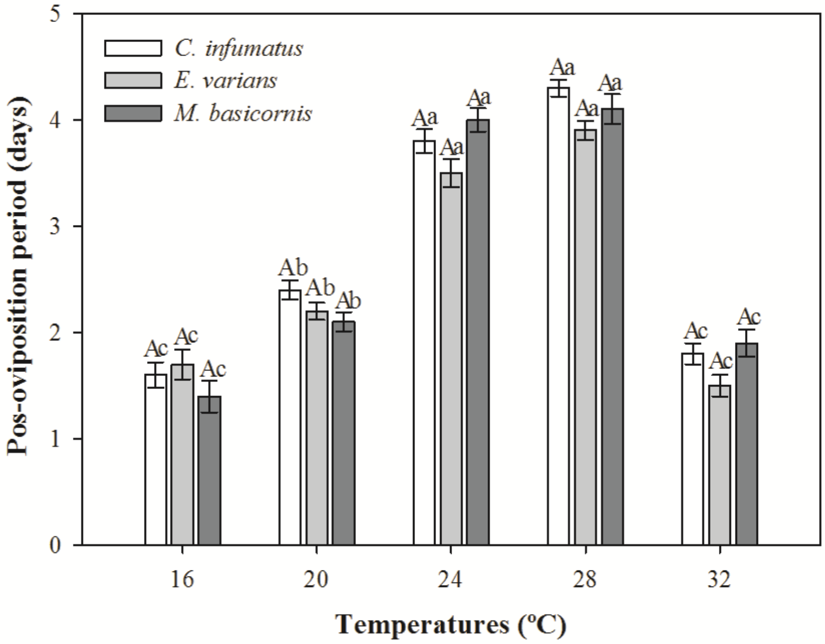

Figure 3: Pos-oviposition period in days (± SE) of *C. infumatus*, *E. varians* and *M. basicornis* at different temperatures, 70±10% RH and 12h photophase.

Means (± SE) followed by same capital letter indicate non-significant differences for the species in same temperature (One-way ANOVA) and same lower case letter (Two-way ANOVA) indicate non-significant differences between the species at different temperatures (Tukey's test ($P \leq 0.05$). n = number of replicates (females) of daily and total fecundity (Table 1).

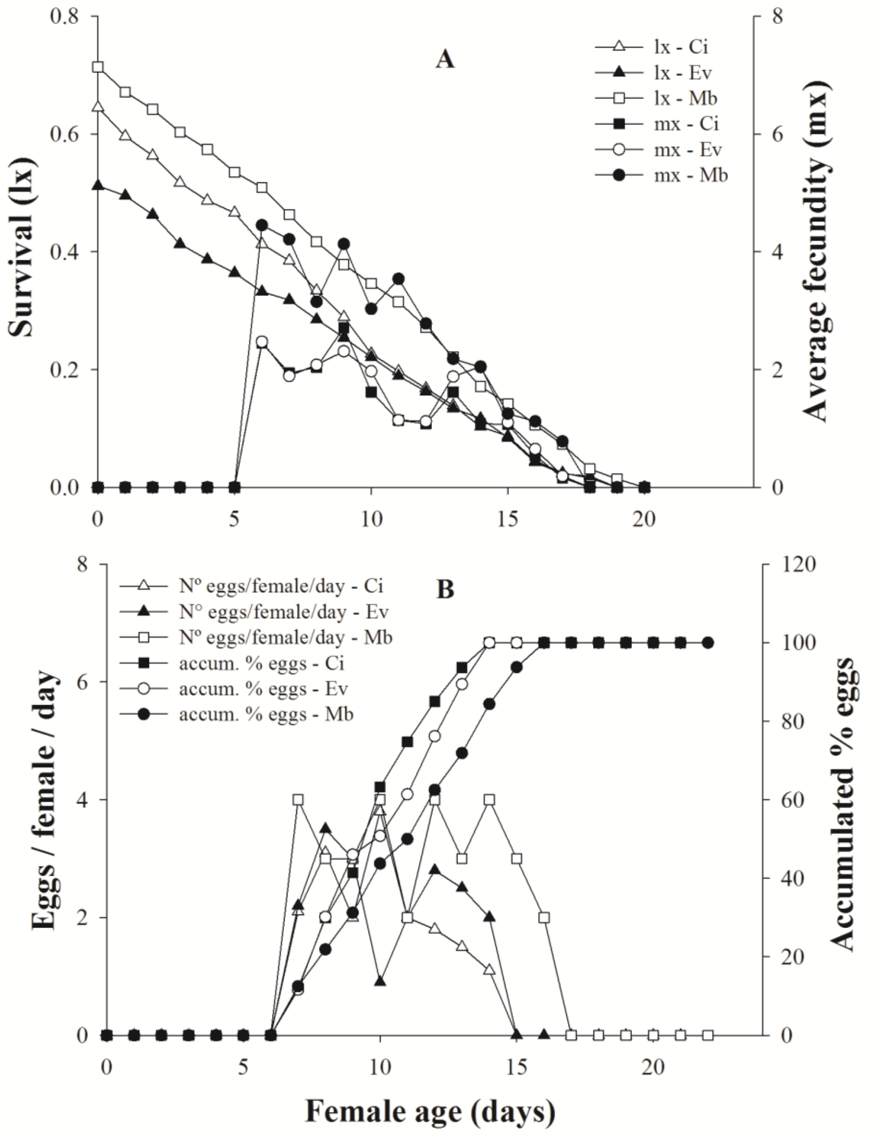

Figure 4: Survival (l_x) and average fecundity (m_x) (A); eggs/female/day and accumulated percentage eggs (B) of *C. infumatus* (Ci), *Engytatus varians* (Ev) and *M. basicornis* (Mb) at 16°C (± 1°C), 70±10% RH and 12h photophase.

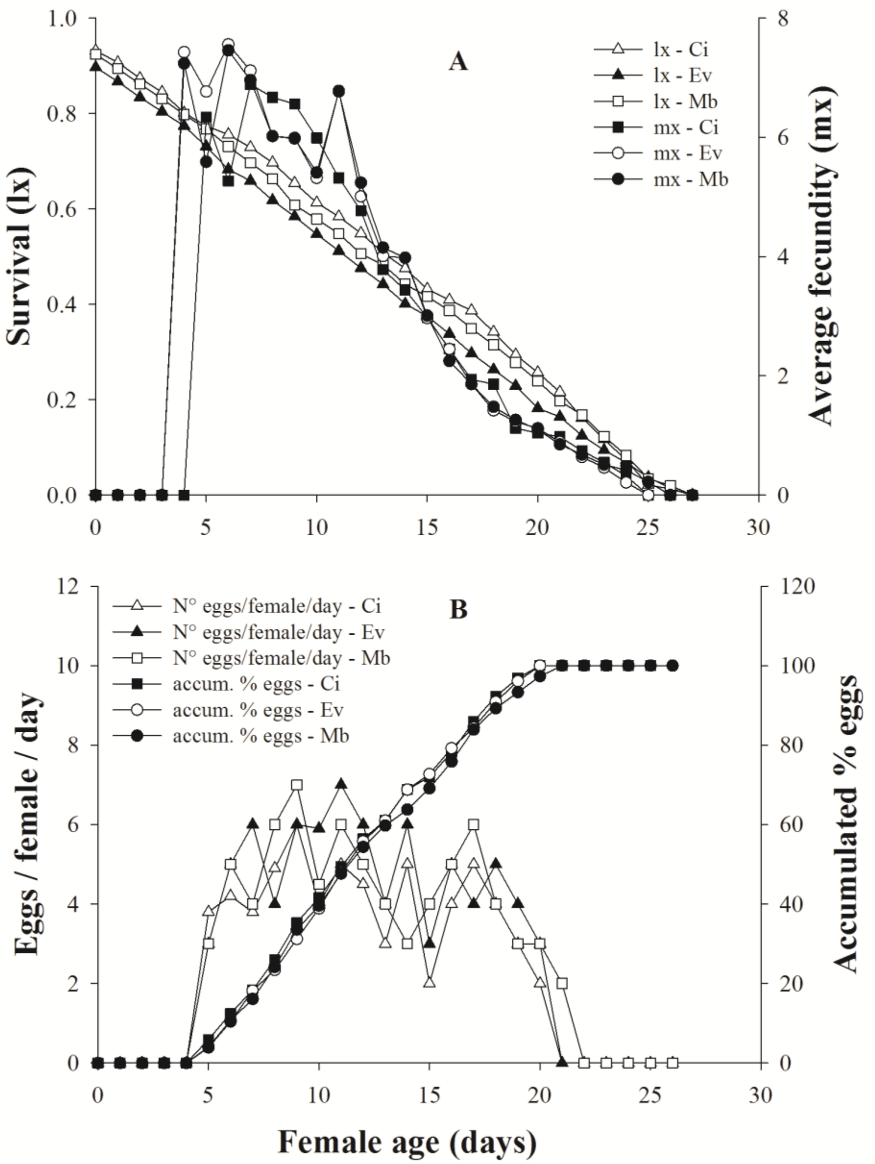

Figure 5: Survival (l_x) and average fecundity (m_x) (A); eggs/female/day and accumulated percentage eggs (B) of *C. infumatus* (Ci), *Engytatus varians* (Ev) and *M. basicornis* (Mb) at 20°C (± 1°C), 70±10% RH and 12h photophase.

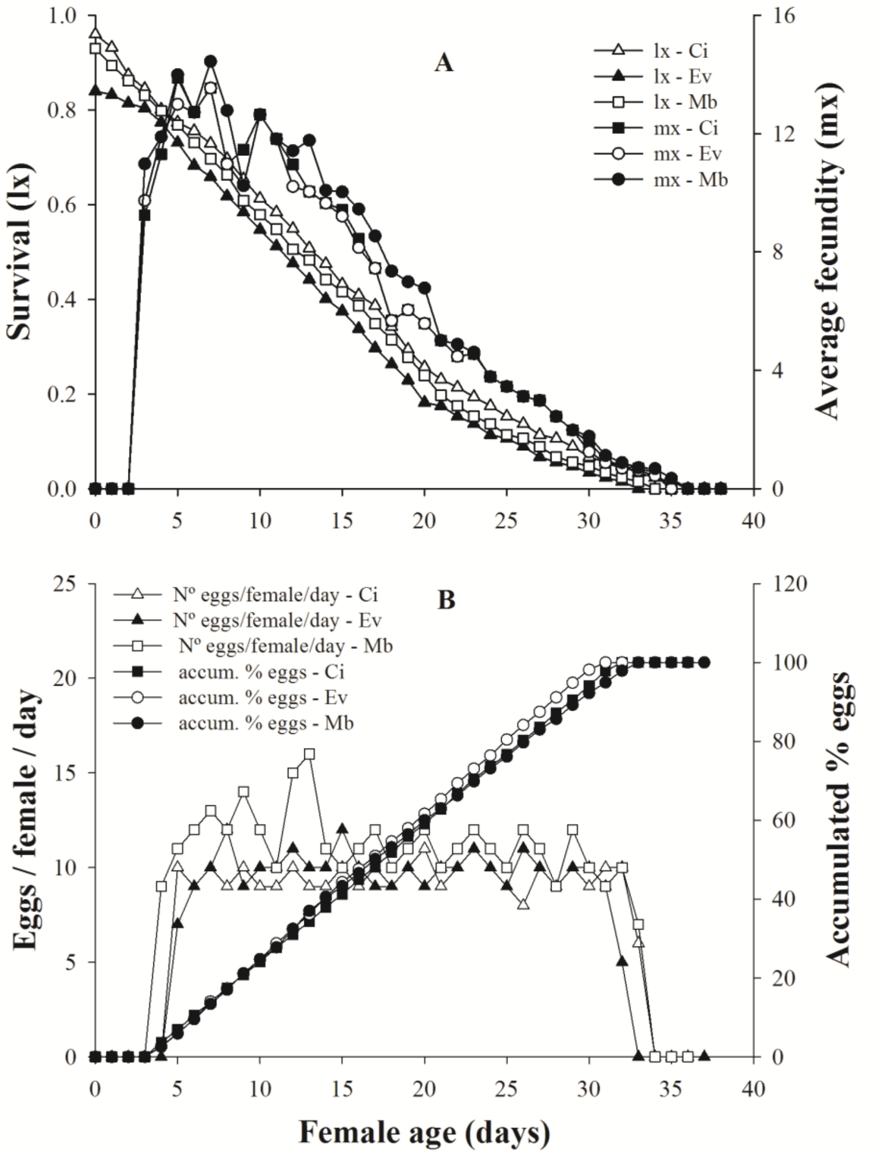

Figure 6: Survival (l_x) and average fecundity (m_x) (A); eggs/female/day and accumulated percentage eggs (B) of *C. infumatus* (Ci), *Engytatus varians* (Ev) and *M. basicornis* (Mb) at 24°C (± 1°C), 70±10% RH and 12h photophase.

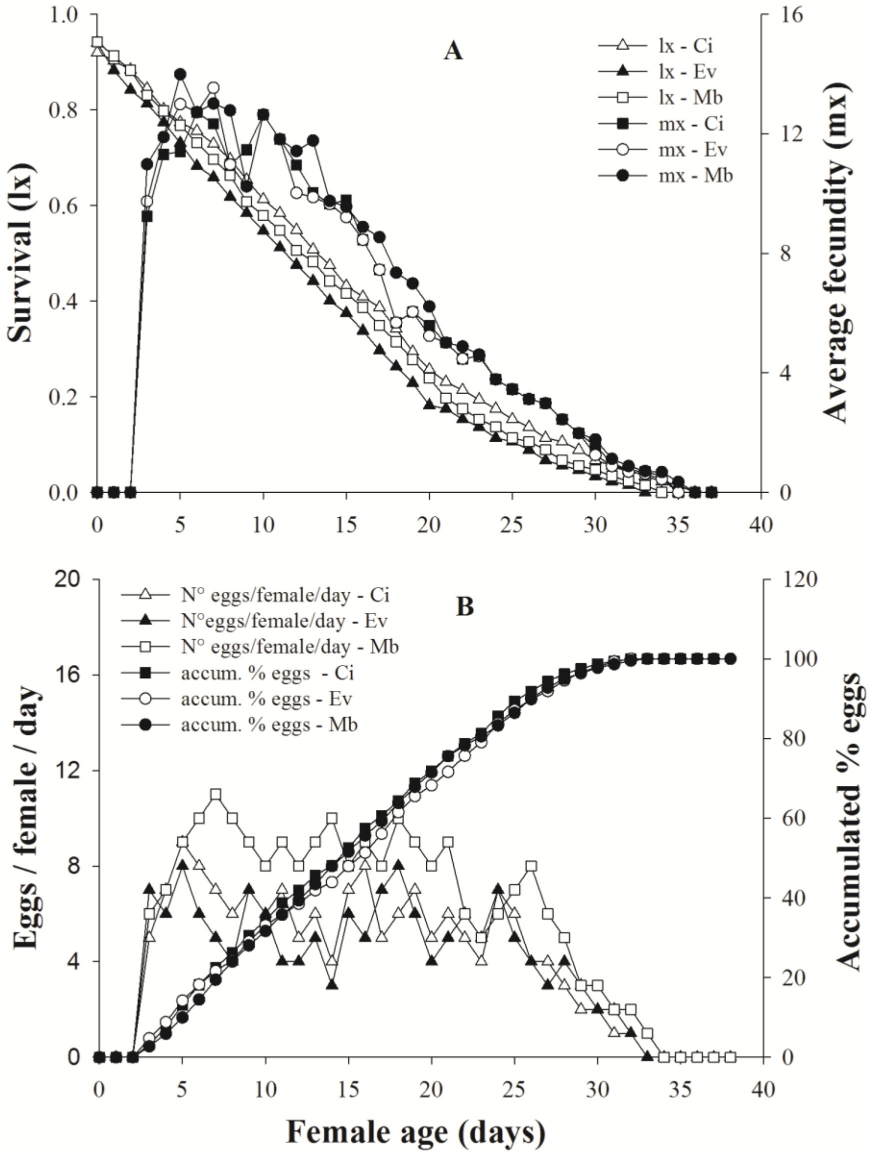

Figure 7: Survival (l_x) and average fecundity (m_x) (A); eggs/female/day and accumulated percentage eggs (B) of *C. infumatus* (Ci), *Engytatus varians* (Ev) and *M. basicornis* (Mb) at 28°C (± 1°C), 70±10% RH and 12h photophase.

