UNIVERSIDADE FEDERAL DE LAVRAS

JURACY CALDEIRA LINS JUNIOR

SEARCH CAPACITY, PREY PREFERENCE, PREDATION RATES AND REACTION TO PREY AND PREDATOR INDUCED VOLATILES OF PREDATORY MIRIDS OF TWO TOMATO PESTS, *Tuta absoluta* (Lep.: Gelechiidae) AND *Bemisia tabaci* (Hem.: Aleyrodidae)

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

Orientadora
Dr. Vanda Helena Paes Bueno

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(CAPACIDADE DE BUSCA, PREFERÊNCIA ALIMENTAR, TAXA DE PREDAÇÃO E REAÇÃO AOS VOLÁTEIS DE PLANTAS INDUZIDOS PELA PRESA E POR FITOFAGIA DE MIRÍDEOS PREDADORES DE DUAS PRAGAS DE TOMATE, *Tuta absoluta* (Lep.: Gelechiidae) E *Bemisia tabaci* (Hem.: Aleyrodidae))

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.

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LAVRAS – MG
2014

A Deus, princípio de toda sabedoria,
que está comigo em todos os momentos
dando-me forças para seguir firme na jornada da vida,
OFEREÇO

Aos meus pais, Juracy e Maria Celeste
e ao meu irmão Nilton,
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GENERAL ABSTRACT

The tomato borer *Tuta absoluta* (Meyrick) and the silverleaf whitefly *Bemisia tabaci* (Gennadius) are key pests of tomato and cause considerable losses in crops when control measures are not adopted. The use of pesticides is the main control strategy of both pests in Brazil, whereas in Europe the predatory mirid bugs *Macrolophus pygmaeus* Rambour and *Nesidiocoris tenuis* (Reuter) are now widely commercialized for control of whiteflies and the tomato borer. In Brazil, three predatory mirid bugs [*Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal)] were found to prey on *T. absoluta* and *B. tabaci* and are being evaluated as biological control agents of tomato pests. In this work we focused in evaluate the effects of glandular trichomes on tomato plants on activities and prey searching capacity of these three Brazilian mirid predators. Next, their preferences for the preys *T. absoluta* and *B. tabaci*, as well as their functional response towards *T. absoluta* eggs were evaluated. Finally, the behavioural responses of naïve and experienced *N. tenuis* and *M. pygmaeus* to uninfested plants, to herbivore-induced plant volatiles from plants infested with various stages of *T. absoluta* and/or *B. tabaci*, to the natural and synthetic pheromone of *T. absoluta*, and to volatiles produced by plants damaged by the predators were investigated in Y-tube olfactometer experiments. Differences in density of glandular trichomes on two tomato lines have no negative effect on movement and prey searching efficiency of *C. infumatus, E. varians* and *M. basicornis* for *T. absoluta* eggs. When presented a choice between the two prey species, *M. basicornis* ate more *T. absoluta* eggs, *C. infumatus* ate more *B. tabaci* nymphs and *E. varians* equally accepted both prey. Predation rates at different egg densities of *T. absoluta* revealed that *C. infumatus* and *M. basicornis* exhibited type II functional response and *E. varians* exhibited type III functional response. Results of Y-tube experiments reveled that *N. tenuis* did not respond to volatiles produced by uninfested plants, whereas *M. pygmaeus* was attracted to them. Both predators were attracted to volatiles from prey-infested plants, and the first evidence that experience affects responses to pest infested plants by of *M. pygmaeus* was provided. Infestation of the same plant by *T. absoluta* and *B. tabaci* resulted in similar responses of both predators as those to plants infested by either herbivore singly. The two predators did not respond to sex pheromones of *T. absoluta. Macrolophus pygmaeus* avoided plants damaged by conspecifics, while *N. tenuis* females were attracted by such plants.

Keywords: Biological control. *Campyloneuropsis infumatus*. *Engytatus varians*. *Macrolophus* spp. Miridae. *Nesidiocoris tenuis*. Tomato.

RESUMO GERAL

A traça-do-tomateiro *Tuta absoluta* (Meyrick) e a mosca-branca *Bemisia tabaci* (Gennadius) são pragas chave na cultura do tomate. O uso de inseticidas é a principal estratégia de controle dessas pragas no Brasil, enquanto que na Europa os mirídeos predadores *Macrolophus pygmaeus* Rambour e *Nesidiocoris tenuis* (Reuter) são comercializados para o controle biológico dessas pragas. No Brasil, foram recentemente encontrados três mirídeos [*Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal)] capazes de predar *T. absoluta* e *B. tabaci,* os quais estão sendo avaliados como agentes de controle biológico de pragas em tomate. Neste estudo foram avaliados os efeitos de tricomas glandulares de tomate em várias atividades e na capacidade de busca dos mirídeos predadores. Em seguida, foram determinadas as preferências alimentares pelas presas *T. absoluta* e *B. tabaci,* assim como as respostas funcionais de cada um dos predadores alimentados com ovos de *T. absoluta*. Também em experimentos com olfatômetro em Y, foram avaliadas as respostas comportamentais de *M. pygmaeus* e *N. tenuis* aos odores de plantas sadias, aos voláteis induzidos por herbivoria a partir de plantas infestadas com vários estágios de *T. absoluta* e/ou *B. tabaci*, ao feromônio natural e sintético de *T. absoluta*, e aos voláteis produzidos por plantas danificadas pelos predadores. Os resultados revelaram que diferenças na densidade de tricomas glandulares em duas linhagens de tomate não tiveram efeito negativo sobre a locomoção e a eficiência de busca dos predadores *C. infumatus, E. varians* e *M. basicornis* por ovos de *T. aboluta.* Nos testes de preferência, quando os predadores estiveram em contato com as duas presas (ovos de *T. absoluta* e ninfas de *B. tabaci*), *M. basicornis* consumiu mais ovos, *C. infumatus* consumiu mais ninfas e *E. varians* consumiu igualmente ambas as presas. Nos testes de predação a diferentes densidades de ovos de *T. absoluta, C. infumatus* e *M. basicornis* exibiram resposta funcional do tipo II. Já *E. varians* exibiu resposta do tipo III. Nos experimentos de olfatometria, *N. tenuis* não respondeu aos voláteis emitidos por plantas sadias, enquanto que *M. pygmaeus* foi atraído por tais voláteis. Ambos predadores foram atraídos pelos voláteis de plantas infestadas pelas presas, e a experiência prévia com a presa afetou as respostas olfativas de *M. pygmaeus*. As respostas dos predadores aos voláteis de plantas infestadas simultaneamente por *T. absoluta* e *B. tabaci* foram similares às respostas obtidas para plantas infestadas por cada herbívoro separadamente. Ademais, os dois predadores não respondem ao feromônio sexual de *T. absoluta*. *Macrolophus pygmaeus* evitou plantas danificadas pelos conspecíficos, enquanto que fêmeas de *N. tenuis* foram atraídas por tais plantas.

Palavras chave: Controle biológico. *Campyloneuropsis infumatus. Engytatus varians. Macrolophus* spp. Miridae. *Nesidiocoris tenuis*. Tomate.

SUMMARY

FIRST PART

GENERAL INTRODUCTION

The tomato *Solanum lycopersicum* L. is the most widely cultivated vegetable in the world, being produced both in the open fields or in greenhouses, either for fresh consumption or for processing (ALVARENGA, 2004). Brazil occupies a prominent place in world production of this vegetable with a cultivated area of 63.218 hectares and production of 4.017.106 ton (AGRIANUAL, 2013).

Tomato is a host plant for a large number of herbivorous insects and all its parts or structures provide food, shelter and micro-habitats for reproduction of various insects pests and their natural enemies (LANGE; BRONSON, 1981). The expansion of tomato crops in Brazil led to the development of various pests that cause severe crop damage, and among these the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most important problems (SOUZA; REIS, 2000). This insect can cause losses up to 100% if control methods are not adopted (GUEDES; PICANÇO, 2012). In Europe *T. absoluta* was accidentally imported and first detected in eastern Spain in 2006 and has since spread to several European, Middle Eastern, Africa North of the Sahel and Asia countries (DESNEUX et al., 2011; DESNEUX et al., 2010), where it has caused considerable yield losses. Another key pest on tomato is the silverleaf whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). This insect is considered one of the most serious threats to crop cultivation worldwide, and causes indirect losses to many cultivated plants, including tomato, by transmitting viruses, and directly causes plant physiological disorders and honeydew contamination that serves as substrate for sooty mould (BARRO et al., 2011).

The use of pesticides is the main control method against *T. absoluta* and *B. tabaci* in Brazil (HAJI et al., 2005; SOUZA; REIS, 2000). For *T. absoluta* control, for example, in a single 12-week production cycle, the crop can be sprayed with pesticides 4-6 times per week (GUEDES; PICANÇO, 2012) resulting in ineffectiveness of this control method by fast development of resistance to these chemicals (SIQUEIRA; GUEDES; PICANÇO, 2000). In addition, the indiscriminate use of pesticides may eliminate the natural enemies in the crop and also cause high pesticide residue levels on the harvested tomato fruits.

In Brazil there is no strategy of biological control for *B. tabaci* (HAJI et al., 2005) and for *T. absoluta*, currently, releases of the egg parasitoid *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) in limited scale can be done (PARRA; ZUCCHI, 2004). However, the controversial efficiency of this strategy in controlling the pest (GUEDES; PICANÇO, 2012) has stimulated the search for new potential biological control agents of *T. absoluta* (BUENO et al., 2013; BUENO et al., 2012). Indeed, in Brazil and other South American countries limited research has been done on predators as biological control agents of *T. absoluta* (DESNEUX et al., 2010; BUENO; LENTEREN, 2012) unlike Europe where the predatory bugs *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* Rambour (Hemiptera: Miridae) are frequently used in the integrated pest management of *T. absoluta* and whitefly species (CASTAÑÉ et al., 2004; SANCHEZ et al., 2009; URBANEJA et al., 2012).

In the State of Minas Gerais – Brazil, the mirid bugs *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal) were reported on *Petunia* sp. and tobacco *Nicotiana tabacum* L. (Solanaceae) (BUENO et al., 2013; BUENO et al., 2012; FERREIRA; SILVA; COELHO, 2001). These mirids are distributed throughout Latin America

(CARVALHO; FERREIRA, 1972; CASTINEIRAS, 1995; FERREIRA; SILVA; COELHO, 2001; FERREIRA; HENRY, 2011; MELO et al., 2004). *E. varians* is reported preying *B. tabaci* and *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) in several crops including tomato (CASTINEIRAS 1995; LUNA et al., 2006). According to Bueno et al. (2012) and Bueno et al. (2013) *C. infumatus, E. varians* and *M. basicornis* are potential natural enemies of *T. absoluta*. The three predator species are able to feed on eggs and larvae of the referred pest; they walk freely on tomato and use this plant as oviposition substrate. In addition, these predators are easily reared at laboratory conditions and have relatively short developmental time and low immature mortality when fed with lepidopteran eggs (MONTES, 2013). However, the successful use of these predators as biological control agents of tomato pests may depend of other criteria used for evaluation of natural enemies.

According to Lenteren and Woets (1988) in the preintroductory evaluation of natural enemies for biological control in greenhouses, the following criteria should be taken into account:

i. *Internal synchronization with the host/prey*. Natural enemy development should be synchronous with that of the pest organism to prevent cyclical outbreaks;
ii. *Climatic adaptation*. Tests should be performed to determine whether the natural enemies are able to develop, reproduce, and disperse in the climatic conditions under which they are to be used;
iii. *No negative effects*. The natural enemy should not attack other beneficial organisms in the same environment or nonpest organisms of importance or interest in the area where it is to be introduced. In addition, mirid predators are zoophytophagous insects, i.e. they feed on both prey and plant, so tests should be performed to

determine whether the plant feeding by these predators cause damage to the plant;

iv. *Good culture method*. Good method for mass production of natural enemies and the costs involved in this process are the basis for a successful biological control program;

v. *High kill-rate potential*. Efficient natural enemies should have a pest kill rate equal to or greater than the potential maximum rate of population increase (r_m) of the pest species;

vi. *Good searching efficiency*. The most difficult parameter to determine. Natural enemies should be able to locate and reduce pest populations before they have crossed economic threshold densities.

In 2011, following the criteria pointed out by Lenteren and Woets (1988), it was started, at Federal University of Lavras, a project of evaluation of the mirid predators *C. infumatus, E. varians* and *M. basicornis* as biological control agents of *T. absoluta* and other tomato pests (BUENO et al., 2013). The data presented here are envolving some criteria of evaluation of these three mirid predadors.

When evaluating natural enemies for biological control of pests in tomato, an important criterion to be evaluated is the ability of the predator/parasitoid in walking and search for the prey/host on hairy surfaces. Trichomes on tomato provide protection against herbivores, but may also have direct and indirect negative effects on natural enemies by irritating, trapping, poisoning them or acting as a barrier to movement or to nutritional tissue (SIMMONS; GURR, 2005). For example, tomato glandular trichomes caused entrapment and mortality of third instar nymphs of the predator *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). Indirectly, trichomes of tomato may also affect the performance of predators. Barbour, Farrar and Kennedy (1993) showed that exudates from glandular trichomes of tomato reduced the number of eggs of *Manduca sexta* (L.) (Lepidoptera: Sphingidae) consumed by *Geocoris punctipes* (Say) (Hempitera: Geocoridae).

A generalist predator that overcome the barrier of trichomes of tomato leaves may be faced with more than one prey species because tomato is a host plant for a variety of herbivorous insects (LANGE; BRONSON, 1981). Actually, the pests *T. absoluta* and *B. tabaci* often occur together in tomato (CALVO et al., 2009) and the use of a single species of natural enemy that feeds on these two pests reduces the complexity and costs of biological control (CALVO et al., 2012). Thus, knowledge of prey preference of a predator is important and the presence of various prey species on a host plant can obscure the interpretation of predation results in an experimental setting with a single prey species. Prey preference is defined as disproportionate attack rate on one prey species, when more than one species is available and on the premise that all species have a similar attack rate by the predator (HASSEL; SOUTHWOOD, 1978). Evaluating prey preferences of *M. pygmaeus,* Lykouressis, Perdikis and Gaspari (2007) found that the attack rate of the predator on nymphs of *Myzus persicae* (Sulzer) was higher than on nymphs of *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae). Therefore, it seems likely that *M. pygmaeus* would reduce *M. persicae* numbers more effectively than those of *M. euphorbiae*.

When prey preferences are known for a certain predator, it is also important to obtain information about its predation efficiency which is often expressed as the functional response and describes the relationship between prey density and the number of prey consumed by an individual predator (SOLOMON, 1949). Depending on the predator, the prey and climate conditions, the predation rate in relation to prey density may originate three basic types of response curves. According to Holling (1959) these curves can represent a linear increase (type I), a deceleration (type II), or a sigmoid relationship (type III). In areas with limited variation in the density of prey, it is expected to result in a density-independent prey mortality represented by a linear increase (type I), a density-dependent negative response, represented by a decreasing curve (type II) and positively density-dependent response corresponding to a temporary increase in predation rate (type III). The mirid predator *M. pygmaeus* exhibited type II response when challenged with varying densities of *M. persicae* (FOGLAR; MALAUSA; WAJNBERG, 1990). However, the same predator feeding on different densities of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) responded in a density dependent way and exhibited a typical type III functional response (ENKEGAARD; BRØDSGAARD; HANSEN, 2001). Thus, a predator that impose positively density dependence prey mortality (type III) are supposed to potentially manage the prey population and could be considered as efficient biocontrol agents (LENTEREN; BAKKER, 1975).

Knowing the orientation mechanisms used by predators in finding their prey is an extra step for evaluation/selection of biological control agents and plant volatiles produced in response to attack of herbivores play an important role in prey finding (DICKE; SABELIS, 1988). Herbivore-induced plant volatiles (HIPVs) may be used by predators as cue to locate their prey (DICKE, 1999; VET; DICKE, 1992). There is some, but rather limited, information about the response of mirid predators to HIPVs. It is known that they are attracted by prey-infested plants (INGEGNO et al., 2013; INGEGNO; PANSA; TAVELLA, 2011; MCGREGOR; GILLESPIE, 2004; MOAYERI et al., 2006a; MOAYERI et al., 2006b; MOAYERI et al., 2007; MOLLÁ-HERNÁNDEZ, 2013) but not to volatiles emitted directly by the prey (INGEGNO; PANSA; TAVELLA, 2011; MOAYERI et al., 2006a; MOAYERI et al., 2006b; MOLLÁ-HERNÁNDEZ, 2013). Dealing with complex information becomes even more complicated when predators show plant feeding behavior. *Macrolophus* *pygmaeus* plant feeding does usually not cause problems (CASTAÑÉ et al., 2011). Economic damage by *N. tenuis* to tomato frequently occurs (SANCHEZ; LACASA, 2008), can be observed as necrotic brown rings around the stems and shoots caused by repeated feeding at the same point (ARNÓ et al., 2010) and is inversely proportional to prey availability (SANCHEZ, 2009). Repeated feeding at the same location suggests that *N. tenuis*-damaged plants may produce volatiles which attract conspecific predators to necrotic rings.

In order to provide information about the possible use of the predatory mirid bugs *C. infumatus, E. varians* and *M. basicornis* recently found in Brazil as biological control agents of *T. absoluta* and *B. tabaci* in tomato, we studied the effect of glandular trichomes of tomato plants on various activities and prey search capacity of these predators. Next, the preference of these three mirids for *T. absoluta* and *B. tabaci,* as well as the functional response of the predators towards *T. asboluta* eggs were evaluated. Finally, we investigated the behavioral responses of *M. pygmaeus* and *N. tenuis* to uninfested plants, to herbivore-induced plant volatiles (HIPVs) from plants infested with various stages of *T. absoluta* and/or *B. tabaci*, to synthetic sex pheromone of *T. absoluta,* and to volatiles produced by plants damaged by predators.

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SECOND PART – ARTICLES

ARTICLE 1 Walking activity and prey searching behavior of three mirid predators of *Tuta absoluta* (Lep.: Gelechiidae) on tomato lines with different densities of glandular trichomes

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Abstract. Glandular trichomes on plants may provide protection against herbivores, but may also negatively affect the walking and search efficiency of natural enemies. We investigated the effects of glandular trichomes on tomato plants on various activities and prey searching capacity of three recently found Brazilian mirid predators of *Tuta absoluta* (Meyrick), *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal). The time spent by each predator on walking, grooming, probing and feeding activities as well as prey searching and handling time on *T. absoluta* eggs on leaflets and stems of the tomato breeding lines TOM 587 and TOM 687 were evaluated. TOM 687 had more glandular trichomes compared to TOM 584 on both leaflets and stems. However, this difference did not differently affect grooming, probing and feeding activities and the time spent on searching and handling for *T. absoluta* eggs of the three mirid predators. *E. varians* walked longer on TOM 687 All three predators could climb the stems of both tomato cultivars and find the eggs of *T. absoluta* on leaves in the top of the plants. Differences of more than a factor in two tomato hair density did not differently affect activities and prey searching efficiency of *C. infumatus*, *E. varians* and *M. basicornis*. Thus, we can conclude that the use of tomato plants with glandular trichomes will not reduce biological control of *T. absoluta* by these three mirid predators.

Keywords: Biological control; Miridae; Tomato borer; *Solanum licopersicon*

Walking activity and prey searching behavior of three mirid predators of *Tuta absoluta* (Lep.: Gelechiidae) on tomato lines with different densities of glandular trichomes

Introduction

Plants may defend themselves against herbivores via various mechanisms which include morphological traits such as plant architecture, thorns, trichomes, or surface waxes as well as chemical traits such as secondary plant metabolites (Bennett and Wallsgrove 1994; Levin 1973; Price et al. 1980). Trichomes are found on many plant species and can be glandular i.e., possessing a small membranous head containing toxins and/or adhesives, or non-glandular. Glandular trichomes can operate by irritating, trapping or poisoning arthropods and non-glandular trichomes can act as a barrier to movement or to nutritional tissue (Simmons and Gurr 2005). The secretion of glandular trichomes on leaves of plants from the genus *Lycopersicon* has been proven to be toxic to many insect pests (Kennedy 2003; Price et al. 1980; Simmons and Gurr 2005) suggesting that trichome-based resistance could be an effective tool in a pest management program against a wide variety of arthropod pests.

However, glandular trichomes may also be beneficial for herbivores by negatively affecting the performance of natural enemies on these surfaces (Price et al. 1980). But positive effects of trichomes on the performance of predators have been reported as well, e.g. by Styrsky et al. (2006), who demonstrated that predation rates of herbivores by *Solenopsis invicta* Buren (Hymenoptera: Formicidae) was greater on pubescent compared to glabrous soybean plants. Barbour et al. (1997) showed that there is no incompatibility of host-plant resistance with biological control provided by *Geocoris punctipes* (Say) (Hemiptera: Geocoridae) as in the field collections, the predator was found in higher numbers in trichome-based resistant tomato plants than in susceptible ones. However, reports of negative effects of trichomes and biological control are much more numerous. Glandular hairs on tomato plants are known to negatively affect survival (Barbour et al. 1993; Lambert 2007), movement (Coll et al. 1997; Economou et al. 2006; Lambert 2007), search capacity and prey consumption of heteropteran predators (Barbour et al. 1993; Coll and Ridgway 1995).

Negative effects of trichomes on the leaf surface of tomato on natural enemies are mostly caused by glandular trichomes types I, IV, VI and VII. Glandular trichomes types IV and VI have been associated with high levels of resistance to arthropods (Kennedy 2003). Type IV has a short, multicellular stalk on a monocellular base and produces a droplet of exudate at the tip. High densities of type IV and the presence of high levels of toxic acylsugars in their exudate may play a major role in the resistance of *Lycopersicon* spp. against whiteflies, aphids, leaf miners, spider mites and the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Hartman and St Clair 1999; Hawthorne et al. 1992; Maluf et al. 2010). Type VI trichomes, which have a four-celled glandular head on a short multicellular stalk and a monocellular base, also have been implicated in resistance of several *Lycopersicon* species to a number of arthropod pests (Kennedy 2003). If high densities of glandular trichomes on tomato plants reduce a predator’s walking activity, we can hypothesize that it will result in a lower encounter rate between predator and prey. In turn, this will negatively affect biological control of pests by the predators.

One of the main tomato pests is *T. absoluta*, a devastating pest of tomato in South America (Guedes and Picanço 2012) and invaded Europe, North Africa and Asia in the past decades causing very serious damages to tomato crops (Desneux et al. 2011).Its main control strategy in South America has been the use of chemical insecticides. But the extensive use of these chemicals has led to development of resistant pest populations (Guedes and Picanço 2012). Thus, alternatives for chemical pest control are essential for a sustainable production of tomato and one of these alternatives might be biological pest control.

In the Mediterranean basin (Europe), the predatory bugs *Macrolophus pygmaeus* Rambour (=*M. caligninosus*) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) are successfully used for control of *T. absoluta* (Desneux et al. 2010; Urbaneja et al. 2012) and other pests on tomato. However, until recently, limited research has been done on arthropod predators in South American countries (Bueno and van Lenteren 2012; Desneux et al. 2010). In Brazil, only a few species of predators are listed as natural enemies of *T. absoluta* (Bueno et al. 2013; Bueno et al. 2012; Miranda et al. 2005; Vivan et al. 2002). Of these, only the mirid bugs *C. infumatus, E. varians* and *M. basicornis* have very recently been partly evaluated as candidates for biological control of *T. absoluta* (Bueno et al. 2013; Bueno et al. 2012). They have relatively short developmental times and low immature mortalities (Montes 2013), high fecundities, high *Tuta*-egg predation rates, can use tomato as an oviposition substrate, can walk on tomato and can complete their development on this plant (Bueno et al. 2013; Bueno et al. 2012). Interestingly, mirid predators in general are reported to prefer to colonize pubescent plants rather than hairless plants (Voigt et al. 2007).

In this study, we examined the effect of glandular trichomes of two tomato lines, with contrasting hair density, levels of acylsugar contents and pest resistance, on walking, grooming, probing, feeding activities, as well as on prey searching and handling time for *T. absoluta* eggs of three predatory mirids, *C. infumatus, E. varians* and *M. basicornis.*

Material and methods

Plants and insects

Two pre-commercial breeding lines of *Solanun lycopersicon* L. were used in this experiment. The line TOM 687 was selected for high acylsugar content and has high density of glandular trichomes on the leaves (W. R. Maluf, 2014, personal communication). This line also is mentioned as resistant to *T. absoluta* (Maluf et al. 2010), and was the less preferred for oviposition by *T. absoluta* (Maciel et al. (2011). The line TOM 584 has lower number of hairs and low levels of acylsugars (Resende et al. 2006). Seeds of these two breeding lines supplied by HortiAgro Sementes Ltda were sown in trays. After twenty days, the seedlings were transferred to plastic pots (1.5 L) containing soil. Tomato plants of 45 to 60 days-old of each line were used in the experiments.

Adults of *T. absoluta* were collected in the field and a colony was kept in the laboratory inside cages (90x70x70 cm) on plants of susceptible tomato cv Santa Clara. Adults from this rearing maintenance were used in the experiments. The predators *C. infumatus, E. varians* and *M. basicornis* were collected on tobacco plants in RiberãoVermelho, Minas Gerais, Brazil (Montes 2013). These insects were reared according to the method of Agustí and Gabarra (2009) and Bueno et al. (2013) inside cages (60x30x30 cm) on tobacco plants cv TNN and fed with *Anagasta kuehniella* eggs. Female predators from the 7th laboratory generation were used in the experiments.

Quantification of trichomes and level of acylsugars on two tomato lines

Two completely expanded leaves in the middle portion of the plant, and a small piece of the stems on the apex were cut from each tomato line. The leaves and stems were fixed with alcohol 70% for 72 hours. Then, only the terminal leaflet of each leaf was used for counts of the trichomes. Paradermal cuts of the stems' epidermis and of the upper and lower epidermis of the leaflets were made, mounted and photographed under a stereoscopic microscope (100x magnification). The images were analyzed by Image Tool 3.0 software (Image

Tool for Windows, The University of Texas Health Science Center, San Antonio, TX, USA). The acylsugar level on the leaflets, which were collected on the apex of the plants, was determined by colorimetric method described by (Resende et al. 2002).

Predation rate

In a greenhouse, 45-60 days-old tomato plants from the two lines were kept inside iron cages (60 cm high, 30 cm in diameter) covered with organza tissue. Five early-emerged couples of *T. absoluta* were introduced into the cages and allowed to lay eggs on the plants for 72h. After this period, the leaves from the bottom and medium height of the plants were removed in order to have a better view of the walking activity of the predators. The number of eggs of *T. absoluta* on the apex was counted and then a single female up to 7 days-old of each predator was released on the bottom of the plant inside the cage. After 24h, the number of remaining eggs was counted in order to estimate the number of *T. absoluta* eggs consumed by one female of each predator. The predation rate was estimated by dividing the number of preyed eggs by the total time on search (24 h). Ten replicates for females of each predator and tomato lines were performed.