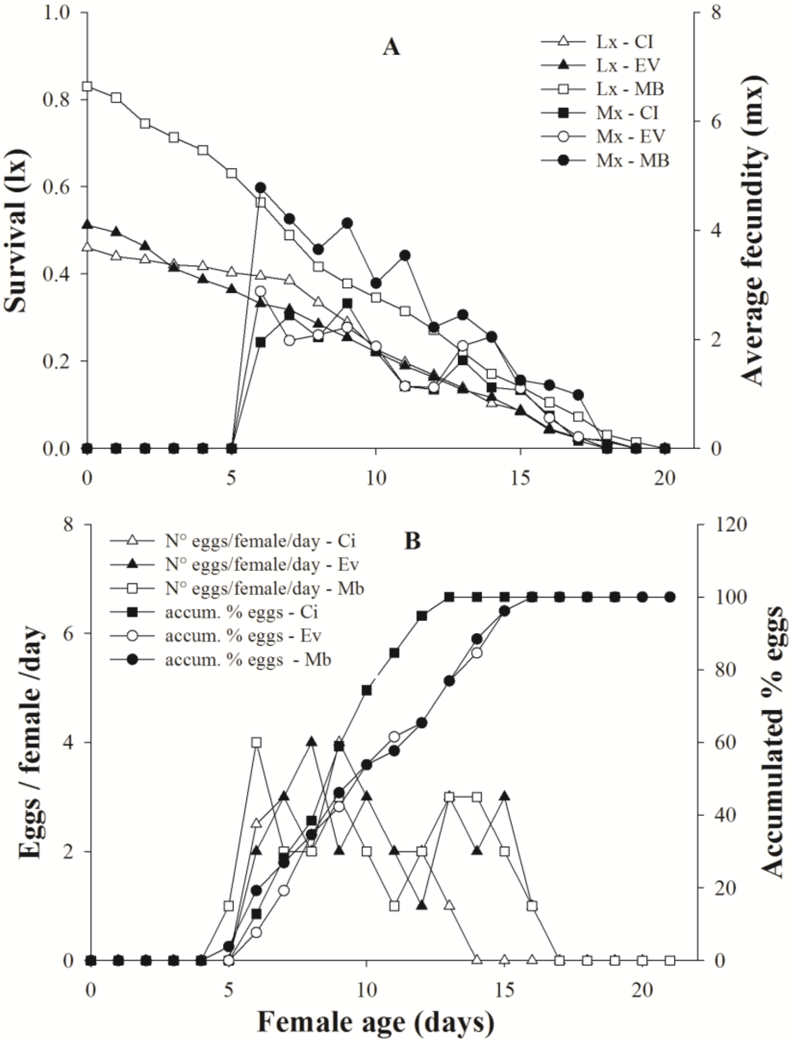

Figure 8: Survival (l_x) and average fecundity (m_x) (A); eggs/female/day and accumulated percentage eggs (B) of *C. infumatus* (Ci), *Engytatus varians* (Ev) and *M. basicornis* (Mb) at 32°C (± 1°C), 70±10% RH and 12h photophase.

(VERSÃO PRELIMINAR)

ARTICLE 2 Prey preference and predation behavior of three mirid predators when offered 1st instar larvae of *Tuta absoluta*, *Neoleucinodes elegantalis* and *Helicoverpa armigera*

Calixto, A.M.[a]
Bueno, V.H.P.[b]
Soares, M.A.[a]
Montes, F.C.[a]
van Lenteren, J.C.[c]

[a] Laboratory of Biological Control, Department of Entomology, Federal University of Lavras, P. O. Box 3037, 37.200-000 Lavras/MG, Brasil;
[b] Corresponding author. Department of Entomology, Federal University of Lavras, P. O. Box 3037, 37.200-000 Lavras/MG, Brasil (e-mail: vhpbueno@den.ufla.br);
[c] Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands.

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Abstract. The knowledge of predation capacity of predators is essential to success of biological control. The species native Brazilian species of mirid predators *(Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal)) are evaluated as biological control agents for control of *Tuta absoluta* (Meyrick) and other tomato pests. The aim of this study was determine their prey preference when fed on 1^{st} instar larvae of *T. absoluta*, *Neoleucinodes elegantalis* (Guenée) and *Helicoverpa armigera* (Hübner) in choice and no-choice tests. Also, behavioural activities where quantified when exposed to *T. absoluta* and *N. elegantalis* in choice and no-choice tests. To determine prey preference, a 24h-starved female of up 5 days old was released in a Petri dishes (9 cm diameter) which contained two tomato leaflets on 1% water-agar solution that were infested with 10 newly-emerged 1^{st} instar larvae of *T. absoluta*, *N. elegantalis* or *H. armigera* in the no-choice tests. For the choice test 5 larvae of two prey species (either *T. absoluta + N. elegantalis or T. absoluta + H. armigera*) were offered. Larval predation by the three mirid species was determined at intervals of 6h, 12h and 24h after their release. For predation behavior tests, a female was released in a Petri dishes (5 cm diameter) containing one tomato leaflet placed on a 1% water-agar layer with 5 larvae of *T. absoluta* and 5 larvae of *N. elegantalis* in the choice test, or 10 larvae of one species in the no- choice test. The predators were observed directly under a stereoscopic microscope for a 60 minutes period to evaluate their behavioral. *C. infumatus, E. varians* and *M. basicornis* consumed an average of 8 larvae of *T. absoluta*, 6 larvae of *N. elegantalis* and 8 larvae of *H. armigera* during 24 hours in the no-choice tests. In choice tests, the three mirid predators preferred *T. absoluta* above the two other preys. Observations of the predation behavior of the three species revealed that they spent most time searching and preying *T. absoluta* in choice and no-choice tests. The species *C. infumatus*, *E. varians* and *M. basicornis* will attack 1st instar larvae of the three pests. However, their prey preference indicates that *T. absoluta* is best attacked and consumed.

Key words: Lepidopteran preys, predators, Miridae, predation rate, behavioral activities.

Prey preference and predation behavior of three mirid predators when offered1st instar larvae of *Tuta absoluta*, *Neoleucinodes elegantalis* and *Helicoverpa armigera*

Introduction

Species of the Family Miridae (Hemiptera: Heteroptera), are omnivores, and comprise species that are important predators of crop pests (Wheeler, 2001). In Europe, the species *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) are used in augmentative biological control against *Tuta absoluta* (Meyrick) (Lep.: Gelechiidae) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in tomato crops (Calvo et al., 2012; Urbaneja, Montón, & Mollá, 2009). Recently in Brazil, studies were initiated to evaluate the three mirid species

Campyloneuropsis infumatus (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal) (Hemiptera: Miridae) (Bueno et al., 2012, 2013) for their potential as biological control agents of important pests in tomato crops.

The tomato borer *T. absoluta*, is one of the most important pests in tomato crops in South America and recently also on other continents (Miranda et al., 2005; Desneux, Luna, Guillermaud, & Urbaneja, 2011). Leaves and fruits are damage by the larvae and can lead to a loss of 100% in tomato production, if no control is applied (Desneux, Luna, Guillermaud, & Urbaneja, 2011; Urbaneja et al., 2012; Bueno et al., 2013). After hatching, the 1st larvae instars scrape the leaf for 20 to 30 minutes before entering the mesophyll and to penetrate it takes approximately 1 hour (Coelho & França, 1987). The tomato fruit borer *Neoleucinodes elegantalis* (Guenée) (Lep.: Crambidae) is also important pest attacking tomato crops. The larva of *N. elegantalis* develops inside of the fruits, feeding on the mesocarp and the endosperm, causing production losses from 50% to 90% (Nunes & Leal, 2001; Miranda et al., 2005). This pest has a great economic importance and rapid spread and recently has been considered a quarantine pest in Europe (EPPO, 2015). The 1st larval instars of *N. elegantalis* penetrate the fruit within 1-2 hours after hatching (Eiras and Blackmer, 2003). The species *H. armigera* is an important polyphagous pest spreading worldwide and can attack approximately 200 plant species including tomato crops (Guo, 1997, Czepak et al., 2013). Although feeding larvae can sometimes be seen on the surfaces of plants, they are often hidden within plant organs (i.e. flowers or fruits), in which case bore holes may be visible (Smith-Pardo, 2014). Since 2013, *H. armigera* is considered quarantine pest in Brazil and was detected in tomato crops in Espirito Santo state, Brazil (Pratissoli et al., 2015). In Brazil, the control of these three pests still is by use of pesticides and studies on biological control are still limited.

Generalists and omnivorous predators like heteropteran species, among which the mirid predators, can rapidly establish in agro-ecosystems by using alternating preys (Desneux & O'neil, 2008). These predators may feed on any arthropod they are able to subdue, regardless of the nature of the prey. Generally, they may consume species that belong to different trophic levels within a community, acting as a primary predator (consuming herbivores) and as secondary predator (consuming primary predators) (Warren & Lauton, 1987; Polis & Holt, 1992; Strong, 1992) and even of organisms in higher trophic levels. Most of the mirid species, including *N. tenuis* and *M. pygmaeus*, are zoophytophagous, i.e., they can use plants as water source, develop and reproduce on plants in absence of prey by feeding on plants, and can prey on many species of prey in agroecosystems (Lucas & Alomar, 2001).

Due their omnivorous characteristics, many preys can be associate with mirid predators, but little information is available about their prey preference and predatory behavior. Understanding the prey selection process is essential to predict the efficacy of generalist predators as biological control agents and to understand the effects of alternative prey on complex direct and indirect interactions involving predators and their prey species (Eubanks & Denno, 2000). Nymphs of all stages and adults of *N. tenuis* prey on neonate larvae of *Helicoverpa armigera* on tomato plants (Libutan & Bernardo, 1995). Urbaneja et al. (2009) stated that *M.*

pygmaeus and *N. tenuis* can prey *T. absoluta* larvae of all instars. Mollá et al. (2009) and Urbaneja et al. (2012) reported that *M. pygmaeus* preferred 1st larvae instars of *T. absoluta*. However, there are any information about predation rate and behaviour for mirid species, mainly for native species of Brazil, *C. infumatus*, *E. varians* and *M. basicornis*.

The aim of this study was to evaluate the prey preference of *C. infumatus*, *E. varians* and *M. basicornis* when offered 1st larval instars of *T. absoluta*, *N. elegantalis* and *H. armigera* during 24 hours in choice and no- choice tests. Also, the predation behaviour was quantified of these mirid species when exposed to 1st instar larvae of *T. absoluta* and or *N. elegantalis*. For this study, the following hypotheses were formulated: Null hypothesis (H_0): there are differences in prey preference for the three mirid species (*C. infumatus, E. varians and M. basicornis*); these differences occur both in the 24-h predation and in the predation behavior observation tests; Alternative hypothesis (H_1): there are no differences in prey preference for the three mirid species both in the 24-h predation and behavior observation tests. .

Materials and Methods

Rearing of predators and prey

The stock rearings were carried out at the Biological Control Laboratory of Department of Entomology of the Federal University of Lavras (UFLA), Minas Gerais (Brazil) in an acclimatized room at 25 ± 2ºC, 70 ± 10% and 12h photophase.

The mirids *Campyloneuropsis infumatus*, *Engytatus varians* and *Macrolophus basicornis* were collected in tobacco plants and reared on tobacco plants *Nicotiana tabacum* L. cv. TNN as oviposition substrate and eggs of *Anagasta kuehniella* (Zeller) as food, following the methodology described by Bueno et al. (2013), where the details of the rearing can be found.

Adults of *T. absoluta* were collected in the field and taken to the laboratory. A stock colony was kept in plastic cages (60x30x30 cm). Four seedlings of tomato (*Solanum lycopersicum* L.) (Santa Clara cv.) were placed in a plastic pot (140 mL) and used as oviposition substrate for *T. absoluta* females and replaced twice a week. After oviposition, these seedlings were placed in a new cage (60x30x30 cm) until eggs hatched. Then, a piece of moistened foam (phenolic resin - Atlanta®) with tomato leaves was introduced into the cage in order to serve as food for the larvae. The leaves were inserted in the phenolic foam to avoid wilting and whenever necessary new leaves were offered until emergence of adults. After the emergence of adults, new tomato seedlings were introduced into the cage to be used as oviposition substrate to continue the life cycle of *T. absoluta*.

Eggs of *N. elegantalis* were provided by Laboratory of Entomology of Federal University of Viçosa (UFV- Brazil) and placed in glass pots (1.7 L) until egg hatching. Newly-hatched larvae (6 larvae/fruit) were placed on scarlet eggplant *Solanum gilo* Raddi (Solanaceae) and placed in plastic trays (6.5 x 18 x 29 cm) until formation of pupae. Then, pupae were placed in plastic cages (60 x 30 x 30 cm) until the emergence of adults. The cages contained six artificial fruits (see below) used as oviposition substrate and a piece of cotton moistened with a honey solution 10% as food. Artificial fruits were made of Styrofoam® balls (diameter 3.5 cm) dipped in green melted paraffin to produce a thin wax layer that covered the surface simulating natural fruits. After oviposition, the artificial fruits were stored in glass pots (1.7 L) until larval hatching, thus, starting a new life cycle. This methodology was developed by Pontes et al. (2010).

Pupae of *H. armigera* were provided by BUG® Biological Agents (Brazil) and placed in Petri dish (9 cm diameter) until emergence of adults. These pupae were kept in PVC containers (20 cm diameter and 30 cm height) with 30 pupae/container (sex ratio = 0.5). The upper part of the containers was sealed with "voil' tissue allowing aeration, and the bottom part was supported by a plastic dish (28 cm diameter) containing filter paper. All the internal walls of the containers were lined with filter paper, which can be used as oviposition substrate by females. In each container we placed one glass tube (2.5 x 8.0 cm) with a peace of cotton moistened with a honey solution (10%) and other glass tube with moistened cotton and water only. The eggs were collected daily and placed in glass pots (1.7 L) until they hatched. The larvae were reared on a modified artificial diet developed/described by Greene et al. (1976). Larvae (1st instar) were placed in Petri dishes (15 cm diameter) containing artificial diet. Later, 3rd instar larvae were individualized in glass tubes (2.5x 8.0 cm) and fed on artificial diet until pupal formation. The pupae were collected and placed in Petri dishes (9 cm diameter) in the containers until the emergence of adults.

In all tests 1st instar larvae of *T. absoluta, N. elegantalis* and *H. armigera* were used, because these instars cause the lowest amount of damage in tomato. In addition, Urbaneja et al. (2009) found that the mirid predators *N. tenuis* and *M. pygmaeus* kill higher numbers 1st instar *T. absoluta* larvae than of the later stages. The respective sizes of 1st instar larvae were 0.5 mm for *T. absoluta* (Koppert, 2015), 0.8 mm for *N. elegantalis* (Molet, 2012) and 1-1.5 mm for *H. armigera* (DPI & F entomology, 2015).

Predation behavior (choice and no-choice tests)

Twenty females up to 5 days old of the mirid predators *C. infumatus*, *E. varians* and *M. basicornis* were maintained without food for 24 h and then exposed to 1st instar larvae of *T. absoluta* and *N. elegantalis*. Due to time limitation, I could not test the prey *H. armigera*. Each predator female was individualized in glass tubes (2.5 x 8.0 cm) and supplied with water by a piece of moistened cotton. Petri dishes (5 cm diameter) containing one tomato leaflet in a 1% water-agar layer were used as arena. In the choice tests 5 1st instar larvae of each of the two prey species were offered in the following combinations: 5 *T. absoluta* + 5 *N. elegantalis* larvae (3 *N. elegantalis* larvae + 2 *T. absoluta* larvae in one leaflet and 2 *N. elegantalis* larvae + 3 *T. absoluta* larvae in other leaflet). In the no-choice tests 10 1st instar larvae (5 larvae in each leaflet) of *T. absoluta* or of *N. elegantalis* were offered.

To observe the predation behavior, female predators were observed directly under a stereoscopic microscope (Leica EZ4) (20X) and the behavioral were recorded with the use of Etholog software 2.25 (Ottoni, 2000) for a 60-minutes period. Recording started at the first contact of the female with the prey. The following behavioral activities were recorded: moving, searching for prey, encountering prey, inactive, feeding plant (tomato), feeding prey and grooming; for a description of the behavior, see Table 1. And, were recorded too the number of larvae encountered and preyed and percentage of acceptance of each predator and preys offered in choice e no choice tests.