Time allocation of activities

The time allocation of activities of each predators species, such as grooming, walking, probing and feeding were observed based on the description of Economou et al. (2006) (Table 1). The time that each predator stayed immobile on the plant was also measured. Tests were conducted using Petri dishes (9 cm in diameter) as arena and in acclimatized room at 25±2°C, HR 70±10% and 12h photophase. Leaflets from each tomato line were placed with the abaxial surface upwards on a surface of water in Petri dishes. Then, a single female of up to 7 days-old of each predator species was released on the leaflet. The Petri dishes were sealed and the activities of the female predators were monitored by visual observation during 40 minutes with a use of chronometer. Ten replicates for each combination of mirid predator species and tomato lines were performed.

Search and handling time

Three recently-emerged couples of *T. absoluta* were released in small plastic cages (200 mL) containing leaflets of the two tomato cultivars as oviposition substrate. To keep the leaflets turgid, the petioles were placed inside an eppendorf tube containing distilled water. After 24h, the *T. absoluta* couples were removed from the cages and the number of eggs on the leaflets was counted. Fifteen eggs of *T. absoluta* were kept on the lower surface of each leaflet and the excess ones were removed. The leaflets containing eggs were placed with the abaxial surface upwards on a surface of water in Petri dishes. A single female of up to 7 days-old of each mirid predator was released on the leaflets and the Petri dishes were sealed with the lid. The activities of the female predators were monitored by visual observations during 20 minutes. The monitored activities were:

a) searching time – described as the time spent by female predators between the end of the first preyed egg and the beginning of the second preyed egg of *T. absoluta*;
b) handling time – described as the total time spent by predators to consume one egg of *T. absoluta*.

Data analysis

All data were first tested for the presence of normal distributions (Shapiro-Wilk test) and homogeneity of variances (Bartlett's test). If both conditions were met, parametric tests were performed, otherwise, their non-parametric equivalent were performed. The number of glandular and non-glandular trichomes on leaflets and stems from the two tomato lines and the levels of acylsugar in the leaflets were compared by *t* test ($\alpha = 0.05$). The percentage of time spent by each predator species on walking, grooming, probing, feeding activities and when remained immobile were analyzed by a two-way ANOVA; means were separated using the Fisher's LSD test ($\alpha = 0.05$). In order to compare the number of preyed eggs *T. absoluta* by the female mirid predators in the two tomato lines as well as the time spent by the females in searching and handling the prey, the non-parametric Mann-Whitney *U* test was performed. All the statistical procedures were made using the statistical package SPSS v.21.

Results

Trichomes density and acylsugars levels in the two tomato lines

The density of glandular trichomes was significantly different between the two breeding lines TOM 584 and TOM 687 on the leaflets ($t = -4.78$; df = 106.04; $P < 0.001$) and on the stems ($t = -1.45$; df = 115.19; $P < 0.001$) (Table 2). Tomato line TOM 687 showed higher density of glandular trichomes on the leaflets and lower density of non-glandular trichomes compared to line TOM 584 ($t = 4.03$; df = 113.92; $P < 0.001$). There was no significant difference in the amount of non-glandular trichomes on the stems of both tomato lines ($P > 0.05$). TOM 687 showed higher levels of acylsugars (31.27 nmol/cm^2) in the leaflets compared to TOM 584 (21.13 nmol/cm^2) ($t = -3.84$; df = 5.78; $P = 0.009$) (Table 2).

Predation rate

Tuta absoluta laid significantly more eggs on the line TOM 584 (148.9 ± 8.64 eggs) than on the line TOM 687 (112.2 ± 7.34 eggs) (Mann-Whitney *U* test, $Z = -2.59$ $P = 0.09$). The number of preyed eggs by each predator in each tomato line is shown in Figure 1. The three mirid predators were able to walk on the stems of the two tomato lines find and prey *T. absoluta* eggs deposited in the apical part of the plants. There was no significant difference between the number of preyed eggs by each mirid predator either on TOM 584 or TOM 687 tomato lines (Mann-Whitney *U* test; *C. infumatus:* $Z = -0.53$; $P = 0.596$; *E. varians:* $Z = -0.833$; $P = 0.405$; *M. basicornis:* $Z = -0.947$; $P = 0.344$). The predation rates of *C. infumatus, E. varians* and *M. basicornis* on *T. absoluta* eggs were 1.04, 0.81 and 1.25 eggs.h^{-1} on the line TOM 584, and 0.80, 1.21 and 1.06 eggs.h^{-1}, respectively, on the line TOM 687, respectively.

Time allocation of activities and searching and handling time

The percentage of total time spent in each activity (walking, grooming, probing and feeding) or when immobile, by females of each mirid predator in each tomato line is shown in Figure 2. In spite of different density of glandular trichomes on the two tomato lines there was no effect on percentage of time spent in each monitored activity for any of the mirid species tested (Table 3). However, the time spent on grooming, probing and feeding activities were different among the three mirid predators (Table 4). *Macrolophus basicornis* spent more time on feeding and probing activities than *C. infumatus* and *E. varians* regardless of the two tomato lines (Figure 3). Only for time spent in walking activity there was a significant interaction between mirid predator species and tomatolines (Table 4). *Engytatus varians* spent more time walking on the line TOM 584 than on TOM 687, *M. basicornis* spent more time walking on TOM 687 than on TOM 584 and there was no difference between the two tomato lines on the walking activity of *C. infumatus* (Table 5).

The times spent by females of each mirid predators on searching for and handling time of the prey are shown in Figure 4. There was no significant effect of the tomato line on these parameters ($P > 0.05$, Mann-Whitney U test).

Discussion

We found that tomato line with high density of glandular trichomes and high levels of acylsugar reduce the number of eggs laid by *T. absoluta,* but do neither negatively affect the time allocation of activities (walking, grooming, probing and feeding) nor the searching and handling capacity of the three mirid predators *C. infumatus, E. varians* and *M. basicornis*. Mirid bugs inhabit pubescent, particularly glandular plant surfaces, which are avoided by many other entomophagous arthropods (Voigt et al. 2007). Our results suggest that a neutral effect of leaf texture and exudates of trichomes on tomato plants on search efficiency of the mirid predators, an indication that glandular trichomes and their exudates will not reduce the control of *T. absoluta* by these predators. Previous studies on predatory mirid bugs also showed that the density of glandular trichomes on plants do not affect their locomotion (Economou et al. 2006) and their attachment ability on these surfaces (Voigt et al. 2007). According to Southwood and Juniper (1986) mirid bugs have a small contact area with the plant surface because they have long legs. The hind tibiae are especially long, so that the hind femur is situated above body height. Moving one leg, the femur is raised and the tibia moved almost vertically, without contacting any trichome. In addition, insects belonging to the tribe Dicyphini (Hemiptera: Miridae, Bryocorinae), like these mirid predators, possess specialized tarsi that enable them to walk on surfaces with sticky material from glandular trichomes (Schuh and Slater 1995).

Although some predators like mirids have adaptations that allow them to exploit plants with high densities of glandular trichomes, there are reports that tomato glandular trichomes and its exudates negatively affec some heteropteran predators. Exudates from type VI glandular trichomes of tomato plants increased the mortality rate of nymphs and adults of *Geocoris punctipes* (Say) (Hemiptera: Geocoridae) and reduced the egg consumption of *Manduca sexta* (L.)

(Lepidoptera: Sphingidae) (Barbour et al. 1993). Tomato glandular trichomes caused entrapment and mortality of third instar nymphs of *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) (Lambert 2007). Nymphs of *Orius niger* Wolff (Hemiptera: Anthocoridae) spent most part of their time grooming their tarsi and rostrum when exposed to a tomato cultivar with high density of glandular trichomes (Economou et al. 2006). *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) walked less on tomato than on sweet corn and Lima bean plants and the reduced search efficiency of this predator for different preys in tomato plants was attributed to the presence of glandular trichomes (Coll et al. 1997).

Glandular trichomes on tomato did not affect the time spent o walking by the predators *C. infumatus* and *M. basicornis* whereas *E. varians* spent less time walking on the tomato line with high density of glandular trichome. In a similar study, walking activities of nymphs of *M. pygmaeus* was also not affected by density of glandular trichomes on different tomato cultivars (Economou et al. 2006). Also several reports on the role of glandular trichomes on predators' locomotion are mentioned in the literature (Barbour et al. 1993; Coll et al. 1997; Economou et al. 2006; Lambert 2007; Voigt et al. 2007). But, most of the studies are focused on the effect of trichomes present on the leaves and related to the nymphs of the predators, whereas the effects of trichomes on the stems on locomotion of adult predators are neglected. In fact, walking on stems is challenging for many natural enemies and entrapments caused by exudates of glandular trichome may occur mostly in this part of the plant (Kennedy 2003; Lambert 2007). In this study, we found that the density of glandular trichome on stems of both lines is higher than the density found on the leaves and we hypothesized that the predators would have more difficulty of walking on stems than on leaves. However, contrarily, the three mirid predators walked freely on stems of both tomato line and were able to find and prey *T. absoluta* eggs deposited on the apical part of the potted plant in the greenhouse.

Also the grooming activity helps the predators to continue to walk on surfaces with glandular trichome. However, the time spent by predators in cleaning activities might reduce the time available for searching for the prey (Eigenbrode et al. 1996). In this study, the time spent by the *C. infumatus*, *E. varians* and *M. basicornis* on grooming activity was not affected by tomato lines with different densities of glandular trichomes. Within species, *E. varians* spent more time on grooming (27.9% or 11.1 min) than *C. infumatus* (21.9% or 8.7 min) and *M. basicornis* (15.0% or 6.0 min) but, this activity did not affect their time spent in searching for eggs of *T. absoluta* as discussed below. In a similar study, Economou et al. (2006) found that nymphs of the mirid predator *M. pygmaeus* groomed significantly more on the tomato cultivar H30 with high density of glandular trichomes in their leaves (43.6 trichomes/mm^2) than on Elxis and Dombito cultivars (13.3 and 19.0 trichomes/mm^2, respectively), which have less amounts of glandular trichomes compared to H30.

In this study, *M. basicornis* probes (12.0% or 4.8 min) and feeds (25.8% or 10.3 min) on the tomato plant more than *C. infumatus* and *E. varians*. Predators belonging to the tribe Dicyphini show zoophytophagy, i.e. they feed on both prey and plant (Albajes and Alomar 2005; Coll and Guershon 2002). Although the main function of plant feeding by Dicyphini predators is water supply (Hamdi et al. 2013), in the absence or scarcity of prey, plant-feeding by these predators might cause some damage to the plant (Gabarra et al. 1988). In addition, studies conducted with *N. tenuis* revealed that economic damage caused by this predator on tomato plant is inversely proportional to prey availability (Calvo et al. 2009; Sanchez 2009b). However, economic damage caused by predatory mirid bugs in tomato plants is not the rule into Dicyphini species, and according to Castañé et al. (2011) the mirid predator, *M. pygmaeus*, also feed on tomato but it does not cause economic damage to the plant. Studies concerning to the injuries and potential damage caused by *C. infumatus, E.*

varians and *M. basicornis* in tomato are in progress in our lab (Silva et al, in preparation).

Predation rate data suggest that hairiness and presence of toxic exudates of the glandular trichomes on tomato plants do not reduce encounter rate between the mirid predators and prey. The density of trichomes on *Salix cinerea* L. (Salicaceae) leaves also did not affect search efficiency of *Ortothylus marginalis* L. (Hemiptera: Miridae) and *Anthocoris nemoraris* L. (Hemiptera: Anthocoridae). Both predators were able to find and prey eggs and larvae of *Phratora vulgatissima* L. (Coleoptera: Chrysomelidae) on clones of *S. cinerea* differing in trichome density (Björkman and Ahrné 2005). However, reduced foraging efficiency of predators on plants with glandular trichomes has been reported. For example, the acylsugar from glandular trichomes of *Datura wrightii* L. (Solanaceae) acts directly by deterring and hampering movement of *Nabis americoferus* Carayon (Hemiptera: Nabidae), *Geocoris pallens* Stal (Hemiptera: Geocoridae) and *Chrysoperla* spp. (Neuroptera: Chrysopidae) and indirectly by reducing their foraging efficiency for the prey *Lema daturaphila* Kogan and Goeden (Coleoptera: Chrysomelidae) and *Tupiocoris notatus* Distant (Hemiptera: Miridae) (Gassmann and Hare 2005).

The negative impact of glandular trichomes on natural enemies suggests that herbivores might experience natural selection to utilize glandular plants because they provide enemy-free space (Jeffries and Lawton 1984). This may explain why *T. absoluta* prefers solanaceous plants as host (Desneux et al. 2010), specially tomato which is well defended by glandular trichomes (Kennedy 2003; Simmons and Gurr 2005). Consequently, the impact of glandular trichomes is expected to be greater on natural enemies than on *T. absoluta.* However, mirid bugs including the three species studied here show a certain degree of specialization on tomato plants with glandular trichomes. Our results show that glandular trichomes on tomato plants (on stems and leaflets) have no negative effect on movement and prey searching efficiency for *T. absoluta* eggs of the mirid predators *C. infumatus, E. varians* and *M. basicornis*. Thus, we can conclude that there would not be any incompatibility between the use of tomato plants with glandular trichomes and/or high acylsugar content as factors of plant resistance and biological control of *T. absoluta* mediated by these three mirid predators.

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Tables

Table 1. Monitored activities of the predators (Economou et al. 2006)

Activities	**Definition**
Immobile	Standing immobile without probing, feeding, grooming
Moving	Forward motion
Grooming	Rubbing its tarsi over each other, or over other part of the body (rostrum, antennae, abdomen)
Probing	Moving the rostrum on the plant surface, while standing immobile or showing small movements
Feeding	Piercing the rostrum in the plant and sucking fluid

Table 2. Number/cm^2 (± SE) of glandular and non-glandular trichomes on upper and lower surface of leaflets and on stems and acylsugar levels (± SE) in the leaflets of TOM 584 and TOM 687 tomato lines.

Tomato lines	Trichomes/cm^2				Acylsugar ($nmol/cm^2$)
	Leaflets		Stems		
	Glandular	Non-glandular	Glandular	Non-glandular	
TOM 584	134.53 ± 25.84 b	4540.55 ± 251.38 a	1383.78 ± 134.50 b	7865.49 ± 360.21 a	21.13 ± 3.35 b
TOM 687	399.67 ± 36.65 a	2692.88 ± 304.47 b	2483.21 ± 157.41 a	8497.97 ± 320.04 a	31.27 ± 4.06 a

Average followed by the same letter in the columns did not differ by *t* test ($P < 0.05$).

Table 3. *F* and *P*-values of the effects of tomato lines and predator species on each activity of *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* (ANOVA two-way).

Effect	**Df**	**Walking**		**Grooming**		**Probing**		**Feeding**		**Immobile**	
		F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Cultivar	1	0.506	0.480 ^{NS}	0.432	0.514 ^{NS}	0.685	0.15 ^{NS}	0.198	0.659 ^{NS}	0.664	0.419 ^{NS}
Species	2	2.752	0.073 ^{NS}	4.292	0.019*	5.911	0.005*	7.311	0.002*	1.998	0.145 ^{NS}
Cultivar*Species	2	4.332	0.018*	0.414	0.663 ^{NS}	0.127	0.881 ^{NS}	0.017	0.983 ^{NS}	1.443	0.245 ^{NS}

* Significant ($P < 0.05$)

Table 4. Total time spent (%) in walking activity by the predators *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* on TOM 584 and TOM 687 tomato lines.

Tomato lines	Species		
	C. infumatus	*E. varians*	*M. basicornis*
TOM 584	11.69bA	19.12aA	12.05Ba
TOM 687	8.91bA	13.36bB	18.37aA

Average followed by the same lower case letter in the rows and capital letters in the columns did not differ by Fisher’s LSD test ($P < 0.05$).

Figures

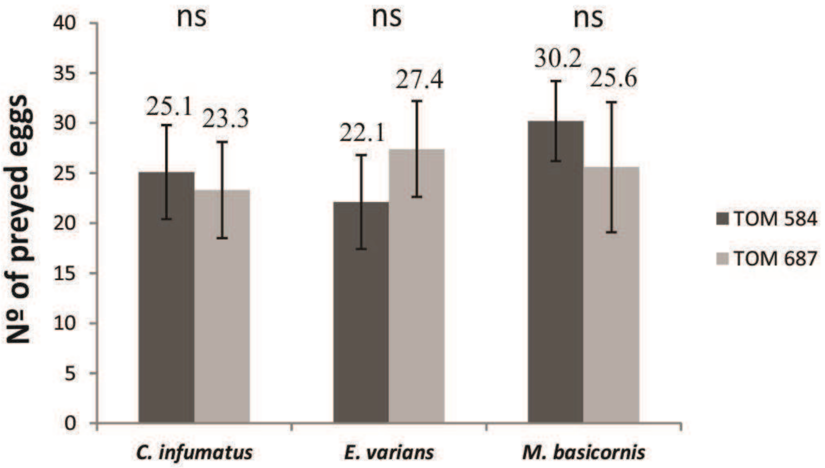

Figure 1. Number of *Tuta absoluta* eggs (± SE) consumed by *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* on TOM 584 and TOM 687 tomato lines.

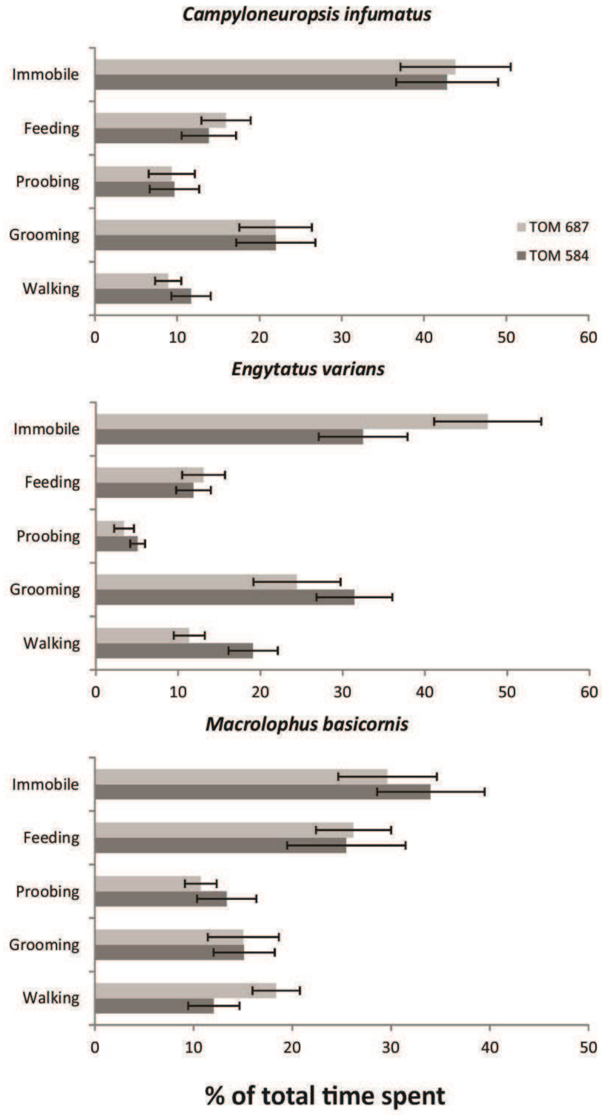

Figure 2. Total time spent (%) (± SE) by *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* on walking, grooming, probing, feeding and immobile on TOM 584 and TOM 687 tomato lines.

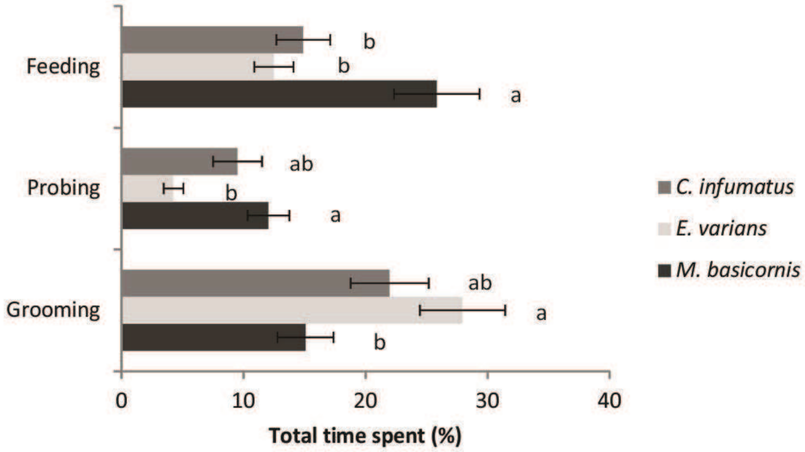

Figure 3. Time spent (%) (± SE) by *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* on feeding, probing and grooming activities, regardless of tomato line effect. Columns followed by different letters differ significantly (Fisher's LSD test, $P < 0.05$).

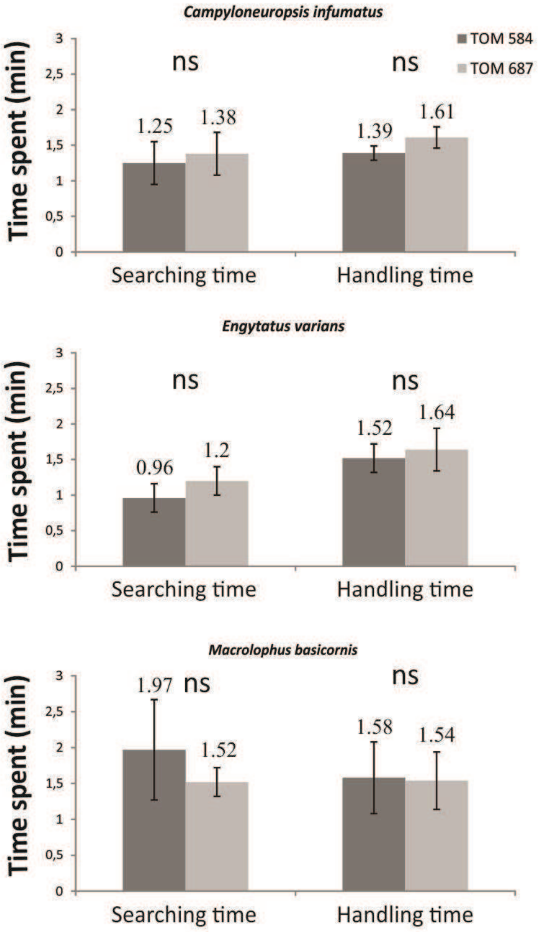

Figure 4. Time spent (in minutes) (±SE) by *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* on prey-searching and handling time for *Tuta absoluta* eggs on TOM 584 and TOM 687 tomato lines (ns = not significant, Mann-Whitney *U* test).

ARTICLE 2 Predation rates at different densities of *Tuta absoluta* eggs and prey preference for *Bemisia tabaci* or *Tuta absoluta* by three South American mirid predators

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Abstract. Prey preference and predation rates play a role in the natural enemy selection as biological control agent, concerning to mainly to the efficiency and target in biological control programs. Prey acceptance of the mirid predators *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal) for the preys *Tuta absoluta* (Meyrick) and *Bemisia tabaci* (Gennadius) were evaluated, as well as their functional response towards *T. absoluta* eggs. The study was conducted at laboratory conditions (25°C, RH 70±10% and 12h photophase). Both prey species were accepted by the three mirid predators. When presented a choice between the two prey species, *M. basicornis* ate more *T. absoluta* eggs, *C. infumatus* ate more *B. tabaci* nymphs and *E. varians* equally preyed on both prey. Predation rates at different egg densities of *T. absoluta* revealed that *C. infumatus* and *M. basicornis* exhibited type II functional response and *E. varians* exhibited type III functional response. Handling times (T_h) were estimated to 47 s, 11.1 min and 26 s for *C. infumatus, E. varians* and *M. basicornis*, respectively. The highest theoretical maximum predation per unit of time was found for *M. basicornis* (94.8 eggs/24h) followed by *E. varians* (94.3 eggs/24h) and *C. infumatus* (48.7 eggs/24 h). Prey type affects the prey selection and preference of the three mirid predators and *E. varians* and *M. basicornis* show highest predation rates on *T. absoluta* eggs. The prey preference and predation efficiency are a positive signal for the potential use of these mirid predators as biological control agent of *T. absoluta*.

Keywords: *Campyloneuropsis infumatus; Engytatus varians; Macrolophus basicornis;* Feeding preference; Functional response; Biological control; Tomato

Predation rates at different densities of *Tuta absoluta* eggs and prey preference for *Bemisia tabaci* or *Tuta absoluta* by three South American mirid predators

Introduction

The predation rate of a predator is an important indicator of its possible effectiveness in suppressing pest populations. However, in the environment, generalist predators can forage on a wide range of prey and the simultaneous presence of various prey species can obscure interpretation of predation results in an experimental setting with a single prey species. Therefore, knowledge of prey preferences of a predator is important. Facing two preys, optimal foraging theory predicts that a predator will select the more profitable one to maximize its net energetic gain (Charnov 1976; Stephens and Krebs 1986).

Prey preference is defined as disproportionate attack rate on one prey species, when more than one prey species is available (Hassell and Southwood 1978) and on the premise that all prey species have a similar encounter rate by the predator. However, many other factors may result in disproportional attack rate between a generalist predator and its prey complex, such as nutritional quality (Dicke and De Jong 1988), mobility of the prey (Eubanks and Denno 2000), defense of the prey (Moitoza and Phillips 1979), prey size (Fantinou et al. 2009), odour (Venzon et al. 1999b) and so on. Also most predaceous insects use one or more of these characteristics in the prey selection process. Predators from Tribe *Dicyphini* (Hemiptera: Miridae), frequently show a clear preference for one prey species (Bonato et al. 2006; Foglar et al. 1990; Ghabeish et al. 2010; Lykouressis et al. 2007; Provost et al. 2006) or a switching behaviour when the predator eats disproportionately more of the more abundant prey (Enkegaard et al. 2001; Jaworski et al. 2013). However, generalist predators may also show an antiswitching behaviour when the predator eats disproportionately more of the less abundant prey. Also, they may show no preference, when the consumed prey ratio is equal to prey individuals ratio in the environment (i.e. null switching) (Chesson 1984). Prey preference data are essential to predict the efficiency of generalist predators as biological control agents and to understand the effects of alternative prey on complex interactions involving those predators and their prey species (Eubanks and Denno 2000). For example, prey switching behavior exhibited by *Macrolophus pygmaeus* (Rambour) (=*M. caliginosus*) (Hemiptera: Miridae) when foraging in patches with disproportionate densities of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Bemsia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) may help regulating both prey populations simultaneously to low densities if prey switching is maintained at larger scales in the agro-ecosystem (Jaworski et al. 2013).

When prey preferences are known for a certain predator, it is also important to obtain information about its predation efficiency. Predation efficiency is often expressed as the functional response and describes the relationship between prey density and the number of prey consumed by an individual predator. Holling (1959) described three possible responses, in which, the number of prey consumed rises linearly (type I), hyperbolically (type II), or sigmoidally (type III). This could further be simplified in terms of density dependence. That is, they result in a density-independent prey killing represented by a linear increase (type I), a density-dependent negative response, represented by a decreasing curve (type II) and positively density-dependent response corresponding to a temporary increase in predation rate (type III). Usually, a type III response does not exhibit positive density dependence throughout the range, as only a portion, i.e. at the initial level, shows a sigmoidal increase; later on, it also exhibits negative density dependence due to satiation. Therefore, it is important to differentiate type II and III at relatively low prey values. The generalist predator *M. pygmaeus* exhibited type II response when challenged with varying densities of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Foglar et al. 1990). However, the same predator feeding on different densities of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) responded in a density dependent way and exhibited a typical type III functional response (Enkegaard et al. 2001). Therefore, a predators that impose positively density dependence prey mortality (type III) are supposed to potentially manage the prey population and could be considered as efficient biocontrol agents (van Lenteren and Bakker 1975). However, Hughes et al. (1992) pointed out that predators exhibiting type II response have been successfully established and managed prey populations.