Predation rate (no-choice test and choice tests)

Female predators of up to 5 days old were individualized in glass tubes (2.5 x 8.0 cm) supplied with water by a moistened cotton piece, and maintained without food for 24h. In Petri dishes (9 cm diameter) two tomato leaflets were placed on 1% water-agar solution and infested leaflets with newly emerged 1st instar larvae of the prey. One female predator was released in a Petri dish containing prey for 24h. The Petri dish was sealed with parafilm. The number of prey eaten was determined at intervals of 6h, 12h and 24h after their release. Preys were introduced in the Petri dish at 8:00 a.m. and after 1 hour the predator were introduced. Then, at the intervals of 6h (15:00 p.m.), 12h (21:00 p.m.) and 24h (next morning 9:00 a.m.) the number of prey eaten was counted by using a stereoscopic microscope (Leica EZ4) (20X). In the climate room the photoperiod was kept on 12L (06:00 – 18:00) :12D (18:00 – 06:00). It was checked before releasing of mirid predators if all *T. absoluta* 1st larvae instar were already inside the mines. Choice (prey preference) and no-choice (predation rate) tests were done. In the choice tests 5 1stinstar larvae of two lepidopteran prey species were offered in the following combinations: 5 *T. absoluta* + 5 *N. elegantalis* and 5 *T. absoluta* + 5 *H. armigera*. In case of the choice tests either 2 *T. absoluta* larvae + 3 *N. elegantalis* larvae or *H. armigera* larvae, and in the other leaflet 3 *N. elegantalis* or *H. armigera* larvae + 2 *T. absoluta* larvae were offered. In the no-choice test 10 1st instar larvae were offered: 10 *T. absoluta*, 10 *N. elegantalis* or 10 *H. armigera*. Twenty-five replicates for each mirid predator were evaluated in the choice and no-choice tests. The number of prey used in these experiments was determined in pre-tests. These tests showed that neither of the predators consumed more than 9 1st instar larvae instars of any prey during 24h.

Data analysis

Predation Behavior

The Etholog 2.25 program registered (Ottoni, 2000) the time (in seconds) spent by each mirid predator in each behavioral activity during 60 minutes. The time for each behavioral activity in seconds was transformed in time percentage (0 – 100).These data did not show a normal distribution (Shapiro-Wilk test) ($p \geq 0.05$). Also the numbers of larvae consumed by the predators were also not normally distributed. To compare the mean the non-parametric Kruskal-Wallis test ($p \leq 0.05$) was used with the statistical software R Development Core Team, 2014. The data about percentage of acceptance in no-choice and choice tests were not presented normal distribution and were compared with Chi-square test (χ^2) ($p \leq 0.05$) (R Development Core Team, 2014).

Predation rate (no-choice and choice tests)

The number of larvae which were preyed after 6h, 12h and 24h by each predator in each treatment of predation rate in choice and no-choice tests was recorded.

The data of predation in the no-choice tests were not normally distributed according Shapiro-Wilk test ($p \geq 0.05$). Therefore, to compare the means the non-parametric Kruskal-Wallis test ($p \leq 0.05$) was used with the statistical software R Development Core Team, 2014. Data of predation rate (preference) in choice of each specie for *T. absoluta* + *N. elegantalis* or *T. absoluta* + *H. armigera* were not normally distributed according Shapiro-Wilk test ($p \geq 0.05$) and to compare the means was used Chi-square test (χ^2) ($p \leq 0.05$).

Results

Predation Behavior

The percentage of acceptance in choice tests (60-min) was higher for *T. absoluta* than *N. elegantalis* to three mirid predators (H = 12.133, df = 2, p<0.001) (Table 2). In no-choice tests there was not difference in percentage of acceptance for mirid predators preyed *T. absoluta* (H = 11.133, df = 2, p = 0.002) or *N. elegantalis* (H = 17.445, df = 2, p<0.001).

And, in choice and no-choice tests the larvae of two preys offered were not resistant to predation by mired predators.

During the 60-min direct observation experiments, predators spent most of their time searching for prey and feeding on *T. absoluta* larvae (H = 0.567, df = 6, p = 0.002). The time spent on each of the different activities was similar for the three predators (H = 0.645, df = 2, p = 0.124) (except for a slight but significant difference for the time spent on being inactive (H = 2.345, df = 2, p = 0.004) (Figure 1).

Predators spent similar amounts of time for all activities in the arena with *N. elegantalis* larvae, except for the time being inactive where small differences were found(H = 0.098, df = 6, p = 0.023) (Figure 2).

When the larvae of *T. absoluta* and *N. elegantalis* were offered together in a choice test, *C. infumatus*, *E. varians* and *M. basicornis* spent more time feeding on *T. absoluta* larvae (H = 3.589, df = 7, p = 0.017) than for *N. elegantalis* (Figure 3) showing a preference for *T. absoluta* larvae. *E. varians* spent a less time inactive than the other species (H = 1.621, df = 2, p<0.001). The mirid predators spent a similar time in the activities feeding *N. elegantalis*, feeding plant and grooming (H = 0.986, df = 7, p = 0.231) and a less time into encountering the preys (H = 5.487, df = 2, p = 0.002) (Figure 3).

Predation rate (choice and no-choice tests)

Significantly different number of *T. absoluta* prey were consumed by three mirid species after 6h, 12h and 24h in the no-choice test, whereas after 24h the three mirid species consumed the highest number of 1st larvae instar of *T. absoluta* (H = 4.931, df = 2, p = 0.004). For each time interval (6, 12 and 24 hours) the number of prey consumed was the same for the three species of predators (6h interval: H = 0.943, df = 2, p = 0.624; 12h interval H = 0.366, df = 2, p = 0.833; 24h interval: H = 2.118, df = 2, p = 0.347) (Table 4).

Also the predation rates of *N. elegantalis* in the no-choice test by the three predators were significantly different at the different time intervals (H = 2.251, df = 2, p = 0.002) and after 24h the mired predators consumed the highest number of 1st larvae instar of *N. elegantalis*. And also for *N. elegantalis* predation rates at each time interval (6h, 12h or 24h) were similar (6h interval: H = 0.240, df = 2, p = 0.887; 12h interval: H = 2.053, df = 2, p = 0.358); 24h interval: (H = 4.230, df = 2, p = 0.121) (Table 4).

Similar results were found when were offered *H. armigera* as prey for *C. infumatus*, *E. varians* and *M. basicornis* in each time interval. There were significant differences among the time intervals (6h, 12h and 24h) (H = 2.953, df = 2, p = 0.006).

For each time interval (6, 12 and 24 hours) the number of 1st larvae instar of *H. armigera* consumed was the same for the three species of predators (6h interval: H = 0.073, df = 2, p = 0.964; 12h interval: H = 8.372, df = 2, p = 0.115; 24h interval: H = 11.331, df = 2, p = 0.103) (Table 4).

There was significant difference in predation rate of mirid predators in choice tests (preference) when offered *T. absoluta* and *N. elegantalis*. *C. infumatus*, *E. varians* and *M. basicornis* preferred *T. absoluta* than *N. elegantalis* (Table 5).

Also when was offered *T. absoluta* and *H. armigera* only *C. infumatus* in time intervals 12h and 24h preferred *T. absoluta* than *H. armigera* (Table 6).

Discussion

This is the first study on potential prey preference and predation behavior of *C. infumatus*, *E. varians* and *M. basicornis* when preying on *T. absoluta*, *N. elegantalis* and *H. armigera.*

The feeding preference of a predator is largely governed by the ratio of energy obtained relative to the costs of searching and consuming the prey (Krebs & McCleery, 1984). These costs are determined by prey size but also by prey behavior. In this study, the number of *T. absoluta* 1^{st} instar larvae consumed by the three mirid species was higher than values obtained to *M. pygmaeus* (2.0 larvae) and *N. tenuis* (2.5 larvae) per 24h (Urbaneja et al., 2009). Mirid predators preferred neonate larvae (Mollá et al., 2009; Urbaneja et al., 2012). The mirid *N. tenuis* is reported to feed preferentially on neonate larvae (<8 h old) of *Spodoptera litura* in tobacco plants (Torreno 1994).

Other important point is that mired predators can prey *T. absoluta* larvae inside of mines in all tests evaluated. And size of larvae did not influence the predation on *T. absoluta* and *H. armigera* by mirid predators that preyed similar number of larvae of both species.

Concerning to *N. elegantalis* although this larvae were not preferred prey, the three mirid predators consumed similar number of as *T. absoluta* larvae consumption when the prey were offered in no-choice test. This is important finding as the 1st instar larvae of *N. elegantalis* is also like *T. absoluta* a critical point as a pest. Female adult oviposit directly on the fruit or on the underside of the calyx, and after emergence, the larvae spent 74 minutes to enter on the fruit (Eiras & Blackmer, 2003). Sandoval and Manzano (2012) reported that *N. elegantalis* 1st instar larvae remained on the surface of fruit from 2 to 2.8 hours, thus becoming vulnerable to control. The species *H. armigera* is also reported as prey to mirid predators (Libutan & Bernardo, 1995). Thus, *N. elegantalis* and *H. armigera* could be target preys to these three mirid predators when released against *T. absoluta* on tomato crops.

The release of natural enemies that could prey simultaneously several target prey could be advantageous and preferable in augmentative biological control. According to Wheeler (2001), the trophic flexibility of mirid predators should enhance their value as biological control agents and help to clarify their role in agroecosystems, and this important to biological control. But the results may also be influenced by defence and escape characteristics of different prey species and in that case we cannot speak about prey preference. The 1st instar larvae of *T. absoluta* penetrate in the mesophyll (mines) and can only move in a limited way. *N. elegantalis* and *H. armigera* are not yet in the leaf or fruit and can more easily defend themselves. Although *N. elegantalis* usually penetrate to the fruit, the larvae were out during the tests as the tomato leaves were used as support in the release arena in this study.

The species *C. infumatus*, *E. varians* and *M. basicornis* are generalist predators (Bueno et al., 2013). Generalist predators often have discernible preferences for prey, frequently attacking certain insect species more while avoiding others (Richards, 1982; Wyatt & Foster, 1989; Digweed, 1993). In the prey selection process, predators can prefer one type of prey based on its mobility, size, color, odour and taste (Awan, 1985; Digweed, 1993). Then, probably, size, color, odor or taste could be involved in attracting these three mirid predators to the preys, but these cues need to be better investigated to point their role in the interaction - *C. infumatus*, *E. varians* and *M. basicornis*- prey.

The predation behaviors observed on the three mirid species were in accordance to the prey preference tests. The mirid predator's *C. infumatus*, *E. varians* and *M. basicornis* exposed to *T. absoluta* and or *N. elegantalis* larvae showed clearly prey preference for *T. absoluta* than *N. elegantalis*. And there are not preference between *T. absoluta* and *H. armigera* larvae. The three mirid predators spent more time among their different activities in searching for and preying *T. absoluta*.

Conclusion

The three mirid predators can find and prey *T. absoluta, N. elegantalis, H. armigera*. The 1st instar larvae of *T. absoluta* is more often consumed than those *N. elegantalis* and *H. armigera*, and this might be the result of the impossibility for *T. absoluta* larvae to defend themselves in the mine when attacked. The three mirid species show potential to be used as biological control of the three pest species although when all three occur simultaneously *T. absoluta* control might be better than that of the other two species. The Null hypotheses (H0) were confirmed: there are differences in predation rate which might be related to prey preference exhibited by the three mirid species (*C. infumatus, E. varians and M. basicornis*); these differences occur both in the 24-h predation and in behavioral observation tests.

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Tables

Table 1: Description of behavioral activities of *C. infumatus*, *E. varians* and *M. basicornis* when exposed to 1st instar larvae of *T. absoluta*, *N. elegantalis* and *H. armigera* (adapted of Economou et al., 2006).

Activity	**Definition**
Moving	Forward, backward or sideward movement
Searching for prey	Moving the rostrum and antennae in arena with slow movements
Encountering prey	Touching the prey with rostrum
Inactive	Standing immobile without probing, feeding, grooming
Feeding plant (tomato)	Inserting the rostrum in the plant and sucking fluid
Feeding prey	Inserting the rostrum in the prey integument and sucking fluid
Grooming	Rubbing the tarsi over each other, or over other parts of the body (rostrum, antennae, abdomen)

Table 2: Acceptance (%) of *C. infumatus*, *E. varians* and *M. basicornis* fed on 1st instar larvae of *T. absoluta* and *N. elegantalis* during 60 minutes in choice test at 25°C (± 2°C), 70±10% RH and 12h photophase.

Mirid predators	*T. absoluta*			*N. elegantalis*		
	Larvae encountered	Larvae preyed	Acceptance (%)	Larvae encountered	Larvae preyed	Acceptance (%)
C. infumatus	61	48	79.6 ± 0.34A**a*	32	20	57.3 ± 0.35Bb
E. varians	62	44	73.0 ± 0.41Aa	41	23	57.4 ± 0.31Bb
M. basicornis	68	45	68.3 ± 0.45Ab	38	26	70.2 ± 0.44Aa

*Means (± SE) followed by same lower case letter in columns indicate non-significant differences for species to same prey offered (Kruskal-Wallis test (P≤0.05). **Means followed by same capital case letter in the lines indicate non-significant differences between each specie for two prey offered (Chi-square test (χ^2) (P≤0.05).

Table 3: Acceptance (%) of *C. infumatus*, *E. varians* and *M. basicornis* fed on 1st instar larvae of *T. absoluta* and *N. elegantalis* during 60 minutes in no-choice tests at 25°C (± 2°C), 70±10% RH and 12h photophase.

	T. absoluta			*N. elegantalis*		
Mirid predators	Larvae encountered	Larvae preyed	% acceptance	Larvae encountered	Larvae preyed	% acceptance
C. infumatus	96	73	77.4 ± 0.38a*	57	36	68.2 ± 0.33a
E. varians	94	71	76.7 ± 0.34a	59	34	61.3 ± 0.31a
M. basicornis	103	74	73.3 ± 0.41a	65	36	60.7 ± 0.37a

*Means (± SE) followed by same lower case letter in columns indicate non-significant differences for species to same prey offered (Kruskal-Wallis test (P≤0.05).

Table 4: Predation rate of *C. infumatus*, *E. varians* and *M. basicornis* fed on 1st instar larvae of *T. absoluta*, *H. armigera* and *N. elegantalis* in no-choice tests at 25°C (± 2°C), 70±10% RH and 12h photophase.

	T. absoluta	*N. elegantalis*	*H. armigera*
C. infumatus – 6h	2.8 ± 0.44c*	2.2 ± 0.36c	2.68 ± 0.45c
E. varians – 6h	2.72 ± 0.39c	2.24 ± 0.39c	2.72 ± 0.47c
M. basicornis –6h	2.6 ± 0.42c	2.16 ± 0.33c	2.68 ± 0.53c
C. infumatus – 12h	5.04 ± 0.55b	3.88 ± 0.45b	4.92 ± 0.54b
E. varians – 12h	4.92 ± 0.51b	4.04 ± 0.51b	4.44 ± 0.51b
M. basicornis – 12h	4.96 ± 0.48b	4.16 ± 0.46b	5.04 ± 0.56b
C. infumatus – 24h	7.37 ± 0.58a	5.4 ± 0.52a	7.2 ± 0.59a
E. varians – 24h	7.72 ± 0.53a	5.64 ± 0.54a	06.6 ± 0.61a
M. basicornis – 24h	7.72 ± 0.61a	5.92 ± 0.57a	7.48 ± 0.57a

*Means (± SE) followed by same lower case letter in columns indicate non-significant differences for species to same prey offered in different time intervals (Kruskal-Wallis test ($P \leq 0.05$).

Table 5: Predation rate of *C. infumatus*, *E. varians* and *M. basicornis* fed on 1st instar larvae of *T. absoluta* and *N. elegantalis* in choice (preference) tests at 25°C (± 2°C), 70±10% RH and 12h photophase.