Thus, functional response studies are essential for the successful use of biocontrol agents in augmentative releases and furthermore is a starting point for predator-prey dynamics quantification (Wiedenmann and Smith 1997). However, laboratory-measured functional response may bear little resemblance to those measured in the field conditions (Wiedenmann and O'Neil 1992) as it is affected by several experimental conditions such size of foraging arenas, non-realistic prey densities, time of exposure, prey type, plant characteristics and temperature (Hemerik and Yano 2011).

Biological control of *T. absoluta* and *B. tabaci* are still a challenge in Brazil. The parasitoids *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) and *Encarsia* spp. (Hymenoptera: Aphelinidae) are reported as natural enemies of *T. absoluta* and *B. tabaci*, respectively in Brazil (Oliveira et al. 2003; Parra and Zucchi 2004). Of these, only *T. pretiosum* is used on a small scale, but its efficiency for *T. absoluta* control is still discussed (Guedes and Picanço 2012). Based on the successful use of the predatory bugs *Nesidiocoris tenuis* (Reuter) and *M. pygmaeus* (Hemiptera: Miridae) in integrated pest management of whiteflies and *T. absoluta* in Europe (Urbaneja et al. 2012; van Lenteren 2012), Bueno et al. (2012) started a search on solanaceus crops for natural enemies of *T. absoluta* in the State of Minas Gerais–Brazil. The mirid predators *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal) (Hemiptera: Miridae) were found and now they are been evaluated as biological control agents of key pests in tomato in Brazil. These three mirid bugs have relatively short developmental time, high predation rates and low immature mortality. Also they can use tomato plants as oviposition substrate and complete their development on this plant, are easily reared in laboratory conditions and are able to feed on eggs and larvae of *T. absoluta* (Bueno et al. 2013).

Because prey choice of a generalist predator may directly affect the control efficiency of its various preys, the objective of this work was to evaluate the potential prey preference of *C. infumatus, E. varians* and *M. basicornis* for eggs of *T. absoluta* and nymphs of *B. tabaci*. Also the functional response of these predators when offered different densities of *T. absoluta* eggs was evaluated.

Material and methods

Tuta absoluta was reared under laboratory conditions (25 ± 2ºC, 60 ± 10% R.H. and a 12 h photophase) inside cages (90x70x70 cm) on tomato plant cv. Santa Clara. *Bemisia tabaci* was reared in a greenhouse compartment (27 ± 5ºC, 70 ± 10% R.H. and a 12 h photophase) on tomato cv. Santa Clara.

The predators *C. infumatus, E. varians* and *M. basicornis* were field collected in Ribeirão Vermelho, State of Minas Gerais (Brazil) on tobacco plants (Bueno et al. 2012) and reared according to Agustí and Gabarra (2009) and Bueno et al. (2013) in a climate room at 25 ± 2ºC, 60 ± 10% R.H. and a 12 h photophase. The predators were kept inside acrylic cages (60x30x30 cm) with a tobacco plant cv. TNN as oviposition substrate and eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) as food.

Prey use

To determine if each predator is able to feed on *B. tabaci* nymphs and *T. absoluta* eggs, they were presented to each pest separately in no-choice tests. Tomato leaflets containing 2nd and 3rd nymphs of *B. tabaci* were collected from the stock rearing and placed in Petri dishes (9 cm diameter) on a layer of agar-water 1%. Fifty nymphs were kept on each leaflet, excess nymphs were removed. Similarly, fifty eggs of *T. absoluta* were collected from the stock rearing with a thin brush and transferred to a clean tomato leaflet kept in a Petri dish on a layer of agar-water 1%. Females of each mirid predator up to 7-days-old were starved for 24 h prior to the tests and then released into the Petri dishes, which were kept in climate chamber at 25 ± 1ºC, 60 ± 10% R.H. and 12 h photophase. After 24 h the number of *B. tabaci* nymphs and *T. absoluta* eggs consumed by each predator were counted under a stereomicroscope. Tests for each predator-prey combination were replicated 25 times.

Prey preference

To evaluate the potential prey preferences of the three mirid predators for *B. tabaci* nymphs and *T. absoluta* eggs, a choice experiment was performed (cafeteria experiment, Krebs (1999)). Females of each predator were presented to both preys simultaneously. Tomato leaflets containing nymphs of *B. tabaci* were collected from the rearing and placed in Petri dishes (9 cm diameter) on a layer of agar-water 1%. Thirty nymphs were kept on each leaflet and the excess was removed, and thirty eggs of *T. absoluta* were added to/on the same leaflet. Females of each predator up to 7-days-old were starved for 24 h prior to the tests and then released in the Petri dishes, which were kept in a climate chamber at 25 ± 1ºC, 60 ± 10% R.H. and a 12 h photophase. The numbers of prey consumed by each female 2, 4, 8 and 24 hours after predator release were counted under a stereomicroscope. Prey was not replaced during the experiment. For each mirid predator species the experiment was replicated 20-24 times.

Functional response

Tomato seedlings (approximately 10 cm in height and with 3-4 leaves) were infested with different density of *T. absoluta* eggs and offered to the predators. The seedlings were grown in plastic cups (50 mL) and after *T. absoluta* infestation they were put inside cylindric plastic cages (20 cm high, 10 cm diameter) covered with organza tissue. Females of each predator species up to 7-days-old were starved for 24 h before the tests. Prey densities were determined through preliminary tests to ensure that maximum levels of predation could be reached for each predator, which resulted in densities of 2, 4, 8, 16, 32, 64, 128 and 256 eggs of *T. absoluta*/tomato seedling. As we observed in our preliminary tests that *C. infumatus* consumed fewer eggs than the other two mirids, the density of 256 eggs was not evaluated for this predator species. A single female of each predator was released in each cage and the number of consumed eggs was counted after 24 h. The experiment was carried out in a climate room at 25 ± 3ºC, 60 ± 10% R.H. and a 12 h photophase. Preys were not replaced during the tests, as we offered an excess of prey. The test consisted of 25 replicates for each prey density and for each mirid predator. A control treatment without predators was done, consisting of 10 arenas for each prey density to determine the natural mortality.

Data analysis

Predation data were tested for normal distribution results (Shapiro-Wilk test) and homogeneity of variance (Levene's test). A non-parametric Mann-Whitney *U*-test to compare the number of each prey consumed by each predator separately was used. Potential prey preferences were evaluated using the Rodgers index, which ranges from zero to one where a value of one represents strongest preference (Krebs 1999). The Rodgers index was calculated as R*i* = A*i*/max(A*i*), where "A*i*" is the area under the curve of the proportion of species, "*i*" is the prey items consumed over time and "max(A*i*)" is the largest value of "A*i*" per experimental unit. For each individual predator, the most eaten prey was given a preference score of 1.0 and the least eaten prey was awarded the lowest preference score. Rodgers index means were checked for normality and homogeneity of variance and next the Mann-Whitney *U*-test was used to compare the differences in the prey indices of each predator separately. SPSS version 21.0 (SPSS Inc., Chicago, USA) package was used for these statistical procedures.

The type of the functional response was determined using regression analysis of the proportion of prey eaten "N_a" in relation to the number of prey offered "N_o". When the proportion of prey eaten declined monotonically with prey density the functional response was assumed to be type II, but when the proportion of prey eaten was density dependent over part of the range of prey density, the functional response was assumed to be type III.

Handling times "T_h" which is defined as the time required by predator to pursue, capture and consume the prey and attack rates "a" is defined as the number of prey consumed by predator during a certain amount of time. These parameters associated with a type II functional response were estimated using Holling's disc equation modified by a reciprocal linear transformation (formula 2), after the shape of the functional response curves was determined (Livdahl and Stiven 1983).

Type II:
$$\frac{1}{N_a} = \frac{1}{aTN_o} + \frac{T_h}{T} \tag{1}$$

The data were also fit using a type III functional response model (formula 2), where "N_a" is the number of prey attacked per predator, "N_o" is the initial prey density, “a” is the attack rate, “T” is the time that predator and prey are exposed to each other, and “T_h” is the handling time associated with each prey consumed (Hassell 1978). In this type III functional response model, “b”, “c”, and “d” are constants from the function that relate a and N_o: $a=\left(d+bN_o\right)/\left(1+cN_o\right)$. Parameters were obtained by fitting observed data to the models above using non-linear least square regression.

Type III:

$$N_a = N_o\left\{1-\exp\left[\frac{\left(d+bN_o\right)\left(T_hN_a-T\right)}{\left(1+cN_o\right)}\right]\right\} \qquad (2)$$

The regression analysis was performed using the software Microsoft Excel and parameters of attack rate and handling time of the prey were estimated using software R version 3.0.2 (R Development Core Team, 2013).

Results

Prey use

The three mirid predators *C. infumatus, E. varians* and *M. basicornis* were able to prey on *T. absoluta* eggs and *B. tabaci* nymphs (Figure 1). There were no significant differences in the number of eggs and nymphs consumed by *E. varians* and *M. basicornis* (P < 0.05). However, *C. infumatus* consumed more eggs of *T. absoluta* than nymphs of *B. tabaci* (Mann-Whitney *U*-test, Z = -3.875, P = 0.0001) (Figure 1).

Prey preference

The cumulative numbers of consumed prey by the three mirid predators over time up to 24 h are shown in the Figure 2. *Tuta absoluta* eggs were the most consumed prey by *M. basicornis* throughout the time evaluated: at each evaluation the number of consumed eggs was more than twice of that of *B. tabaci* nymphs. Rodger’s index of prey preference showed that *T. absoluta* eggs was the preferred prey by *M. basicornis* (Mann-Whitney *U*-test: $Z = -5.123$, $P < 0.0001$) (Table 1). *Engytatus varians* and *C. infumatus* ate more *B. tabaci* nymphs than *T. absoluta* eggs during 3 of the 4 evaluations done. According to the Rodger's index, *C. infumatus* showed a significant preference for *B. tabaci*, (Mann-Whitney *U*-test: $Z = -3.064$, $P = 0.002$) and *E. varians* showed no preference ($P = 0.688$) for both preys.

Functional response

Prey mortality in absence of the predators was negligible. Percentages of dead eggs (by manipulation or natural mortality) in the treatment control ranged from 0 to 1.12%. A regression analysis showed that the proportion of prey attacked (N_a) in relation to the number of prey offered (N_o) declined with increasing prey density for the predators *C. infumatus* (Figure 3a) and *M. basicornis* (Figure 5a). Thus, these predators exhibited a type II functional response (Figures 3b and 5b for *C. infumatus* and *M. basicornis*, respectively). A sigmoidal proportion of prey attacked (N_a/N_o) corresponding to a temporary increase in predation rate proportion was found for the predator *E. varians* (Figure 4a) which resulted in a type III functional response (Figure 4b).The estimated values for attack rates and handling times of the three mirid predators are shown in Table 2. The estimation of handling time (T_h) by the Holling disc equation (2) was 0.0131 h (47 s) for *C. infumatus,* 0.1853 (11.1 min) h for *E. varians* and 0.0071 h (26 s) for *M. basicornis*. The attack rate (*a*) obtained for *C. infumatus* and *M. basicornis* through the same equation were 1.0353 and 1.3662 eggs of *T. absoluta*/h, respectively. The attack rate for *E. varians* was a function of prey density ($a = bN$) with *b* equal to 0.0068.

The highest theoretical maximum predation, limited by the upper asymptote defined by the ratio T/T_h (Hassell 1978), of the predators *C.*

infumatus, E. varians and *M. basicornis* were estimated to 48.7, 94.3 and 94.8 eggs/24 h, respectively.

Discussion

Prey use and prey preference

Our results show that in no choice test, the three mirid predator species are able to attack and feed on both *B. tabaci* nymphs and *T. absoluta* eggs. The amount of *T. absoluta* eggs consumed by each mirid was higher than the consumption rate found for *Diciphus errans* (Wolf) (Hemiptera: Miridae) (Ingegno et al. 2013) and similar to the consumption rate of *M. pygmaeus* and *N. tenuis* (Urbaneja et al. 2009). The number of *B. tabaci* nymphs consumed by predators in this study ranged from 15 to 28 nymphs/female. These values are higher than those found for *M. pygmaeus* (≅ 8 nymphs/24 h) and *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) (≅ 12 nymphs/24 h) (Barnadas et al. 1998).

The results in this study suggest that *B. tabaci* nymphs could be considered as an equally suitable prey as *T. absoluta* eggs for the predators*, E. varians* and *M. basicornis*, whereas *C. infumatus* shows a lower predation rate on *B. tabaci* nymphs. These findings are in accordance with the previous studies reporting that *T. absoluta* and *B. tabaci* are suitable preys to predatory mirid bugs *M. pygmaeus, N. tenuis, Dicyphus tamaninii* Wagner and *Dicyphus errans* (Wolf) (Barnadas et al. 1998; Calvo et al. 2009; Urbaneja et al. 2009; Ingegno et al. 2013).

In the choice tests, each predator species behaved differently in relation to the number of each prey consumed. Based on Rodger's index, the predation rates for *T. absoluta* eggs over *B. tabaci* nymphs was highest in the case of *M. basicornis*. Contrarily, *C. infumatus* showed highest predation rates for *B. tabaci* nymphs and *E. varian*s ate similar numbers of both preys. Mollá (2013) showed that both nymphs and adults of the mirid bug *N. tenuis* did not display a preference for eggs of *T. absoluta* or *B. tabaci* nymphs. Predation rates of *T. absoluta* eggs by *M. pygameus* were highest when this food was the most abundant prey and vice-versa when *B. tabaci* was the most abundant prey. However, when evaluating several prey combinations, *T. absoluta* larvae appeared the less preferred prey (Jaworski et al. 2013).