	T. absoluta	*N. elegantalis*	χ^2	*p*
C. infumatus – 6h	1.76 ± 0.22A[**]c[*]	0.44 ± 0.09Bb	11.7	0.002
E. varians – 6h	2.24 ± 0.19Ac	0.44 ± 0.11Bb	17.4	0.012
M. basicornis –6h	2.32 ± 0.21Ac	0.6 ± 0.12Bb	21.8	0.003
C. infumatus – 12h	3.08 ± 0.24Ab	1.56 ± 0.15Bb	12.3	0.004
E. varians – 12h	3.44 ± 0.29Ab	1.48 ± 0.13Bb	15.9	0.011
M. basicornis – 12h	3.68 ± 0.31Ab	1.4 ± 0.21Bb	19.2	<0.001
C. infumatus – 24h	4.32 ± 0.34Aa	2.53 ± 0.19Bb	20.5	<0.001
E. varians – 24h	4.44 ± 0.39Aa	2.36 ± 0.22Bb	22.6	0.017
M. basicornis – 24h	4.6 ± 0.37Aa	2.32 ± 0.17Bb	23.1	<0.001

[*] Means followed by same capital letter in the lines indicate non-significant differences between each specie for two prey offered in each time interval (Chi-square test (χ^2) (P≤0.05). [**] Means (± SE) followed by same lower case letter in columns indicate non-significant differences for species to same prey offered in different time intervals (Kruskal-Wallis test (P≤0.05).

Table 6: Predation rate of *C. infumatus*, *E. varians* and *M. basicornis* fed on 1st instar larvae of *T. absoluta* and *H. armigera* in choice (preference) tests at 25°C (± 2°C), 70±10% RH and 12h photophase.

	T. absoluta	*H. armigera*	χ^2	*p*
C. infumatus – 6h	1.48 ± 0.27a	1.32 ± 0.22a	9.47	0.122
E. varians – 6h	1.4 ± 0.24a	1.88 ± 0.23a	11.8	0.234
M. basicornis –6h	1.36 ± 0.20a	1.6 ± 0.26a	13.4	0.117
C. infumatus – 12h	2.96 ± 0.18a	2.04 ± 0.32b	16.9	<0.001
E. varians – 12h	2.44 ± 0.25a	2.6 ± 0.34a	18.5	0.215
M. basicornis – 12h	2.36 ± 0.28a	2.48 ± 0.29a	20.3	0.126
C. infumatus – 24h	4.12 ± 0.31a	3.36 ± 0.35b	21.9	<0.001
E. varians – 24h	3.36 ± 0.33a	3.6 ± 0.39a	19.1	0.072
M. basicornis – 24h	3.56 ± 0.36a	3.88 ± 0.37a	22.6	0.131

[*]Means followed by same capital letter in the lines indicate non-significant differences between each specie for two prey offered in each time interval (Chi-square test (χ^2) (P≤0.05). [**]Means (± SE) followed by same lower case letter in columns indicate non-significant differences for species to same prey offered in different time intervals (Kruskal-Wallis test (P≤0.05).

Figures

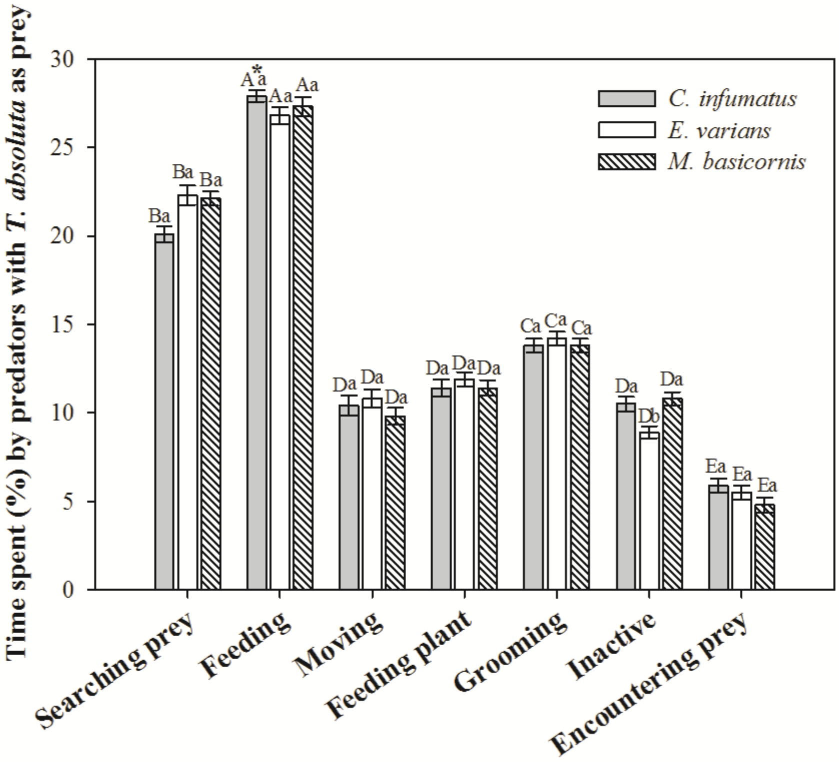

Figure 1: Time spent (%) (± SE) by *C. infumatus*, *E. varians* and *M. basicornis* with *T. absoluta* 1st instar larvae as prey during 60 minutes (no-choice test) in each behavioral activity.

*Means followed by same capital letter (difference for a certain species in all behavior activities) and lowercase letter (difference between species for a certain behavioral activity) in bars do not differ by non-parametric Kruskal-Wallis test (P≤0.05).

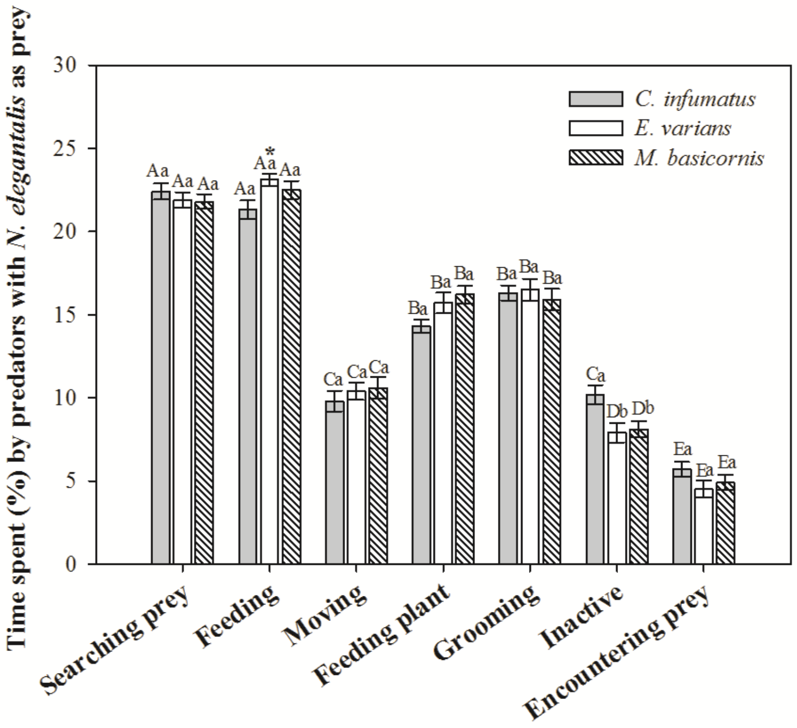

Figure 2: Time spent (%) (± SE) by *C. infumatus*, *E. varians* and *M. basicornis* with *T. absoluta* 1st instar larvae as prey during 60 minutes (no-choice test) in each behavioral activity.

[*] Means followed by same capital letter (difference for a certain species in all behavior activities) and lowercase letter (difference between species for a certain behavioral activity) in bars do not differ by non-parametric Kruskal-Wallis test ($P \leq 0.05$).

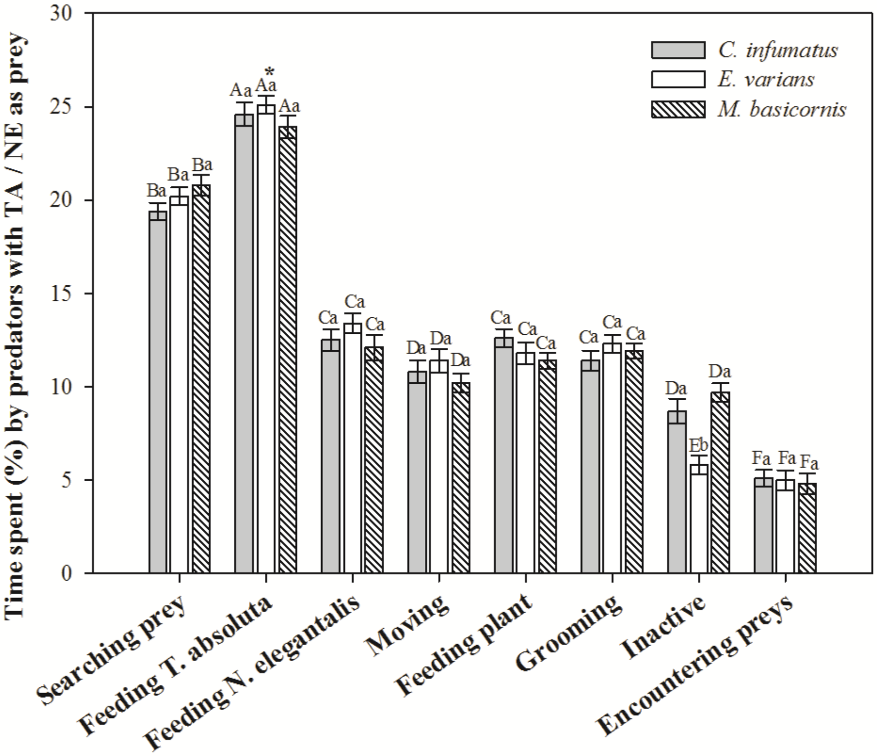

Figure 3: Time spent (%) (± SE) by *C. infumatus*, *E. varians* and *M. basicornis* with *T. absoluta* 1st instar larvae as prey during 60 minutes (choice test) in each behavioral activity.

*Means followed by same capital letter (difference for a certain species in all behavior activities) and lowercase letter (difference between species for a certain behavioral activity) in bars do not differ by non-parametric Kruskal-Wallis test ($P \leq 0.05$).

(VERSÃO PRELIMINAR)

ARTICLE 3 Population dynamics of the predators *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* (Hem.: Miridae) and their prey *Tuta absoluta* (Lep.: Gelechiidae) on tomato plants in greenhouse cages

Calixto, A.M.[a]
Bueno, V.H.P.[b]
Montes, F.C.[a]
Soares, M.A.
van Lenteren, J.C.[c]

[a] Laboratory of Biological Control, Department of Entomology, Federal University of Lavras, P. O. Box 3037, 37.200-000 Lavras/MG, Brasil;
[b] Corresponding author. Department of Entomology, Federal University of Lavras, P.O. Box 3037, 37.200-000 Lavras/MG, Brasil (e-mail: vhpbueno@den.ufla.br);
[c] Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands.

Artigo preparado de acordo com a norma do periódico BioControl

Abstract. Augmentative biological control is currently used to control many pests in several crops worldwide. Mirid predators are commercially used with success in augmentative biological control against *Tuta absoluta* (Meyrick) and *Bemisia tabaci* (Gennadius) (Hem.: Aleyrididae) in tomato in Europe. In Brazil, the mirid species *Campyloneuropsis infumatus*, *Engytatus varians* and *Macrolophus basicornis* are now evaluated as biological control agents for control of tomato pests. The aim of this study was to follow the population dynamics of these mirid predators when introduced together with *T. absoluta* in large cages in a greenhouse with tomato plants. Five periods of testing were conducted in four cages in this greenhouse. In each test four treatments were carried out: *T. absoluta* (control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. In the 1st and 2nd tests 1 couple of *T. absoluta*/plant + 1 couple of mirid predator/plant were released. In the 3rd, 4th and 5th tests 1 couple of *T. absoluta*/plant + 2 couple of mirid predators/plant were released. During 9 weeks the numbers of *T. absoluta* eggs and larvae per leaf, the number of mirid predators per leaf, the percentage of damaged leaves, the numbers of aborted flowers and attacked fruits, and the weight of fruits were recorded. In the 3rd and 4th tests the number of *C. infumatus* and *M. basicornis* per leaf were higher, the number of *T. absoluta* eggs per leaf was lower and the damaged leaves, flowers and fruits by *T. absoluta* were lower compared to the data found in 1st , 2nd and 5th tests. This shows that a release rate of 2 couples per plant is better than release of 1 couple per plant. The lower successes of releases in the 1st and 2nd tests are the result of a lower number of predators released. In the 5th test the temperature conditions and the quality of the plants were poor, resulting in a low success of releases. The results indicate that *C. infumatus* and *M. basicornis* attack and reduce *T. absoluta* numbers and can reproduce in a tomato crop; these species did also not seriously damage tomato plants, flowers or fruits as a result of plant feeding. The species *E. varians* did not establish in any of the tests.

Keywords: Predator release rate, tomato borer, Heteroptera, biological control, mirid predators.

Population dynamics of the predators *Campyloneuropsis infumatus*, *Engytatus varians* and *Macrolophus basicornis* (Hem.: Miridae) and their prey *Tuta absoluta* (Lep.: Gelechiidae) on tomato plants in greenhouse cages

Introduction

The tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is native to South America and is considered to be one of the most important pests in tomato crops (Guedes and Picanço, 2012). This pest was introduced in Spain in 2006 and since then invaded almost all continents (CABI 2015), attacking leaves, flowers, stems and fruits and in absence of control strategies causing high economic losses (up to 100%

crop losses) (Estay, 2000; Desneux et al., 2011; Terzidis et al., 2013; Urbaneja-Bernat et a., 2015).

In South America this pest is controlled by chemical pesticides (Siqueira et al., 2001; Zappala et al., 2011). This approach not only increases the production costs of tomato, but also results in the development of pesticide resistance and elimination of natural enemies. Thus, there are good reasons for searching for novel control of *T. absoluta*, including biological control with predatory mirid bugs, which are of in increasing interest in augmentative biological control of this pest (van Lenteren 2012).

Mirid predators are zoophytophagous, feeding both on plant and prey, and they can be used against pests in greenhouse vegetable crops through augmentative biological control (Castané et al., 2004; Calvo et al., 2012; van Lenteren, 2012; Sanchez et al., 2014). Two mirid bugs, *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) (Heteroptera: Miridae), are commercially used to control *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Tuta absoluta* in Europe (Sanchez and Lacasa, 2008; Mollá et al., 2011; Calvo et al., 2012b, van Lenteren, 2012). Based in this success, various other mirid species are being evaluated, as they can be associate with several preys, as aphids (Messelink et al., 2015), thrips (Gabarra et al., 1995; Riudavets and Castané, 1998), spider mites (Hansen et al., 1999) and eggs and larvae of lepidopteran species (Calvo et al., 2012a; Sanchez et al., 2014).

In Brazil, *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal) (Hemiptera: Miridae) might be potential natural enemies of *T. absoluta* (Bueno et al., 2013). However, no information is available concerning to the population dynamics of these predators when released against *T. absoluta*. It is already known that these mirid species are able to walk on the leaflets and stems with sticky hairs, can reproduce on tomato plants and consume a lot of *T. absoluta* eggs, which are all positive characteristics (Bueno et al 2013).

However, many other factors can influence the effectiveness of biological control agents, and timing, number and method of releases, synchrony with the pests, environmental conditions are important ones affecting the success of a biological control program (DeBach, 1964; Lester et al., 1999; Collier and van Steenwyk, 2004). In addition, knowledge of an adequate release rate of natural enemies is essential in augmentative biological control (van Lenteren, 2010). In Europe, the mirid *N. tenuis* is usually introduced three of four weeks after transplanting tomato seedlings, with a release rate of 1-2 individuals per plant and results in sufficient control of *T. absoluta* (Calvo et al., 2009). However, despite good pest control, *N. tenuis* causes damage in the form of necrotic rings to tomato plants if the predator population becomes large and insufficient prey is available. Thus, information about potential damage caused by mired predators is also important.

The rate of release of two *M. pygmaeus* adults per m^2 to control of *T. absoluta* were reported to be successful by Urbaneja et al. (2012).

Five tests were conducted in cages in a greenhouse with the objectives to determine the population dynamics of three species of mirid predators released for control of *T. absoluta*. Also the damage to tomato plants, flowers and fruits by *T. absoluta* in the presence of the predators, and the damage caused by mirids to the tomato plants were investigated. Control tests were also performed with introductions of only *T. absoluta* in the cages with tomato plants.

This study was done with as Null hypotheses (H_0), (1) Mirid predators reduce *T. absoluta* numbers and (2) there are significant differences in the predation capacity between three mirid predators species, (3) Mirid predators cause damage to tomato plants and (4) different release rates of mirid predators do result different levels of control of *T. absoluta*. The Alternative hypotheses were (H_1): (1) Mirid predators do not reduce *T. absoluta* numbers and (2) predation capacities the three mirid species on *T. absoluta* are similar (3) Mirid predators do not cause damage to tomato plants (4) different release rates of mirid predators do not result in different levels of control of *T. absoluta*.

Materials and Methods

Experimental greenhouse and tomato plants

The tests were conducted in an experimental greenhouse (20 m long x 5 m wide) located at Campus of Federal University of Lavras in the municipality of Lavras, Minas Gerais state, Brazil (21°14’43”S, 44°59’59”W and 919m latitude). In this greenhouse 4 cages of 3.8 m long x 2.5m wide x 2.5 m high were constructed of “anti-thrips” polyethylene (50 mesh) screen walls; and the cages could be accessed each by a separate door secured with a zipper (Figure 1). Each cages contained a metal table (2 x 1 m) (w x l) with two rows of six tomato plants (*Solanum* *lycopersicon* L, Santa Clara cv.), so a total of 12 plants/table/cage. These plants had an average height of 30 cm. Seeds of tomato cultivar Santa Clara were sown in polystyrene trays of 128 cells containing the commercial organic substrate Tropstrato® (composed by pinus bark, peat, expanded vermiculite, enriched with macro- and micronutrients). The seedlings were transplanted to 10cm plastic pots filled with 3 liters of organic substrate + soil (same composition as mentioned above?). The plants were fertilized and irrigated whenever necessary, and no pesticides were used.

Temperature and relative humidity inside the cages in the experimental greenhouse were monitored and measured hourly during all tests by a Datalogger (USB Datalogger, model KG 100) (Table 1).

Rearing insects

Predators: The mirids *Campyloneuropsis infumatus*, *Engytatus varians* and *Macrolophus basicornis* were reared using tobacco plants *Nicotiana tabacum* L. cv. TNN as substrate for oviposition, and eggs of *Anagasta kuehniella* (Zeller) as food (Bueno et al., 2013). Females and males from the 11th generation of the stock colony were used in the release tests.

Tuta absoluta: Adults of *T. absoluta* were collected in the field and taken to the laboratory. A stock colony was kept in plastic cages (60x30x30 cm). Four seedlings of tomato (*Solanum lycopersicum* L., cv Santa Clara) placed in plastic jar (140 mL) and replaced twice a week were used as oviposition substrate for *T. absoluta* females. After oviposition these seedlings were placed in a new cage (60x30x30 cm) until eggs hatched. Then, a piece of moistened foam (phenolic resin used in floriculture) with tomato leaves was introduced into the cage in order to serve as food to the larvae. The leaves were inserted in the phenolic foam to avoid wilting and whenever necessary new leaves were offered until the emergence of adults. After emergence of adults, new seedlings of tomato were introduced into the cage for oviposition and thus, the cycle was continued, resulting in a stock colony of *T. absoluta*. Adults (male and female) from the 11th generation of this stock colony were used in the release tests.

Predator release experiment

A complete randomized block design with five tests with release of predators and *T. absoluta* over time and four treatments were evaluated: 1) *T. absoluta* (control); 2) *T. absoluta* + *C. infumatus*; 3) *T. absoluta* + *E. varians*; 4) *T. absoluta* + *M. basicornis* (Table 2). The numbers of *T. absoluta* (control) and mirid predators on the tomato plants in the four cages in the experimental greenhouse were counted during 9 weeks (Table 2). At the start of the tests the tomato plants were approximately 30 cm height (15 days after transplanting). In the first weeks of each test *T. absoluta* (control) or *T. absoluta* + mirid species + *A. kuehniella* eggs (food to the predators) were introduced in the cages (Table 2). In the 1st and 2nd tests, 1 couple of *T. absoluta/*plant was released in week 1 in all cages (labeled as week 1, the week corresponding to 5 days before the first counting), week 2 (labeled as week2, the week corresponding to 1 day after first evaluation) and week 3 (labeled as week 3, the week corresponding to 1 day after the second evaluation). The release rates of *C. infumatus*, *E. varians* and *M. basicornis* were: 1 couple of predators/plant in week 1 plus *A. kuehniella* eggs. In the 3rd, 4th and 5th tests, the procedure of release was the same as in the 1st and 2nd tests, but instead of 1, we released 2 couples of predators/plant. The predators and *T. absoluta* were placed separated into a glass pot (1.7 L) sealed with plastic wrap in the center of the table with tomato plants. Next, the glass pot was opened allowing insects to fly to tomato plants. Eggs of *A. kuehniella* (0.01g) were sprinkled in adhesive tape and placed on four tomato plants (in two plants in each line placed on the table; i. e. plant 2 and plant 4) in each cage at the same time of the release of the mirids in week 1, week 2 and week 3. To reduce the risk of accidental contamination, we always first counted insects in the control cage with *T. absoluta* (control) and after that the cages with *T. absoluta* + the mirid predators.

Sampling of *T. absoluta* and mirid predators

Five tests were conducted each during 9 weeks (Table 2). The order of the treatments per cage in each test was reversed in five different periods (Table 2). In all tests and treatments, five tomato plants from each cage were randomly selected each week and on these plants the number adults and nymphs of the predators and the number of *T. absoluta* larvae on five leaves (three leaves from the upper, one from the middle and one from the bottom third of the plant) were counted. Also, the numbers of *T. absoluta* eggs were counted on five leaves selected form the upper third of five selected plants. Flowers of five plants were inspected and classified as damaged or not by *T. absoluta*, weekly. Ten leaves of each plant the degree of mining caused by *T absoluta* larvae were recorded too in each week. Finally, fruits of all plants were collected, counted, weighed and classified as damaged or not by *T. absoluta* in the end of experiments. The damage in leaves, flowers and fruits were rated as 0, 1, 2, 3, 4, or 5 when the mined area was 0, 1–25%, 26–50%, 51–75%, 76–99%, or 100% of the leaf surface, respectively, according to methodology proposed by Calvo et al., (2012).

Data analysis

The effects of treatments in each test on the number of *T. absoluta* eggs and larvae/leaf, number of *C. infumatus* and *M. basicornis* per leaf, and percentage of flower abortion were analyzed using a linear mixed effects model with repeated measures with time (weeks) as a random factor nested in blocks to correct for pseudo replication for repeated measures (Crawley, 2002). Linear mixed effects models are extensions of linear regression models for data that are collected and summarized in groups. These models describe the relationship between a response variable and independent variables, with coefficients that can vary with respect to one or more grouping variables. A mixed effects model consists of two parts, fixed effects and random effects. Fixed effects terms are usually the conventional linear regression part, and the random effects are associated with individual experimental units drawn at random from a population. The random effects have prior distributions whereas fixed effects do not. Mixed-effects models can represent the covariance structure related to the grouping of data by associating the common random effects to observations that have the same level of a grouping variable (Crawley, 2002). Treatments were compared through model simplification by combining treatments (Crawley, 2002). These models were analyzed with R software with the functions included in the packages *nlme* and *lme4* (R Development Core Team, 2015). The percentage of flower abortion and attacked fruits were arcsin $\sqrt{}$ x + 1 transformed (for proportion data) prior to analysis to normalize the data. The number of *T. absoluta* eggs and larvae/leaf, number of *C. infumatus* and *M. basicornis* were log (x + 1) (for data near zero) prior to analysis to normalize the data. The weight of fruits presented a normal distribution (Shapiro-Wilk test – $P \geq 0.05$). Percentage of attacked fruits by *T. absoluta* and weight of fruits was compared among treatments using a one-way ANOVA and Tukey's test for mean separation ($p \leq 0.05$) (R Development Core Team, 2015). Untransformed values are given in tables and figures.

Results

***Tuta absoluta* eggs/leaf**

First test: The numbers of *T. absoluta* eggs/leaf were lowest at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{3,135} = 3.457$, $p<0.001$), and no significant differences were observed for the test with *C. infumatus* and *M. basicornis* ($F_{1,71}$ = 5.139, p = 0.123). The number of *T. absoluta* eggs/leaf in the treatment with *E. varians* + *T. absoluta* was not significantly different from that in treatment *T. absoluta* (control) ($F_{1,71}$ = 0.824, p = 0.234). The maximum number of *T. absoluta* eggs/leaf reached 35 and 34 eggs in the 5th week at treatments with *T. absoluta* and *E. varians* + *T. absoluta*, respectively (Figure 1A).

Second test: The number of *T. absoluta* eggs/leaf were lowest at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* (maximum 20 eggs and 19 eggs, respectively) ($F_{3,144}$ = 12.133, p = 0.002) and no significant differences between treatments with these species ($F_{1,74}$ = 10.325, p = 0.255). *E. varians* + *T. absoluta* was not significantly different from *T. absoluta* ($F_{1,74}$ = 7.663, p=0.081). In the 3rd week the numbers of eggs at treatment T. absoluta were 46 eggs/leaf. At treatment *E. varians* + *T. absoluta* was observed a peak of oviposition of *T. absoluta* (32 eggs/leaf) in the 5th week. At treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* the number of eggs of *T. absoluta* were low (less than 20 eggs/leaf) in all weeks of evaluations (Figure 1B).

Third test: At treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta*, the number of *T. absoluta* eggs/leaf were lowest (around 10 to 15 eggs/leaf) ($F_{3,154}$ = 7.665, $p<0.001$), and no significant differences between both treatments with these two released species ($F_{1,81}$ = 14.726, p = 0.144). *E. varians* + *T. absoluta* was not significantly different at treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{1,81}$ = 2.887, p = 0.278). The numbers of eggs of *T. absoluta* were approximately 50 eggs in 3rd week (Figure 2A).

Fourth test: The number of *T. absoluta* eggs per leaf were highest at treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{1,167}$ = 13.852, p = 0.013), and reached a peak (more than 40 eggs) during the 3rd week after releasing.

No significant difference in the number of eggs of *T. absoluta* eggs/leaf at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* were found ($F_{1,84}$ = 4.622, p = 0.355). And, the treatment *E. varians* + *T. absoluta* was not significantly different of *T. absoluta* ($F_{1,84}$ = 6.447, p = 0.114) (Figure 2B).

Fifth test: The number of *T. absoluta* eggs/ leaf was lower and similar at both treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{3,121}$ = 3.177 p = 0.025). Both treatments *T. absoluta* and *E. varians* + *T. absoluta* were not significantly different ($F_{1,69}$ = 14.437, p = 0.210). The number of eggs of *T. absoluta* reached a peak of 30 eggs in the 5th week at treatments *T. absoluta* and *E. varians* + *T. absoluta* (Figure 3).

***Tuta absoluta* larvae/leaf**

First test: The number of *T. absoluta* larvae increased from 1st week in all treatments. No differences were found between treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{1,71}$ = 18.631, p = 0.133). In the 5th week 18 larvae/leaf were found in the *T. absoluta* treatment and 15 larvae/leaf at treatment *E. varians* + *T. absoluta*. No differences were found in the number of *T. absoluta* larvae/leaf in treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{1,71}$ = 21.091, p = 0.315) and after the 4th week, the number of *T. absoluta* larvae decreased and then stabilized at less than 10 larvae/leaf (Figure 4A).

Second test: *T. absoluta* larvae increased from 4th week at treatments *T. absoluta* and *E. varians* + *T. absoluta*, and no differences between these two treatments ($F_{1,74}$ = 12.521, p = 0.112) were found. The number of larvae/leaf reached 26 in the 6th week at treatment *T. absoluta* and 24 at treatment *E. varians* + *T. absoluta*. No differences in the number of *T. absoluta* larvae/leaf at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{1,74}$ = 11.447, p = 0.230); and after 7th week a decrease of the number of *T. absoluta* larvae (less than 10 larvae/leaf) was observed (Figure 4B).

Third test: No differences between treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{1,81}$ = 6.187, p = 0.224). The number of *T. absoluta* larvae increased from 2nd to 6th week. Peaks of larvae/leaf at treatment *T. absoluta* (control) (32 larvae/leaf) and 29 larvae/leaf at treatment *E. varians* + *T. absoluta* occurred in the 6th week. No difference in number of *T. absoluta* larvae/leaf at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{1,81}$ = 8.954, p = 0.119). After the 2nd week there was an increase and after the 5th week a decrease in the number of *T. absoluta* larvae (less than 10 larvae/leaf) (Figure 5A).

Fourth test: The number of *T. absoluta* larvae increased from 3rd week at treatments *T. absoluta* and *E. varians* + *T. absoluta* and no difference between treatments ($F_{1,84}$ = 3.169, p = 0.158) were found. During the 6th week the number of larvae reached a peak of 34 larvae/leaf at treatment *T. absoluta* and 32 larvae/leaf at treatment *E. varians* + *T. absoluta*. No difference in the number of *T. absoluta* larvae/leaf at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{1,84}$ = 17.033, p = 0.204) and after the 5th week the number of *T. absoluta* larvae (less than 10 larvae/leaf) decreased (Figure 5B).

Fifth test: The number of *T. absoluta* larvae increased from 4nd week to 7th week. No differences between treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{1,69}$ = 5.062, p = 0.112). A peak of 32 larvae/leaf at treatment *T. absoluta* and 31 larvae/leaf at *E. varians* + *T. absoluta* were found in the 7th week. No differences in the number of *T. absoluta* larvae/leaf at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* (around 10 larvae/leaf) ($F_{1,69}$ = 7.336, p = 0.320) (Figure 6).

Percentage leaf area attacked by *T. absoluta*

First test: The percentage of leaf area attacked by *T. absoluta* larvae was highest at treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{3,135}$ = 6.052, $p<0.001$). The leaf damage increased from 4th week and reached more than 80% in the 9th week for these two treatments. There was no difference at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{1,71}$ = 11.658, p = 0.072) and the damage caused by *T. absoluta* larvae was below 60% at the end of this test for these predators (9th week) (Figure 7A)

Second test: The percentage of leaf affected by *T. absoluta* larvae was highest at treatment *T. absoluta*; the same occurred at treatment *E. varians* + *T. absoluta*, and no significant differences between these two treatments were found ($F_{3,144}$ = 3.171, p = 0.248). The leaf damage increased over the week's evaluation and reached more than 80% in the

9th week at these treatments. There were not differences at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{1,74}$ = 8.914, p = 0.064) and the damage by *T. absoluta* larvae was below 60% in the 9th week (Figure 7B).

Third test: The percentage of leaf affected by *T. absoluta* larvae was highest at treatments *T. absoluta* and *E. varians* + *T. absoluta* than other two treatments ($F_{3,154}$ = 22.183, p = 0.005). The leaf damage increased from 2nd week and reached around 80% to 9th week for these treatments. The damage caused by *T. absoluta* larvae were not different at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{1,81}$ = 3.668, p = 0.201) and damages were below 50% in the end of the test (Figure 8A).

Fourth test: The percentage of leaf affected by *T. absoluta* larvae was highest at treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{1,167}$ = 12.588, p = 0.425). The leaf damage increased from 2nd week and reached more than 80% to 9th week at these treatments. The damage caused by *T. absoluta* larvae at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* were below 60% in the 9th week and were not significantly different ($F_{1,84}$ = 15.894, p = 0.227) (Figure 8B).

Fifth test: The percentage of leaf attacked by *T. absoluta* larvae were highest at treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{3,121}$ = 5.646, $p<0.001$). The leaf damage increased from 2nd week and reached approximately 60% to 9th week. No difference at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* ($F_{1,69}$ = 11.128, p = 0.087), and the damage by *T. absoluta* larvae was below 40% in the end of test (9th week) (Figure 9).

Miridae/leaf

No damage were caused by *C. infumatus, E. varians and M. basicornis* on tomato leaves, stems, flowers and fruits at the five tests. The species *E. varians* remained in plants only in the 1st week after released and did not reproduce in the conditions evaluated in all five tests.