Many factors may be involved in the selection decision of a predator. Optimal foraging models based on the caloric and nutritive value of prey predict that nutritional quality to foraging costs should be the most important criterion used by predators in prey selection (Stephens and Krebs 1986). The protein content in eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) is in average 16.6% of the fresh weight (Ferkovich et al. 2007) whereas in *B. tabaci* is 10.8% of the fresh weight (Cohen and Byrne 1992). In this study, although we did not measure the individual quality of both preys offered, we can presume that *T. absoluta* eggs are nutritionally superior to *B. tabaci.* Our results also suggest that *M. basicornis* may have selected the more nutritional prey *T. absoluta* eggs over *B. tabaci* nymphs. Actually, lepidopteran eggs represent good quality food for mirid predators (Castañé and Zapata 2005; Montes 2013) and according to Alomar et al. (2006), fertility of *M. pygmaeus* is higher when fed on eggs of *E. kuehniella* than on *B. tabaci* nymphs indicating that lepidopteran eggs maybe the prey of superior quality for this predator. Foglar et al. (1990) also showed that *M. pygmaeus* may exhibit preference for the nutritional superior prey, the aphid *M. persicae* over the spider mite *Tetranichus urticae* Koch (Acari: Tetranychidae). The intrinsic rate of increase (r_m) reported for this predator on these preys are 0.097 (Perdikis and Lykouressis 2002) and 0.031 day^{-1} (Hansen et al. 1999), respectively.

Prey of highest nutritive value increases predator's fitness, however this prey may not always be preferred (Eubanks and Denno 2000). This is the case of *C. infumatus* that showed preference for *B. tabaci* nymphs. It is possible that prey size can be other criterion involved in prey selection, and we hypothesized that prey size may play a role in prey preference of *C. infumatus*. Nymphs of *B. tabaci* are larger prey (2nd instar: 0.31 mm in length, 0.19 mm in width; 3rd instar: 0.44 mm in length, 0.29 mm in width) compared to *T. absoluta* eggs (0.33 mm in length and 0.22 in diameter). Thus, the encounter rate might be higher on large prey than on small one. Fantinou et al. (2009) reported that *M. pygamaus* used prey size in its foraging decisions, and demonstrated that, contrarily to *C. infumatus* in this study, *M. pygmaeus* prefered the smaller prey, 2nd instar of *M. persicae*. This may be explained by the fact of larger aphid may defend themselves against the predator better than small nymphs. However, predator's biomass gain tends to be reduced when consuming small sized prey (Fantinou et al. 2009). Moreover, predators have to consume more amounts of prey in order to meet their nutritional needs. This may explain why in the no-choice tests C. *infumatus* consumed larger quantities of the less preferred prey (*T. absoluta* eggs).

In this study both preys, *T. absoluta* eggs or *B. tabaci* nymphs offered to the predators, were sessile. Prey mobility rather than prey nutritional quality can be another important criterion used by some predators in their prey selection (Eubanks and Denno 2000). Eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (sessile prey) are nutritionally superior than the aphid *Acyrthosiphum pisum* (Harris) (Hemiptera: Aphididae) (mobile prey) as prey for *Geocoris punctipes* (Say) (Heteroptera: Geocoridae), however, this predator consistently attacked the nutritional inferior prey more frequently than the nutritionally superior prey (Eubanks and Denno 2000). Prey preferences of *D. tamaninii* was also affected by prey mobility since sessile nymphs of *T. vaporariorum* were the less preferred prey in comparison to nymphs of *Aphis gossypii* Glover (most preferred) and *M. persicae* and adults of *T. urticae* (Ghabeish et al. 2010). Prey preferences of *C. infumatus, E. varians* and *M. basicornis* should be also evaluated concerning to mobile preys, as in this study the preys offered were sessile. Visually oriented generalist predators may be employed more effectively against highly mobile prey species in biological control programmes or against relatively sessile prey species when more mobile prey are not abundant (Eubanks and Denno 2000).

Functional response

Campyloneuropsis infumatus and *M. basicornis* showed a type II functional response to the offered prey *T. absoluta* whereas *E. varians* showed a type III. Predation curves for arthropod predators at different densities of prey frequently fit a type II functional response (Holling 1959). In this situation, predators increase their prey consumption with increasing prey availability at a decreasing rate until reaching a plateau (Juliano 2001). Also, the type II functional responses from varying densities of different preys have been reported for the mirids *D. tamaninii* and *M. pygmaeus* on *T. vaporariorum* and *Frankliniella occidentalis* (Pergande) (Montserrat et al. 2000), *A. gossypii* (Alvarado et al. 1997), *M. persicae* (Fantinou et al. 2008; Foglar et al. 1990), and *T. urticae* (Foglar et al. 1990) indicating that these predators might remain effective at low prey densities. However, frequent releases of predators would be necessary to control the pest.

According to Juliano (2001) and van Lenteren and Bakker (1975), a predator with a type III functional response is density dependent and increases its searching efficiency on higher densities of the prey. The essential premise of the type III response is that the predator is able to intensify its searching activity when prey densities increase. *Engytatus varians* showed a positive density-dependent response by increasing its search efficiency until the threshold density of 64 eggs of *T. absoluta*/plant. At higher prey densities the search efficiency of this predator tended to decrease suggesting that *E. varians* would be more effective in regulating the pest at low prey densities. Enkegaard et al. (2001) reported a type III functional response for *M. pygmaeus* fed on first instar of *T. vaporariorum* and this predator killed more prey at low densities.

Differences in the shape of functional responses of predators may depend on prey species, size and appearance (Hassell et al. 1977; Streams 1994), prey distribution (Hemerik and Yano 2011), availability of alternative prey as well as the predator's age and hunger level (Holling 1959). According to Hemerik and Yano (2011) experimental conditions may also have influences on functional responses of predators. Most experimental studies done in very small homogeneous spaces such as a Petri dish or a plastic vial result in type II functional response of mirid predators (Alvarado et al. 1997; Fantinou et al. 2008; Montserrat et al. 2000). However, in the current study, the use of a larger foraging arena (cylindric plastic cages, 20 cm high x 10 cm diameter) resulted in a type III functional response for *E. varians*. According to van Lenteren and Bakker (1977) larger foraging arenas may reflect the field situation better by allowing predators to emigrate from the site and search for another one. Enkegaard et al. (2001) using an arena similar in size (14 cm high x 10 cm diameter) to that used in this study also found a type III functional response for *M. pygmaeus* on *T. vaporariorum.* The authors observed that females of *M. pygmaeus* left the plant at lower prey densities. This behavior can be interpreted as an attempt by the females to find more rewarding prey patches, thus indicating that *M. pygmaeus* females require a certain threshold prey density to stay in a patch. Also, observing the behavioral responses of *D. tamaninii* and *M. pygmaues* to different densities of *T. varporoariorum* in a non-enclosed plant, Montserrat et al. (2004) found that *D. tamaninii* frequently left the plant at low prey densities and *M. pygmaeus,* however, tended to stay in a patch independently of prey density. These authors also found that both predators spend a considerably amount of time in non-searching activities like oviposition and feeding on the plant. Although, in this study we did not make a direct observation of non-searching activities, we can suppose that *E. varians* may also have left the plant at lower densities of *T. absoluta* eggs resulting in a type III functional response.

Lower handling time observed by *C. infumatus* and *M. basicornis* in comparison to handling times of *E. varians* suggest that *C. infumatus* and *M. basicornis* eat *T. absoluta* eggs faster than *E. varians*. The handling times by *C. infumatus* (47 s) and *M. basicornis* (26 s) are shorter than the handling times by *D. tamaninii* fed on *T. vaporariorum* (22.8 min), *F. occidentalis* (12.8 min) (Montserrat et al. 2000), *A. gossypii* (8.046 min) (Alvarado et al. 1997) and also shorter than the handling times of *M. pygmaeus* feeding on *T. vaporariorum* (73.7 min) and *F. occidentalis* (20.46 min) (Montserrat et al. 2000), on *M. persicae* (79 min) and *T. urticae* (36 min) (Foglar et al. 1990). When considering the type III functional response, the handling times obtained in our study for *E. varians* (11.1 min) is larger than the value reported for *M. pygmaeus* fed on first instar of *T. vaporariorum* (1 min) (Enkegaard et al. 2001). Handling time, defined as the time required by predator to capture and consume the prey, may indicate a limitation of predation when it takes too long by reducing the probability of capture a new prey by the predator (Giller 1980). However, it is important to note that values for attack rate and handling time should be used only as comparative values since the calculations assume that the individuals in the tests are always searching, attacking and consuming prey. But, that is certainly not the case, especially for adult predators. Another important activity for adult females is oviposition, while males spend considerable time just wandering, possibly looking for the females. In addition, Lins et al. (not published yet) in a previous study (see chapter/article 1) showed that *C. infumatus, E. varians* and *M. basicornis* spend a considerable amount of time (14-20 min of the total time 40 min evaluated) staying motionless on the tomato plant. According to Hassell (1978) Handling times (T_h) estimated on the basis of functional response models differ from "true" handling times obtained by direct observations (see chapter/article 1), because the parameter T_h encompasses time spent not only on actual prey handling but also on other non-searching activities (e.g. oviposition, resting, etc).

Search efficiency and prey handling time are not the only factors affecting functional response of predators. Predation may also be limited by an internal physiological state (e.g. satiation) (Mills 1982). Holling disc equation does not discriminate between the process of handling and digesting the prey and satiation level of a predator is assumed to linearly decrease searching activities (Rijn et al. 2005). Thus, when satiation level is not taken into account, results of functional response may often indicate that predators are time-limited. However, according to Jeschke et al. (2002) the vast majority of predators, including predatory insects, seems to be digestion-limited. For example, from Holling disc equation, the predatory mite *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae) requires 5 min to consume a spider mite egg and potentially consume 12 eggs per hour, whereas in reality the predator consumes (under steady-state conditions) maximally one per hour (Sabelis 1986). Jeschke et al. (2002) have also pointed out that resting may be caused by satiation. This may suggest that, as discussed before about resting, the three mirid species studied here are also digestion-limited predators.

Conclusions

Both *T. absoluta* eggs and *B. tabaci* nymphs are acceptable prey to the three mirid predators. *Tuta absoluta* eggs were the most eaten prey by *M. basicornis, B. tabaci* nymphs by *C. infumatus* and *E. varians* equally accepted both prey within a 24 h period. Thus, prey type has effects on foraging decision of the mirid predators.

When fed on *T. absoluta* eggs *C. infumatus* and *M. basicornis* exhibited a type II functional response and *E. varians* exhibited a type III functional response. These results suggest that the predators could be more efficient at regulating high densities of *T. absoluta* eggs, especially *E. varians* that showed positive density-dependent response.

Nevertheless, it is necessary to consider the results with caution because all experiments have been done under simplified laboratorial conditions. In a whole plant system the predators can disperse and interact with other prey and/or predator, which could significantly affect the effectively of the predators. In addition, the effect of these predators in controlling larvae of *T. absoluta* should be evaluated.

Tuta absoluta and *B. tabaci* often appear together in tomato crops and the use of single predator that attack more than one prey species available in the environment can enhance biological control. Thus, we can suggest that *C. infumatus, E. varians* and *M. basicornis* are promising candidates for biological control of *T. absoluta* and other pests of tomato as *B. tabaci*. Further, more research in semi-field and field conditions will be necessary to achieve more conclusive results for the use of these predators as biological control agents of pests in tomato.

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Tables

Table 1. Rodger's Index (± SE) for prey preference of *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* adults in choice tests for the preys *Tuta absoluta* eggs and *Bemisia tabaci* nymphs (Man-Whitney *U* test, α = 0.05).

Predator	N	Prey		*P*
		T. absoluta eggs	*B. tabaci* nymphs	
C. infumatus	24	0.704 ± 0.055	0.928 ± 0.025	0.002186
E. varians	21	0.802 ± 0.054	0.901 ± 0.132	0.688
M. basicornis	20	0.956 ± 0.031	0.341 ± 0.071	< 0.0001

Table 2. R^2-values, types and parameters for the functional response of the predators *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* when exposed to different densities of *Tuta absoluta* eggs (T = 24h).

Predator	**Type**	**a(h^{-1})**	**T_h(h)**	**R^2**
C infumatus	II	1.0353 ± 0.13	0.0131 ± 0.001 (47 s)	0.64
E. varians	III	(1)	0.1853 ± 0.017 (11.1 min)	0.73
M. basicornis	II	1.3662	0.0071 ± 0.0003 (26 s)	0.89

(1) In the best fit type III model, a = bN, b = 0.0068 ± 0.002.

Figures

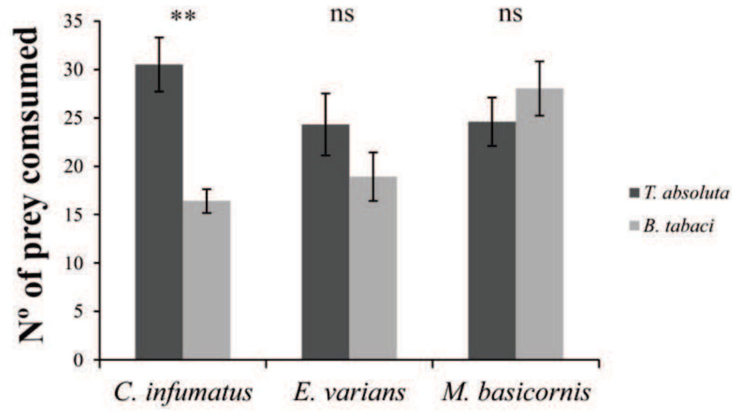

Figure 1. Number of *Tuta absoluta* eggs and *Bemisia tabaci* nymphs (±SE) consumed per 24h by *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* in no-choice tests (n = 25). (25ºC, RH 70±10% and 12h photophase).** = significant (Mann-Whitney *U*-test, $Z = -3.875$, $P = 0.0001$), ns = non-significant.