In the 1st test, the number of *C. infumatus* and *M. basicornis* (adults + nymphs) per leaf increased from 1st week to 8th week, with a decrease in the 9th week. The number of *M. basicornis*/leaf was higher than *C. infumatus*/leaf ($F_{1,56}$ = 6.189, p = 0.002) (Figure 10A).

In the 2nd test, the number of *C. infumatus* and *M. basicornis* (adults + nymphs) per leaf increased from 1st week to 8th week, and after there were a lighting decrease in the 9th week. The number of *M. basicornis*/leaf (maximum 3.5 miridae per leaf) were higher than *C. infumatus*/leaf (maximum 2.6 miridae per leaf) ($F_{1,62}$ = 5.132, $p<0.001$) (Figure 10B).

In the 3rd test, there were an increase in the number of *C. infumatus* and *M. basicornis* (adults + nymphs) per leaf from 1st week to 6th week and after a small decrease in the 7th week. The number of *M. basicornis*/leaf was similar to *C. infumatus*/leaf ($F_{1,88}$ = 25.164, p = 0.265) (Figure 11A).

In the 4th test, the number of *C. infumatus* and *M. basicornis* (adults + nymphs) per leaf increased from 1st week to 6th week and after this, a small decrease in the 7th week were observed. The number of *M. basicornis*/leaf were similar to *C. infumatus*/leaf ($F_{1,91}$ = 13.959, p = 0.312) (Figure 11B).

In the 5th test, the number of *C. infumatus* and *M. basicornis* (adults + nymphs) per leaf increased from 1st week to 4th week and decreased in the 5th week. The number of *M. basicornis*/leaf were higher than *C. infumatus*/leaf ($F_{1,48} = 2.541$, $p<0.001$) (Figure 12).

Weight, damaged fruits, flowers abortion

First test: The tomato fruits were heaviest at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* (68.4 and 74.3g on average, respectively) ($F_{3,126} = 12.344$, $p<0.001$) (Table 3). The percentages of damaged fruits were highest (90%) ($F_{3,126} = 7.045$, $p = 0.015$) (Table 4), and also flower abortion were highest (~ 22%) ($F_{3,140} = 2.388$, $p = 0.003$) at the treatments *T. absoluta* and *E. varians* + *T. absoluta* (Table 5). At treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta,* the percentage of fruit attacked (62.1 and 60.2%, respectively) and abortion flowers were lowest (15.7 % and 14.3%, respectively).

Second test: The fruits originated from the plants in this test were heavier at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* (52.8 and 54.6g on average, respectively) ($F_{3,138} = 13.657$, $p<0.001$) (Table 3). However, in both treatments *T. absoluta* and *E. varians* + *T. absoluta* the percentage of damaged fruits were higher (approximately 90%) ($F_{3,138} = 4.166$, $p<0.001$) (Table 4). Also the percentage of flower abortion were higher in these two treatments ($F_{3,142} = 18.792$, $p = 0.004$) (Table 5). The percentages of abortion flowers were lower at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* (15.5% and 14.7%, respectively) (Table 5).

Third test: In this test, the fruits were heavier at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* (78.3 and 80.3g on average, respectively) ($F_{3,166}$ = 14.135, p = 0.007) (Table 3). The percentage of damaged fruits were higher at both treatments *T. absoluta* and *E. varians* + *T. absoluta* (around 50%) ($F_{3,166}$ = 17.236, p = 0.002) (Table 4). At treatments, *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* the percentage of abortion flowers were lower (14.8% and 13.9%, respectively) than at treatments *E. varians* + *T. absoluta* (20.2%) and *T. absoluta* (19.4%) ($F_{3,161}$ = 7.231, $p<0.001$) (Table 5).

Fourth test: The fruits were heavier at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* (75.6 and 77.8g on average, respectively) ($F_{3,151}$ = 8.017, $p<0.001$) (Table 3) compared to treatments *T. absoluta* and *E. varians* + *T. absoluta*. The percentage of damaged fruits were higher at treatments *T. absoluta* and *E. varians* + *T. absoluta* ($F_{1,151}$ = 16.328, p = 0.011) with a percentage of approximately 80% for both treatments (Table 4). The percentage of flower abortion were higher at treatments *E. varians* + *T. absoluta* and *T. absoluta* ($F_{3,132}$ = 9.589, $p<0.001$) (Table 5) than at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* (percentage of abortion flowers 14.3% and 14.1%, respectively).

Fifth test: At treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta* the fruits were heavier (7.8 and 8.9g on average, respectively) ($F_{3,17}$ = 1.239, p = 0.004) (Table 3). The percentage of damaged fruits were higher at treatments *T. absoluta* (control) and *E.*

varians + *T. absoluta* ($F_{3,17}$ = 3.681, p<0.001) (Table 4). The percentages of attacked fruits were 100% at both treatments. Also the percentage of flower abortion were higher at these both treatments (around 90%) ($F_{3,21}$ = 4.537, p = 0.016) (Table 5). The percentage of abortion flowers were lower (78.7% and 77.9%, respectively) at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta*.

Discussion

During all five tests the number of *T. absoluta* eggs increased during the first weeks after introduction of *T. absoluta* (control) and *E. varians* + *T. absoluta*, and in the end (9th week) *T. absoluta* completed its life cycle. However, at treatments *C. infumatus* + *T. absoluta* and *M. basicornis* + *T. absoluta,* the populations of *T. absoluta* were lower in all tests showing that these two predators can reduce *T. absoluta* populations.

Also was observed that the release rate influenced the action of these two predators during the tests, being the release rate of 2 couples per plant (tests 3-5) instead of 1 couple (tests 1-2) more adequate in reducing *T. absoluta* populations. According to Urbaneja et al. (2012, in tomato greenhouses in Spain, releases of *N. tenuis* (1-2 individuals/m^2) are usually conducted several weeks after transplanting. Calvo et al. (2012a) tested three different release rates (control 0), 0.5, 1.0 and 2.0 individuals per plant) of *N. tenuis* to control *B. tabaci* and found the increase in number of predators after these releases when 2 couple per plant were released and also higher suppression of pest. Avilla et al. (2004) found that the introduction of *M. pygmaeus* (1-2 individuals/m^2) in an experiment in greenhouse with tomato plants reduced the population of *T. absoluta* and damage of the plants. When *M. pygmaeus* was released in Sardinian greenhouses, the weekly release of two predators per m^2 resulted in control of *T. absoluta* (Nannini et al., 2012). In North-east Spain, one to two *M. pygmaeus* per m^2 are released in greenhouses when natural predator populations are low to control of *B. tabaci* (Urbaneja et al., 2012). However, in the case of the three mired predators, more studies are necessary in order to optimize an adequate release rate.

The specie *E. varians* did not establish and reproduce on tomato in any of the tests. *E. varians* did not remain on the tomato plants after release, but flew away to (e.g. to the ceiling) in all five tests. It was unclear why *E. varians* was not going to the plants and consumes *T. absoluta* eggs, because under laboratory conditions this species consumes high numbers of *T. absoluta* eggs and it is able to reproduce on tomato (Bueno et al., 2013). But this could be a characteristic of this species, as show to be very active and mobile inside the rearing cages and in the plants at field conditions.

Although the three mirid predators are known to be zoophytophagous, we found no evidence that *C. infumatus*, and *M. basicornis* caused damage on tomato plants. For *E.varians* it was difficult to conclude if it causes damage, as this species spent little time on tomato plants. According to Castané et al. (2011) and Calvo et al. (2012a, b) *N. tenuis* cause necrotic rings (NR) in the stems and punctures in the leaves of tomato, which initially start as a depression and whitening of affected areas, before swelling and browning. Hemipteran insects show extra oral digestion by injecting saliva with digestive enzymes and lacerate tissues with stylets to liquefy solid nutrients before ingestion (Cohen, 1995; Sanchez et al., 2015).

It is probable in this study that the addition of *A. kuehniella* eggs as food to the predators could assist also in their establishment and reproduction in the greenhouse after released against *T. absoluta*. However, probably this addition of food not appear to interfere in the predation rate of *C. infumatus* and *M. basicornis* on *T. absoluta* but improved their fecundity and longevity. Urbaneja et al., (2009) reported that the fecundity and longevity of *N. tenuis* increased when the predator were fed with *E. kuehniella* eggs. Calvo et al., (2012a,b) stated that the addition of *A. kuehniella* eggs in the first weeks of releasing predators is a normal procedure in practice, and not affecting the predation of *T. absoluta* eggs and larvae.

The difference in temperature and humidity in the greenhouse during the tests also affected the population dynamics of predators and *T. absoluta*. The predators perform best at a temperature interval of 24°C - 28°C (Montes 2013), and *T. absoluta* in the interval of 19°C – 23°C (Cuthbertson et al., 2013). The best results in this study were obtained during the 3rd and 4th tests, when the average temperature approached the optimal temperatures for the predators. Also during these test the release rate of 2 couples of mirid predators improved the reduction of *T. absoluta* numbers and a higher number of mirid predators were found. At high temperatures mirid predators have a high fecundity and longevity (Perdikis and Lykouressis, 2002; Mollá et al., 2014). Also the high temperatures and humidity favored the development and reproduction of *T. absoluta* but, nevertheless the species *C. infumatus* and *M. basicornis* were able to reduce *T. absoluta* numbers and leading to reduced damage in tomato plants.

The 5th test was done at high temperatures and humidities, which caused wilting of plants, abortion of flowers and few fruits after week 5. Still, the species *C. infumatus* and *M. basicornis* reduced *T. absoluta* numbers, which also resulted in lower damage to the tomato plants in comparison to the treatment with *T. absoluta* and *E. varians*.

Conclusion

The hypothesis tested in this study was confirmed. There are differences in predation capacity of mirid predators. The species *C. infumatus* and *M. basicornis* establish in tomato infested with *T. absoluta* and decrease the number of the pest. The species *E. varians* did not establish in tomato in cages in a greenhouse. The release rate of 2 couples of mirid predator/plant gave a better control result than the release of 1 couple/plant. The species *C. infumatus* and *M. basicornis* do not cause damage to tomato plants (Santa Clara cv.).

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Tables

Table 1: Maximum, minimum and average temperatures and humidities in each greenhouse cages during the release of three mirid predators and *T. absoluta*.

1° Test / Release	**Compart. 1**	**Compart. 2**	**Compart. 3**	**Compart. 4**
Maximum temperature (°C)	30.2	29.4	31.8	30.9
Minimum temperature (°C)	16.4	17.1	16.9	17.3
Average temperature (°C)	24.5	23.4	24.7	23.1
Maximum humidity (%)	71.3	70.4	72.7	71.8
Minimum humidity (%)	49.6	50.5	48.9	51.1
Average humidity (%)	70.1	69.7	71.2	70.7

2° Test / Release				
Maximum temperature (°C)	27.6	26.4	28.3	27.9
Minimum temperature (°C)	13.1	12.9	13.5	12.8
Average temperature (°C)	20.7	21.9	21.3	20.9
Maximum humidity (%)	64.8	66.3	65.9	65.2
Minimum humidity (%)	39.7	40.5	38.8	40.2
Average humidity (%)	63.8	64.7	65.6	64.9

3° Test / Release				
Maximum temperature (°C)	31.7	32.3	32.5	32.6
Minimum temperature (°C)	17.4	16.9	17.7	17.8
Average temperature (°C)	22.3	22.5	22.8	21.9

Maximum humidity (%)	72.4	73.1	72.9	73.6
Minimum humidity (%)	55.9	56.4	56.2	55.7
Average humidity (%)	70.8	69.7	71.3	71.6

4° Test / Release				
Maximum temperature (°C)	29.4	28.9	29.4	30.1
Minimum temperature (°C)	16.8	17.1	17.3	17.6
Average temperature (°C)	22.6	23.2	22.8	22.7
Maximum humidity (%)	75.7	76.4	76.3	75.9
Minimum humidity (%)	59.3	58.9	59.2	59.5
Average humidity (%)	72.8	73.4	72.9	73.2

5° Test / Release				
Maximum temperature (°C)	35.7	36.1	35.2	35.9
Minimum temperature (°C)	18.7	19.1	18.4	18.6
Average temperature (°C)	26.7	26.4	25.9	26.2
Maximum humidity (%)	88.4	87.9	88.1	87.8
Minimum humidity (%)	64.5	65.2	64.8	65.1
Average humidity (%)	80.7	81.3	81.5	80.8

Table 2: Overview of 5 tests of four treatments with release of three mirid predatory bugs and *Tuta absoluta* on tomato plants in an experimental greenhouse. Tabs = *Tuta absoluta*; Cinf = *Campyloneuropsis infumatus*; Evar = *Engytatus varians*; Mbas = *Macrolophus basicornis*.

Tests	**Compart. 1**	**Compart. 2**	**Compart. 3**	**Compart. 4**	**Period of evaluation**
1	Control	Mbas + Tabs	Evar + Tabs	Cinf + Tabs	2 April 2014 to 28 May 2014
2	Mbas + Tabs	Evar + Tabs	Cinf + Tabs	Control	25 June 2014 to 20 August 2014
3	Evar + Tabs	Cinf + Tabs	Control	Mbas + Tabs	10 September 2014 to 5 November 2014
4	Cinf + Tabs	Control	Mbas + Tabs	Evar + Tabs	8 December 2014 to 2 February 2015
5	Evar + Tabs	Mbas + Tabs	Cinf + Tabs	Control	18 February 2015 to 15 April 2015

Table 3: Weight of fruits (g) after release experiments in each treatment: 1) *T. absoluta* 2) *C. infumatus* + *T. absoluta*, 3) *E. varians* + *T. absoluta* and 4) *M. basicornis* + *T. absoluta* in greenhouse with tomato plants.

	Weight of fruits (g)							
Test	*T. absoluta*	n	*C. infumatus* + *T. absoluta*	n	*E. varians* + *T. absoluta*	n	*M. basicornis* + *T. absoluta*	n
1°	42.6 ± 0.94b[*]	64	68.4 ± 0.98a	95	44.7 ± 0.89b	70	74.3 ± 0.95a	98
2°	40.3 ± 0.54b	55	52.8 ± 0.66a	86	41.5 ± 0.72b	60	54.6 ± 0.78a	89
3°	51.5 ± 0.47b	84	78.3 ± 0.34a	102	53.4 ± 0.44b	81	80.3 ± 0.55a	107
4°	47.2 ± 0.36b	73	75.6 ± 0.58a	99	48.1 ± 0.77b	78	77.8 ± 0.61a	105
5°	5.9 ± 0.08b	6	7.8 ± 0.05a	8	5.8 ± 0.09b	5	8.9 ± 0.10a	10

[*] Means (± SE) followed by same lowercase letter in lines indicate non-significant differences for species in each test evaluated (Tukey's test ($P \leq 0.05$). n = number of fruits weighed.

Table 4: Damage fruits (%) after release experiments in each treatment: 1) *T. absoluta* 2) *C. infumatus* + *T. absoluta*, 3) *E. varians* + *T. absoluta* and 4) *M. basicornis* + *T. absoluta* in greenhouse with tomato plants.

	Damage fruits (%)							
Tests	*T. absoluta*	n	*C. infumatus* + *T. absoluta*	n	*E. varians* + *T. absoluta*	n	*M. basicornis* + *T. absoluta*	n
1°	91.5 ± 0.75b*	64	62.1 ± 0.88a	95	90.4 ± 0.89b	70	60.2 ± 0.49a	98
2°	89.8 ± 0.57b	55	58.4 ± 0.61a	86	88.5 ± 0.68b	60	55.9 ± 0.64a	89
3°	81.4 ± 0.82b	84	43.7 ± 0.76a	102	78.3 ± 0.77b	81	40.8 ± 0.74a	107
4°	88.9 ± 0.98b	73	47.3 ± 0.56a	99	81.6 ± 0.65b	78	45.4 ±0.52a	105
5°	100 ± 0.66b	6	83.7 ± 0.59a	8	100 ± 0.84b	5	81.3 ± 0.77a	10

*Means (± SE) followed by same lowercase letter in lines indicate non-significant differences for species in each test evaluated (Tukey's test (P≤0.05). n = number of fruits evaluated.

Table 5: Flower abortion by *T. absoluta* (%) after release experiments in each treatment: 1) *T. absoluta* 2) *C. infumatus* + *T. absoluta*, 3) *E. varians* + *T. absoluta* and 4) *M. basicornis* + *T. absoluta* in greenhouse with tomato plants.