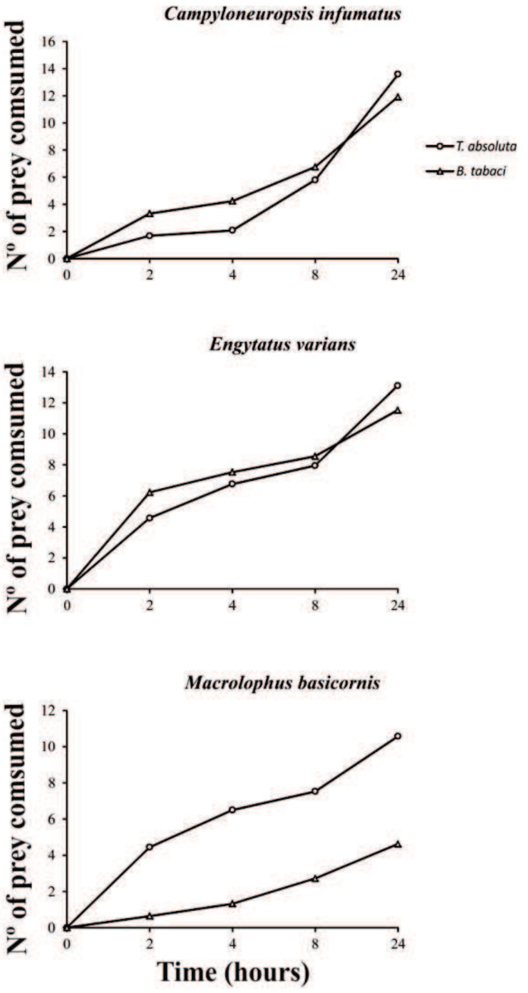

Figure 2. Cumulative number (mean ± SE) of *Tuta absoluta* eggs (○) and *Bemisia tabaci* nymphs (Δ) preyed by *Campyloneuropsis infumatus* (n=24)*, Engytatus varians* (n=21) and *Macrolophus basicornis* (n=20) in choice tests (25ºC, RH 70±10% and 12h photophase).

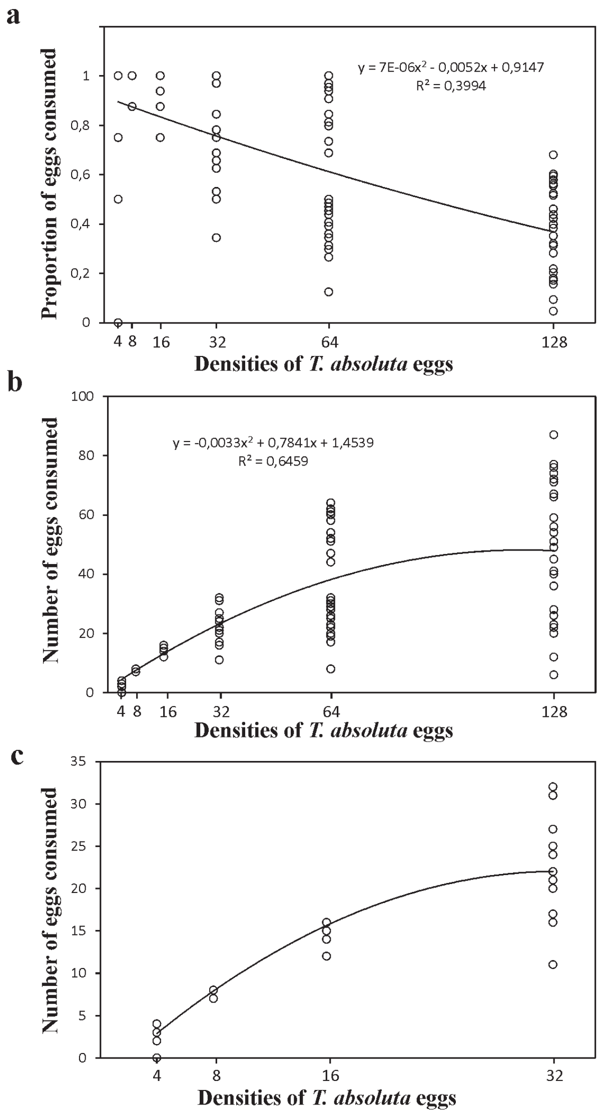

Figure 3. (a) Proportion of *Tuta absoluta* eggs consumed (N_a) in 24 h by *Campyloneuropsis infumatus* in relation to the number of offered prey (N_o); (b) Type II functional response of *C. infumatus* expressed as the number of *T. absoluta* eggs consumed in 24 h in relation to the initial density and (c) type II functional response of *C. infumatus* in detail at low prey densities.

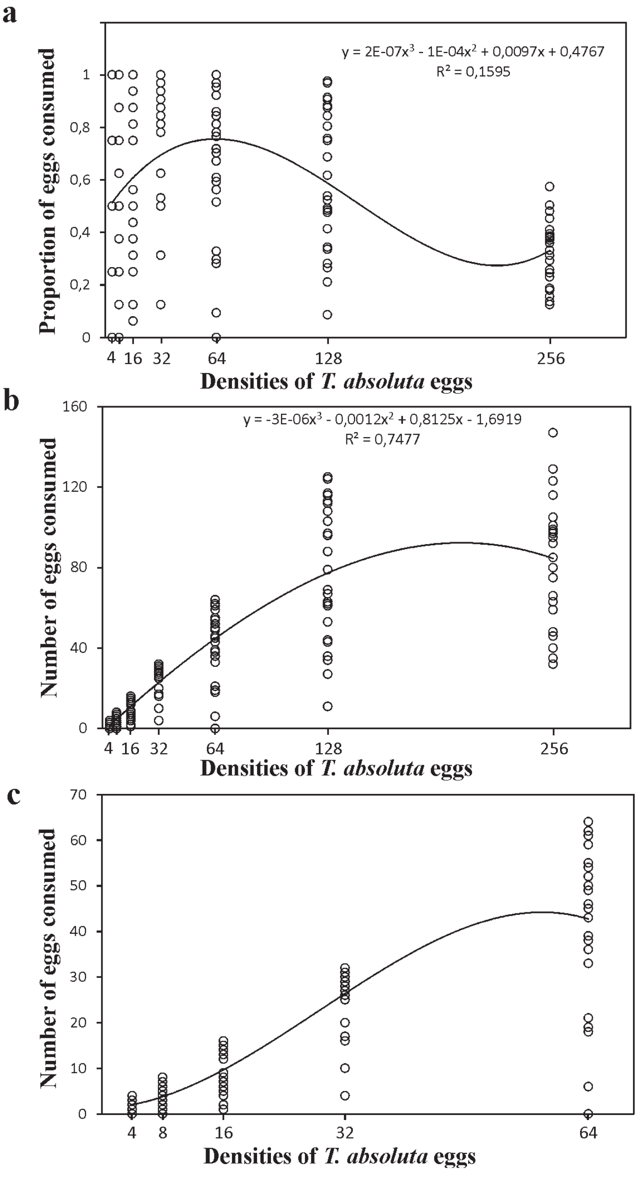

Figure 4. (a) Proportion of *Tuta absoluta* eggs consumed (N_a) in 24 h by *Engytatus varians* in relation to the number of offered prey (N_o); (b) Type III functional response of *E. varians* expressed as the number of *T. absoluta* eggs consumed in 24 h in relation to the initial density and (c) type III functional response of *E. varians* in detail at low prey densities.

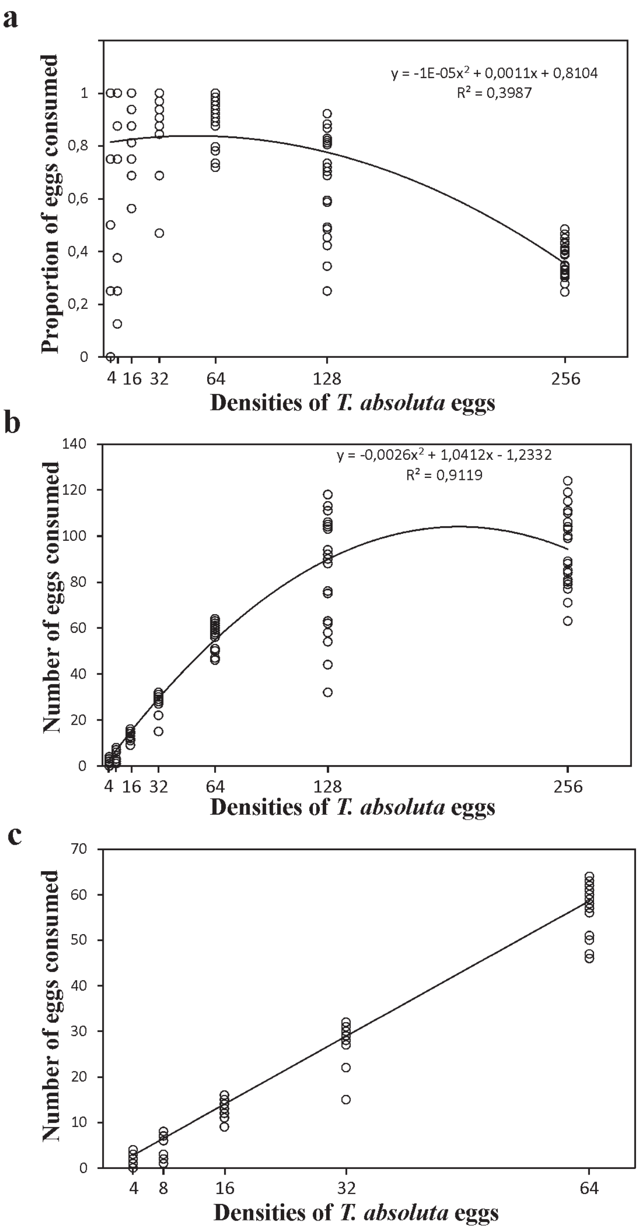

Figure 5. (a) Proportion of *Tuta absoluta* eggs consumed (N_a) in 24 h by *Macrolophus basicornis* in relation to the number of offered prey (N_o); (b) Type II functional response of *M. basicornis* expressed as the number of *T. absoluta* eggs consumed in 24 h in relation to the initial density and (c) type III functional response of *M. basicornis* in detail at low prey densities.

ARTICLE 3 Response of the zoophytophagous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* to volatiles of uninfested plants and to plants infested by prey or conspecifics

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Abstract. The mirid predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae) are used on a large scale for biological control of whitefly and of a recent invader into Europe, the pest *Tuta absoluta* (Lepidoptera: Gelechiidae). Currently, knowledge about the orientation mechanisms used by both predators in finding their prey is limited. In Y-tube olfactometer experiments, we have examined the behavioural responses of naïve and experienced *N. tenuis* and *M. pygmaeus* to uninfested plants, to herbivore-induced plant volatiles (HIPVs) from plants infested with various stages of *T. absoluta* and/or *Bemisia tabaci* (Hemiptera: Aleyrodidae), to the natural and synthetic pheromone of *T. absoluta*, and to volatiles produced by plants damaged by the predators. *Nesidiocoris tenuis* does not respond to volatiles produced by uninfested plants, whereas *M. pygmaeus* is attracted to these plants. Both predators are attracted to volatiles from prey-infested plants, but previous experience with prey only affects the behaviour of *M. pygmaeus*. Infestation of the same plant by *T. absoluta* and *B. tabaci* results into similar responses of both predator species as those displayed to plants infested by either herbivore singly. The two predators do not respond to the natural and synthetic pheromone of *T. absoluta. Macrolophus pygmaeus* females did not respond to early damage by conspecifics but avoided plants after longer term damage. *Nesidiocoris tenuis* females were initially also not attracted to plants previously damaged by conspecifics, but attraction was observed after a longer period of damage. The implications of these results for augmenting the biological control effectiveness of both predators are discussed.

Keywords: Dyciphini; Olfactometer bioassay; Herbivore-induced plant volatiles; Biological control; Tomato

Response of the zoophytophagous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* to volatiles of uninfested plants and to plants infested by prey or conspecifics

Introduction

The tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a devastating pest in tomato crops that may cause losses up to 100% if control methods are not adopted (Guedes and Picanço 2012). This insect is native to South America and recently invaded Europe, Africa and Asia causing very serious damage to tomato crops (Desneux et al. 2010). Another pest that causes severe damage to tomato worldwide is the silverleaf whitefly *Bemisa tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (De Barro et al. 2011). Both pests often occur simultaneously in tomato crops (Calvo et al. 2012) and have shown to be suitable prey for the generalist predators *Macrolophus pygmaeus* (=*M. caliginosus*) Rambour and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) (Barnadas et al. 1998; Calvo et al. 2009; Urbaneja et al. 2009). As a result, these predators are now widely commercialized in Europe for control of whiteflies and the tomato borer (Urbaneja et al. 2012) and they also prey on other tomato pests like aphids, thrips, mites and leafminers.