	Flower abortion (%)							
Tests	*T. absoluta*	n	*C. infumatus* + *T. absoluta*	n	*E. varians* + *T. absoluta*	n	*M. basicornis* + *T. absoluta*	n
1°	22.4 ± 0.44b*	91	15.7 ± 0.43a	75	21.7 ± 0.32b	88	14.3 ±0.38a	78
2°	21.7 ± 0.56b	89	15.5 ± 0.41a	97	22.3 ± 0.44b	85	14.7 ± 0.32a	101
3°	19.4 ± 0.66b	84	14.8 ± 0.35a	99	20.2 ± 0.58b	80	13.9 ± 0.31a	102
4°	18.7 ± 0.72b	81	14.3 ± 0.49a	95	18.5 ± 0.66b	78	14.1 ± 0.29a	99
5°	90.2 ± 0.67b	49	78.7 ± 0.62a	38	89.4 ± 0.74b	45	77.9 ± 0.75a	42

*Means (± SE) followed by same letter in lines indicate non-significant differences for species in each test evaluated by model simplification by combining treatments (Crawley, 2002) ($P \leq 0.05$). n = number of flowers evaluated.

Figures

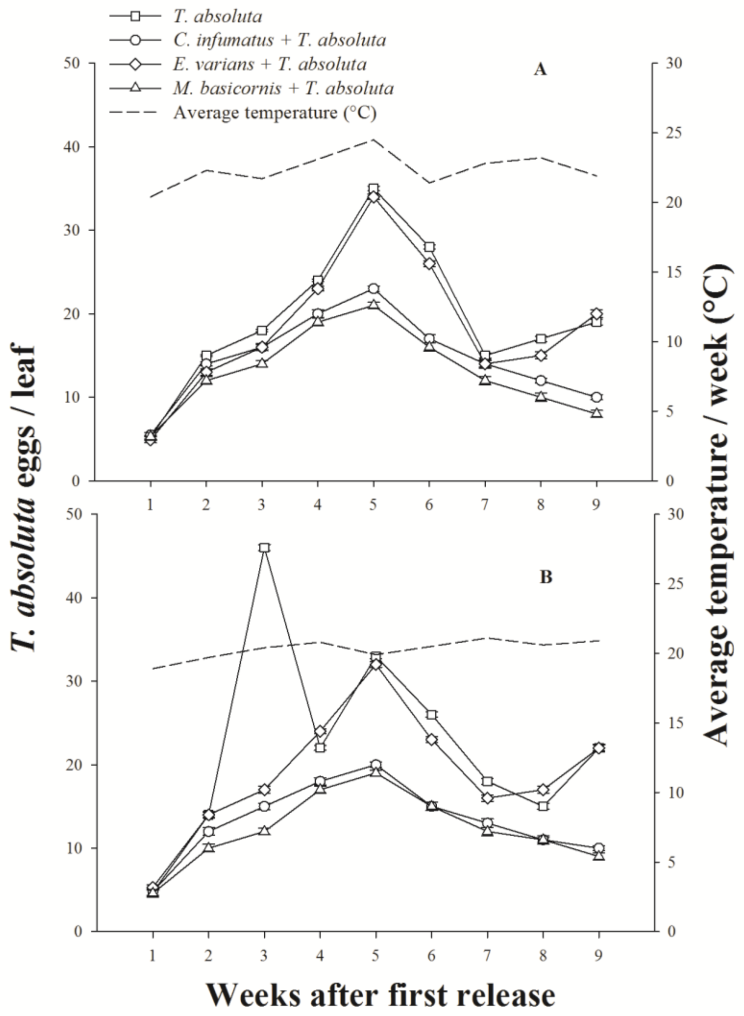

Figure 1: Mean (± SE) number of *Tuta absoluta* eggs per leaf in 1st (A) and 2nd (B) tests per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments (P≤0.05).

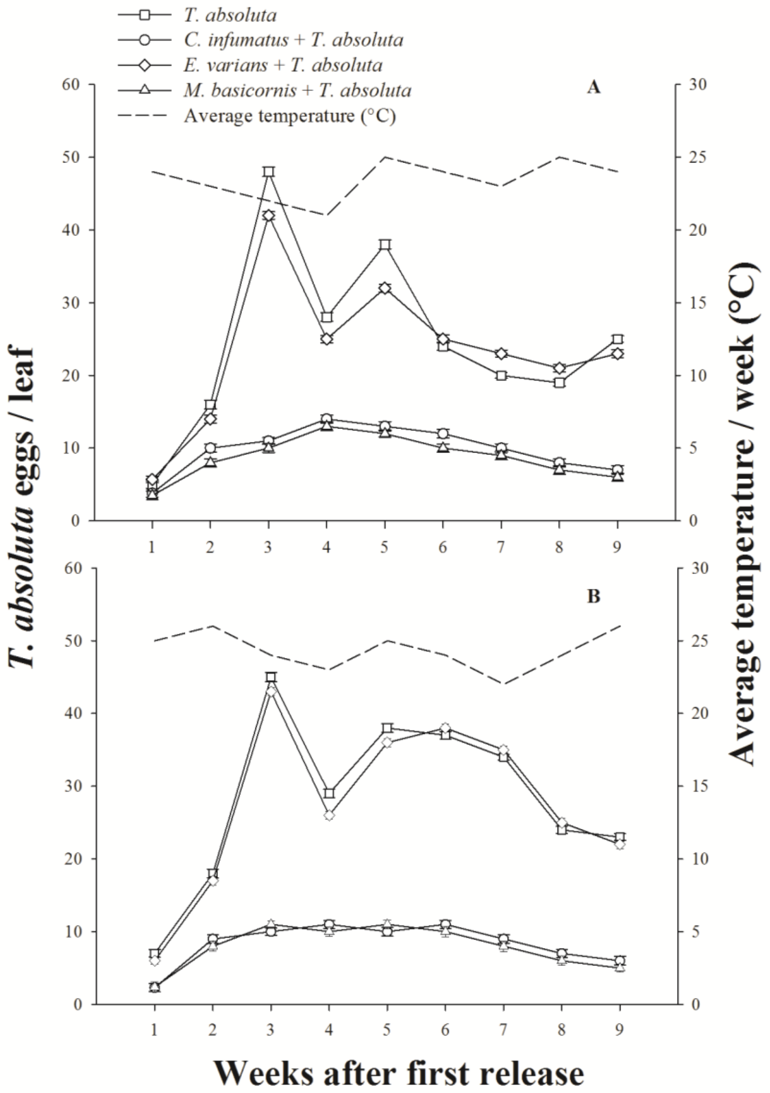

Figure 2: Mean (± SE) number of *Tuta absoluta* eggs per leaf in 3rd (A) and 4th (B) tests per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments (P≤0.05).

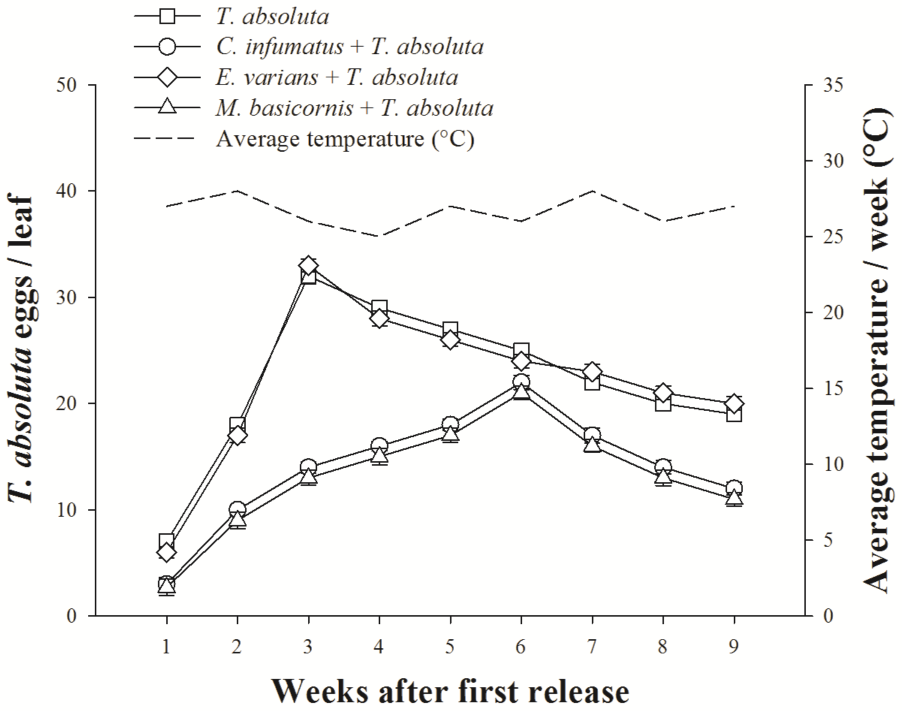

Figure 3: Mean (± SE) number of *Tuta absoluta* eggs per leaf in 5th test per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments (P≤0.05).

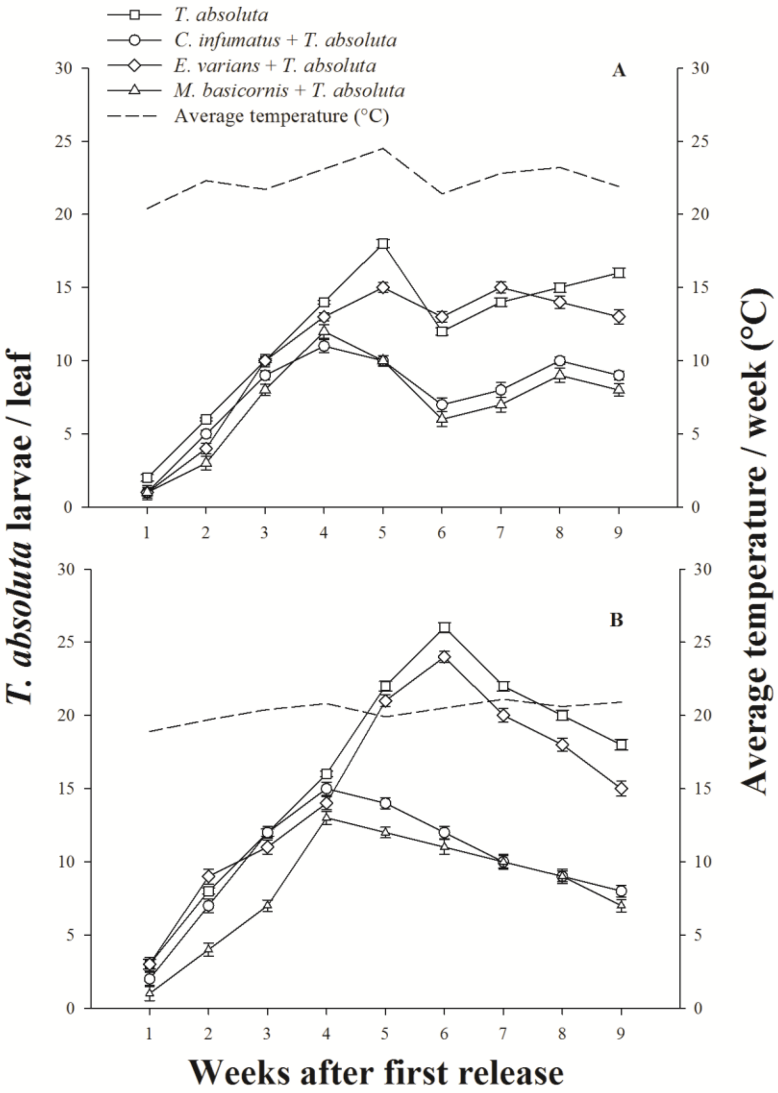

Figure 4: Mean (± SE) number of *Tuta absoluta* larvae per leaf in 1st (A) and 2nd (B) tests per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments ($P \leq 0.05$).

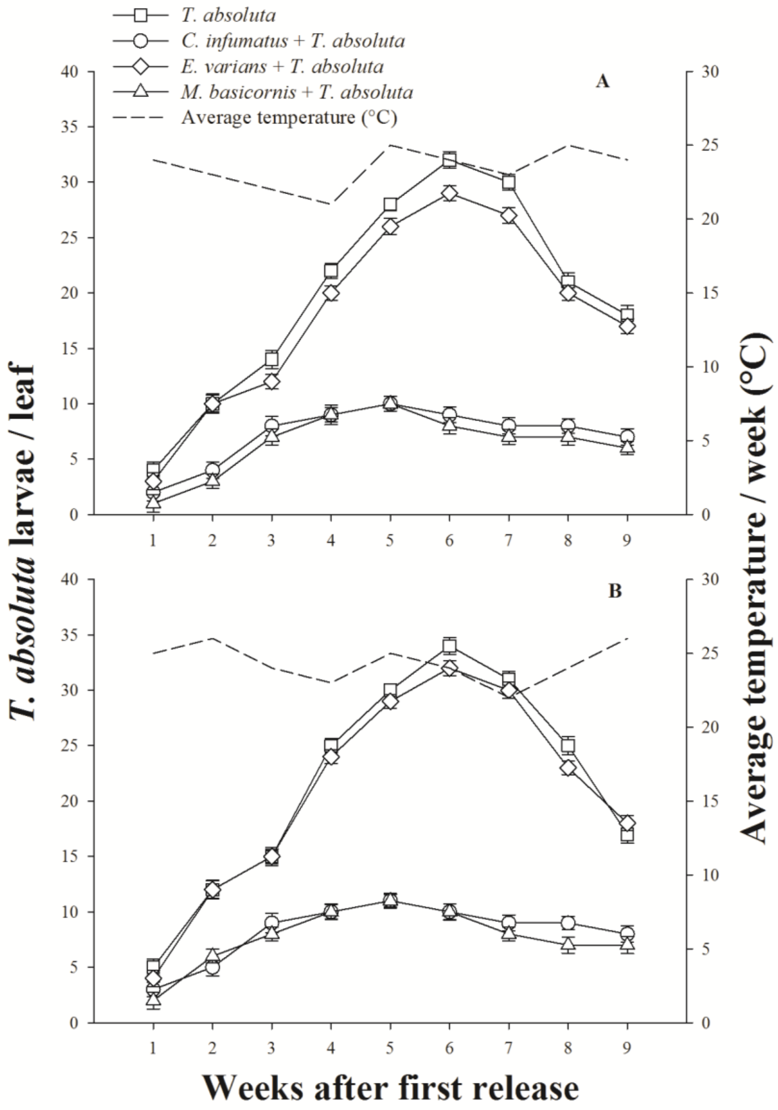

Figure 5: Mean (± SE) number of *Tuta absoluta* larvae per leaf in 3rd (A) and 4th (B) tests per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments (P≤0.05).

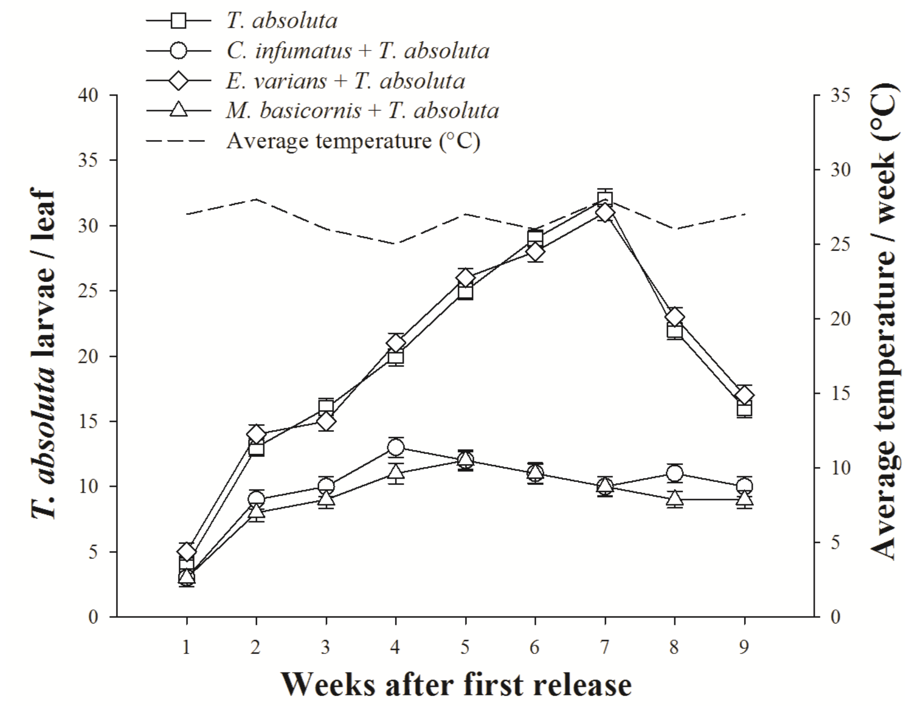

Figure 6: Mean (± SE) number of *Tuta absoluta* larvae per leaf in 5th test per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments (P≤0.05).

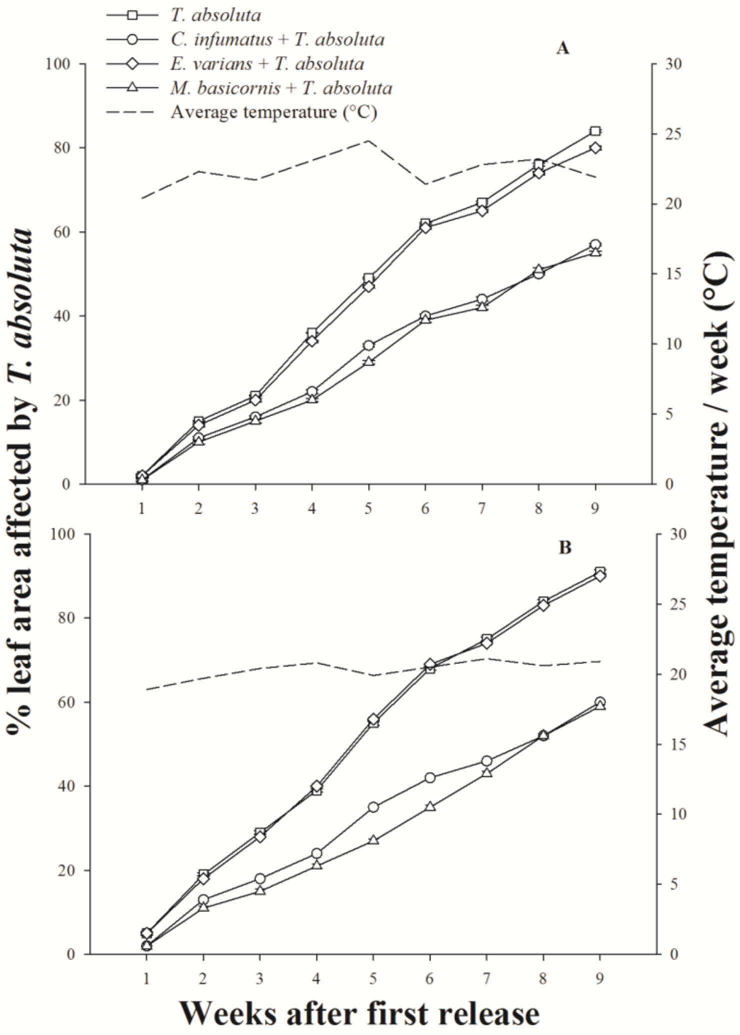

Figure 7: Mean (± SE) percentage of leaf area affected by *Tuta absoluta* in 1st (A) and 2nd (B) tests per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments (P≤0.05).

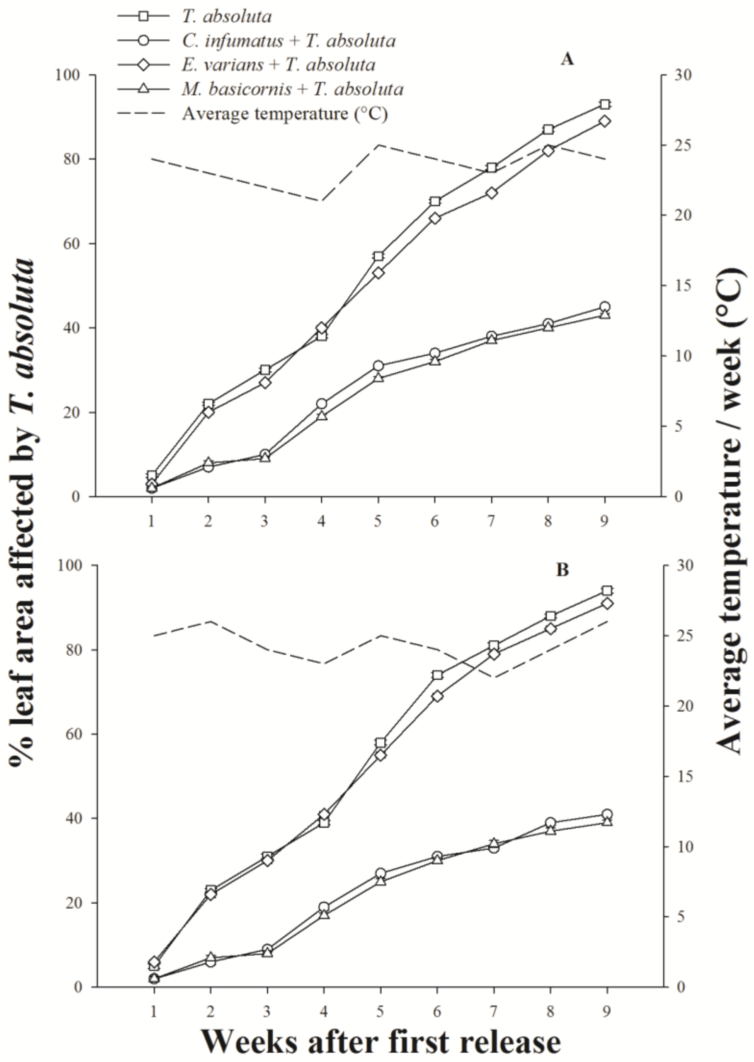

Figure 8: Mean (± SE) percentage of leaf area affected by *Tuta absoluta* in 3rd (A) and 4th (B) tests per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments (P≤0.05).

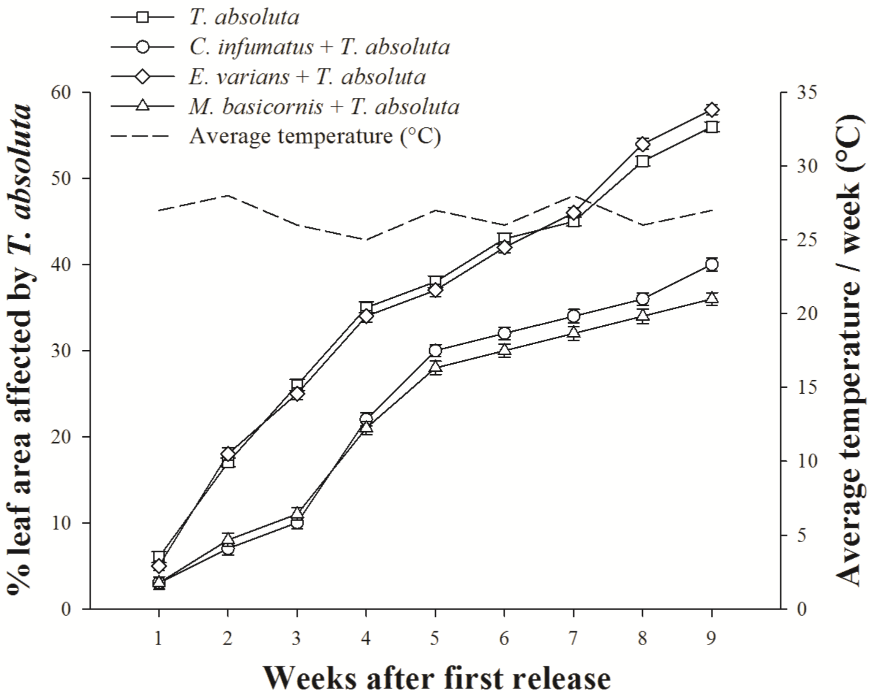

Figure 9: Mean (± SE) percentage of leaf area affected by *Tuta absoluta* in 5th test per week in each treatment: *T. absoluta* (Control), *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta*. Treatments were compared using model simplification by combining treatments (P≤0.05).

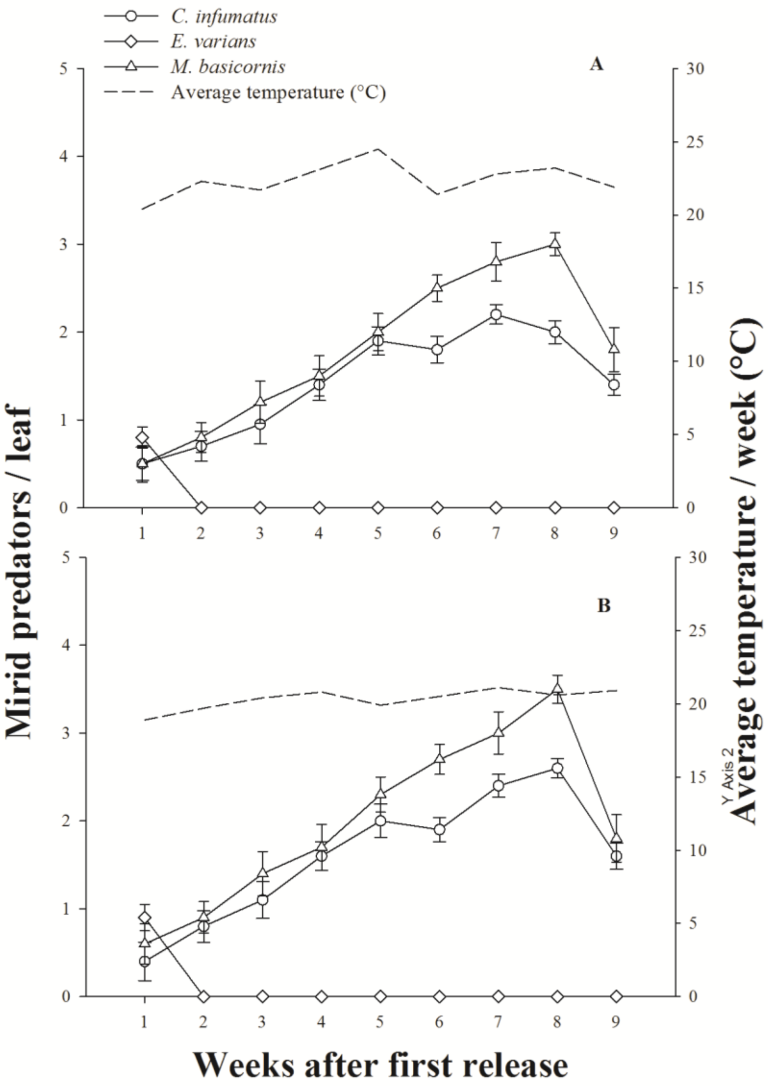

Figure 10: Mean (± SE) number of mirid predators per leaf in 1st (A) and 2nd (B) tests per week in each treatment: *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta* in 3rd Test. Treatments were compared using model simplification by combining treatments (P≤0.05).

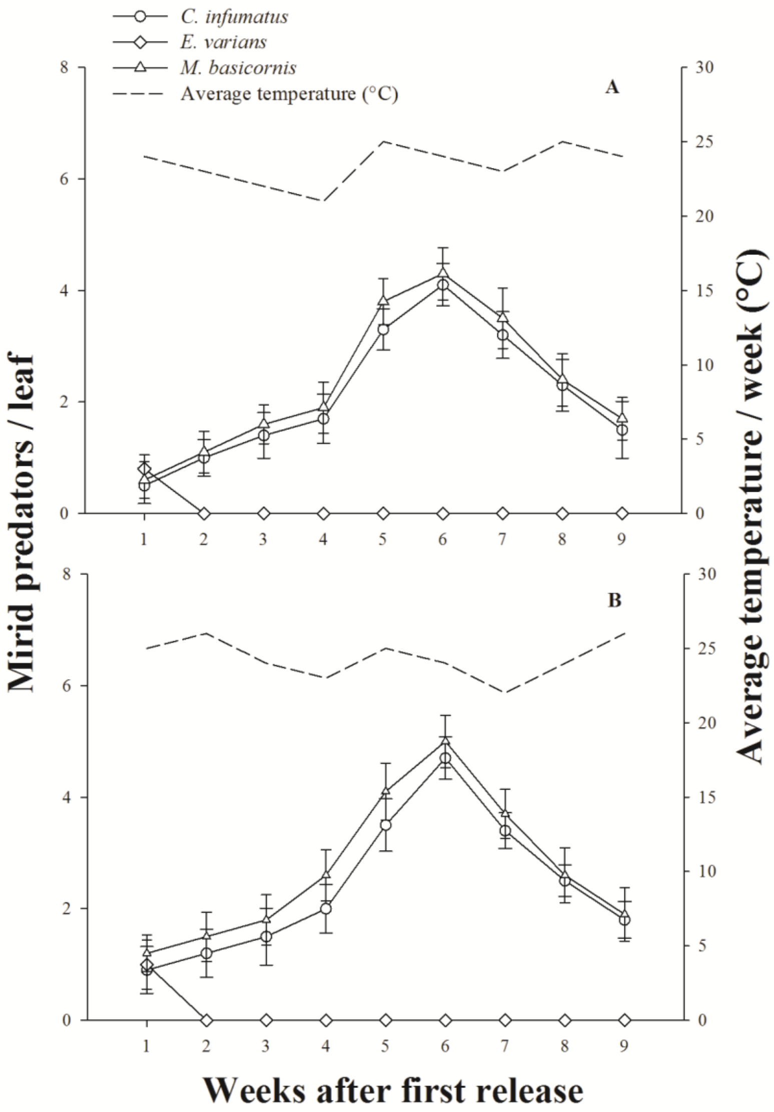

Figure 11: Mean (± SE) number of mirid predators per leaf in 3rd (A) and 4th (B) tests per week in each treatment: *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta* in 3rd Test. Treatments were compared using model simplification by combining treatments (P≤0.05).

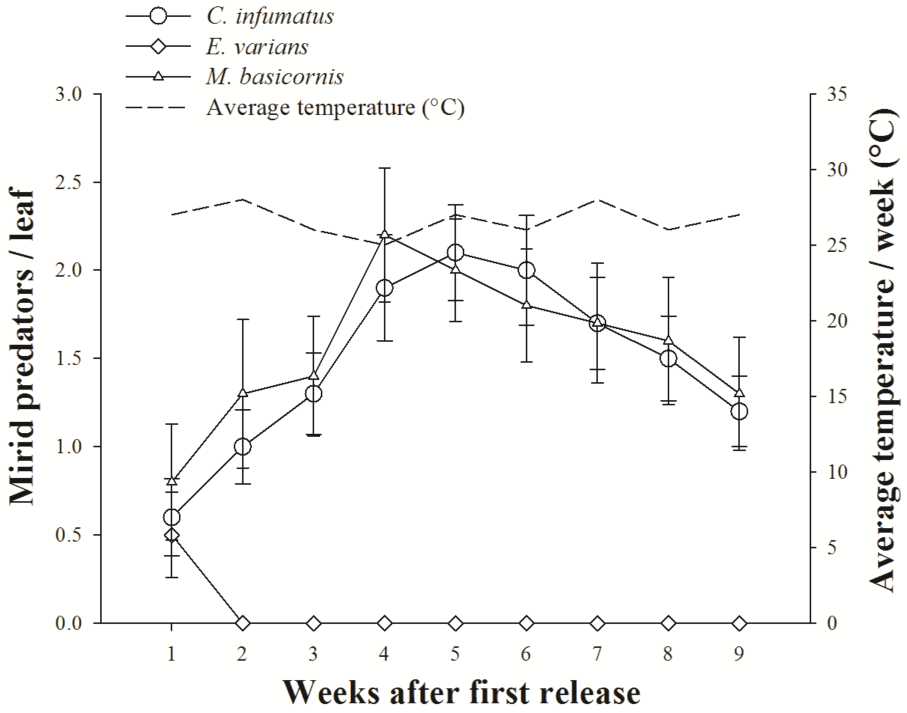

Figure 12: Mean (± SE) number of mirid predators per leaf in 5th test per week in each treatment: *C. infumatus* + *T. absoluta*, *E. varians* + *T. absoluta* and *M. basicornis* + *T. absoluta* in 3rd Test. Treatments were compared using model simplification by combining treatments (P≤0.05).