The use of a single species of natural enemy that feeds on more than one pest reduces the complexity and costs of biological control (Calvo et al. 2012). In addition, having multiple prey species may increase the level of control delivered by generalist biological control agents by providing several food sources to maintain populations and enhance predator performance due to mixed diet effects (Messelink et al. 2008). An important problem in using *M. pygmaeus* and *N. tenuis* to control *T. absoluta* and/or *B. tabaci*, is that during periods of very low prey density they can cause serious feeding damage to the plant and its fruits (Arnó et al. 2010). It is commonly assumed that the main benefit of plant feeding is the availability of alternative food during periods of prey scarcity for the predators (Albajes and Alomar 2005; Bueno and van Lenteren 2012; Coll and Guershon 2002). *Macrolophus pygmaeus* does usually not cause significant damage to tomato (Castañé et al. 2011), but serious economic damage caused by *N. tenuis* to tomato has often been reported (Sanchez and Lacasa 2008). Injury caused by *N. tenuis* is inversely proportional to prey availability (Sanchez 2008, 2009b; Calvo et al. 2009) and can be observed as necrotic brown rings around the stem, shoots and the pedicels of leaves and flowers that are caused by repeated feeding at the same point (Arnó et al. 2010). This repeated feeding at the same location suggests that *N. tenuis*-damaged plants may produce volatiles which attract conspecific predators to necrotic rings, which can explain the observation of Arnó et al. (2010).

Predators may use volatiles produced by plants that are attacked by herbivores as cues to locate their prey (Dicke 1999; Vet and Dicke 1992). Variation in the herbivore-induced plant volatiles (HIPVs) may influence behavioural responses of natural enemies (De Moraes et al. 1998; Takabayashi et al. 1994) and, in some cases, can limit the usefulness of innate responses to volatile blends (Vet and Dicke 1992). Although it seems that the use of infochemicals (Dicke and Sabelis 1988) occurs among all carnivorous arthropods regardless of their specialization, learning to respond to infochemicals is more common in generalists than in specialists (Steidle and Van Loon 2003). Associative learning – i.e. the establishment through experience of an association between two stimuli or between a stimulus and a response (Vet and Dicke 1992) – of volatile cues by generalist predators has been reported for several natural enemies (Drukker et al. 2000; Glinwood et al. 2011). The ability to learn may increase the searching efficiency because generalist predators exploit a range of resources that vary in quality and quantity both in time and space (Glinwood et al. 2011). Plants under multiple attack by different species of herbivores show differences in blends of volatiles they produce (Dicke and van Loon 2000; Dicke et al. 2009; Moayeri et al. 2007b; Ponzio et al. 2013; Zhang et al. 2009), so the predators are supposed to have to deal with highly variable information when exposed to HIPVs (Vet and Dicke 1992). Dealing with complex information becomes even more complicated when predators show plant feeding behaviour. Within the sub-order Heteroptera of the order Hemiptera, phytophagous (herbivorous) and zoophagous (carnivorous or predatory) species occur, as well as predatory species showing omnivorous behavior (i.e. they use food from more than one trophic level). These omnivorous species can be facultatively or obligatorily phytophagous and are often referred to as being zoophytophagaous (Bueno and van Lenteren 2012; Lundgren 2009). Zoophytophagous predators may show advantages for biological control programmes (they can survive when there is no prey) as well as disadvantages (they may cause plant damage as in the case of *N. tenuis*). Experiments concerning the role of volatiles in finding the host-plant and/or prey have been done for only a few heteropteran predators: *Orius* and *Anthocoris s*pp. (Anthocoridae) (Carvalho et al. 2011; Drukker et al. 2000; Venzon et al. 1999a), *Geocoris* spp. (Geocoridae) (Halitschke et al. 2008), *Macrolophus, Nesidiocoris* and *Dichyphus* species (Miridae) (Ingegno et al. 2013; Ingegno et al. 2011; McGregor and Gillespie 2004; Moayeri et al. 2006a, b; Moayeri et al. 2007b; Mollá Hernández 2013), *Perillus* and *Podisus* species (Pentatomidae) (Dickens 1999; Loon et al. 2000; Weissbecker et al. 2000).

In general, zoophagous predators respond to volatiles from prey-infested plants and some predators respond to single compounds present in the blend of volatiles produced by herbivory (Dickens 1999; Drukker et al. 2000; Halitschke et al. 2008; Venzon et al. 1999a). Also mirid predators from the tribe Dicyphini are attracted by prey-infested plants (Ingegno et al. 2013; Ingegno et al. 2011; Lou and Cheng 2003; McGregor and Gillespie 2004; Moayeri et al. 2006a, b;

Moayeri et al. 2007b; Mollá Hernández 2013). There is some, but rather limited, information about the responses of *M. pygmaeus* and *N. tenuis* to HIPVs. It is known that both predators respond to volatiles from prey-infested plants, but do not respond to volatiles emitted directly by the prey (Ingegno et al. 2011; Moayeri et al. 2006a, b; Mollá Hernández 2013). Further, males of *M. pygmaeus* use plant volatiles induced by conspecifics in sexual communication (Moayeri et al. 2007a). Their wide commercial use together with the as yet restricted knowledge made us decide to study the responses of these predators to volatiles produced as a result of the interaction between plants, prey and predators in the context of plants under multiple attack by different herbivores.

In our study system we used two herbivores, belonging to different feeding guilds, as prey. *Bemisia tabaci* is a generalist phloem-feeding herbivore, that feeds on more than 1000 plant species (EFSA 2013), and a rather specialistic leaf miner, *T. absoluta*, which is known to feed on 17 plant species, particularly Solanaceae (Desneux et al. 2010). Based on the very different feeding habits and dietary regimes of these two herbivores, we expect significant differences in the blend of volatiles emitted by attacked plants, because chewing herbivores activate different plant defense pathways than piercing-sucking species. For example, chewing herbivores predominantly activate the jasmonic acid (JA) signaling pathway, whereas phloem-feeding insects frequently activate the salicylic acid (SA) signaling pathway (Walling 2000). Crosstalk between these pathways can mould the final defense response of the plant (Kunkel and Brooks 2002; Thaler et al. 2012), including volatile production that functions as indirect defense by attraction of natural enemies of herbivores. We therefore investigated whether possible variations in HIPVs from plants attacked by these herbivores affected the capacity of *M. pygmaeus* and *N. tenuis* in using such infochemicals in prey-searching behaviour.

In Y-tube olfactometer experiments, we have examined the responses of naïve and experienced *N. tenuis* and *M. pygmaeus* to uninfested plants, and to HIPVs from plants infested with various stages of *T. absoluta* and/or *B. tabaci*. We also studied how experience with the prey affects the response of predators in the context of single and multiple herbivory. Next, we investigated whether the predators responded to the natural and synthetic pheromone of *T. absoluta*. Finally, we assessed if the damage produced by plant feeding by the predators in the absence of prey affected their behaviour.

Material and methods

Plants and insects

Tomato plants *Solanum lycopersicon* L. cv. Moneymaker were grown in plastic pots in a greenhouse compartment (25 ± 2ºC, 70% ± 10% R.H., L16:D8). Plants of 30-35 days-old (5-6 leaves and 20-25 cm in height) were used in the experiments. Plants used for rearing of insects were kept under the same conditions.

Tuta absoluta was kept in cages in a greenhouse compartment (25 ± 2ºC, 60 ± 10% R.H., L16:D8). Adults were kept in a mesh cage (60 x 40 x 40 cm) with a potted tomato plant as oviposition substrate. After egg hatching, the leaves were excised from the stems, placed in glass tubes with water, and kept in plastic cages (30 x 30 x 30 cm) until larval development. Uninfested tomato leaves were introduced into the cages when necessary to ensure *ad libitum* feeding. Pupae were collected from these cages and introduced in the cages with adults.

Bemisia tabaci was reared in another greenhouse compartment (25 ± 2ºC, 60 ± 10% R.H., L16:D8) on tomato cv. Moneymaker. Adults were kept inside cages (60 x 40 x 40 cm) on potted tomato plants as oviposition substrate. Once per week new plants and adults were introduced in the cages.

The mirid predators used in this study were supplied weekly by Koppert Biosystems (Berkel and Rodenrijs, The Netherlands). Nymphs of *M. pygmaeus* and *N. tenuis* were kept in climate cabinets (25 ± 1ºC, 70 ± 5% R.H., L16:D8) inside cages (60 x 40 x 40 cm) containing a potted tomato plant and eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were offered as food.

Y-tube olfactometer set-up

The responses of *M. pygmaeus* and *N. tenuis* females to volatiles emitted by different volatile sources were observed in a two-choice Y-shaped Pirex tube (3.5 cm inside diameter) formed by an entry arm (20 cm in length) and two side arms (23 cm in length, 70º angle). The tube was positioned vertically as in other studies with Dicyphini (Ingegno et al. 2013; Ingegno et al. 2011; McGregor and Gillespie 2004; Moayeri et al. 2006a, b, 2007a; Moayeri et al. 2007b; Mollá Hernández 2013). The two side arms were each connected to a glass container (35 L in volume) harbouring the volatile source. The airflow was provided by an air pump and adjusted with a flow meter to 2.5 L.min^{-1}. Before reaching the glass container the air passed through an activated charcoal filter. The glass containers with volatile sources were kept behind a black panel, preventing insects from visually detecting the plants. A single female predator was introduced at the downwind end of the entry arm and observed until she walked at least 10 cm up in one of the side arms. Females that did not choose a side arm within 10 minutes were considered as having made “no choice” and they were excluded from data analysis. Each female was tested only once and then discarded. For each pair of volatile sources, 50 females were tested during five different experimental days, ten on each day. In experiments *i* and *ii* (see below), ten naïve and ten experienced females of each species of predator were tested during the same experimental day for each pair of volatile sources.

After testing a batch of five females, the volatile sources were switched between the left and right sides of the arms to minimize unforeseen positional bias. After a trial of ten females, the Y-tube and the glass containers were washed with neutral soap and alcohol (70%). Y-tube bioassays were carried out in a climate room at 22 ± 1ºC, 70 ± 10% R.H.

Volatile sources tested

The following experiments were conducted (see also overview of all volatiles tested in Table 1):

i. Response to volatiles emitted by tomato plants infested either by T. absoluta *(eggs or larvae) or by* B. tabaci *(mixed stages).* Tomato plants (30-35 days-old) were covered with organza bags and 5 couples of *T. absoluta* of up to 3 days old were released into each bag. Females were allowed to lay eggs for 48 h, then the adults were removed and the plants infested with eggs were used in the experiments. For larval infestation, the same process was repeated; eggs hatched 4-5 days after oviposition, and larvae were allowed to feed for 72 h before the tests. For the whitefly infestation, plants were placed inside cages (60 x 30 x 40 cm) and 50 adults of *B. tabaci* were released in each cage. Ten days after infestation, the plants with adults, eggs and nymphs were used in the tests.

ii. Response to volatiles emitted by tomato plants infested simultaneously by both pest species. Because damage produced by *T. absoluta* larvae is more severe than damage produced by whiteflies, plants were first infested with *B. tabaci* as described above. After seven days, the plants were infested with 30 1st and 2nd instar larvae of of *T. absoluta.* After three days (10th day after inoculation of *B. tabaci*) plants were used in the experiments.

iii. Response to natural and synthetic sex pheromone of T. absoluta. Pupae of *T. absoluta* were individually put into glass tubes (5.5 x 1.3 cm) covered with Parafilm® and kept in a climate cabinet (25 ± 1ºC, 70 ± 5% R.H., L16:D8) until adult emergence. Female calling behaviour was checked to make sure that they were releasing sex pheromone during the bioassays. During the calling behaviour, females extrude the ovipositor, exposing the pheromone gland. This can easily be observed at 1.5x magnification with a hand lens or stereomicroscope. Ten calling females up to 3 days old were released inside a glass container (volume 1 L) connected to one of the arms of the Y-tube olfactometer. The synthetic sex pheromone of *T. absoluta* was provided by Pherobank B.V. (Wageningen, The Netherlands). The solution contained the main and minor component of the sex pheromone [(3*E*,8*Z*,11*Z*)-tetradecatrien-1-yl acetate and (3*E*,8*Z*)-tetradecadien-1-yl acetate, respectively] in a ratio of 95:5. One mL of solution (containing 1000 ng) was impregnated in filter paper discs (8.4 cm in diameter). After complete evaporation of the solvent (hexane), the filter paper disc was placed inside a glass container of 1L connected to the Y-tube. One mL of pure hexane solution was used as a control treatment.

iv. Response to volatiles emitted by tomato plants damaged by the predators in the absence of the prey. Tomato plants without prey were covered with organza bags and 20 newly emerged adults of either *M. pygmaeus* or *N. tenuis* were released into a bag. The plants were kept in climate cabinets at 25 ± 1ºC, 70 ± 10% R.H., L16:D8. After 4 and 10 days the predators were removed from the cages and the plants were used in Y-tube experiments. The number of feeding lesions caused by the predators on the plants was counted.

Naïve and experienced predators

Predators that did not have contact with *T. absoluta* or *B. tabaci* prey, nor with HIPV before the tests were considered naïve. Predators that were kept together with prey-infested plants during 24 h preceding the tests and had the opportunity to consume prey and to be exposed to HIPVs were considered experienced. For example, in single infestation experiments, experienced predators had opportunity to feed on *B. tabaci* or *T. absoluta* (larvae or eggs) and in double infestation experiment, experienced predators had opportunity to feed on both pest simultaneously. All the predators were kept on tomato plants except the naïve predators used in the test of “uninfested tomato vs clean air”. Those predators had not been in contact with tomato plants before the tests, as they were kept on tobacco *Nicotiana tabacum* L.

Statistical analysis

The responses of *M. pygmaeus* and *N. tenuis* were analyzed by Generalized Linear Models with a binomial distribution and a logit-link function. The response variable was the proportion of insects responding to one of the volatile sources. For the experiments *i* and *ii,* the effect of day and experience with the prey were included in the GLM model. For all experiments, to test whether the choice of the predators was significantly different from a 50% distribution, we fitted a separate binomial GLM to estimate the proportional response of each predator. The significance of the response was tested using a chi-square Wald test. The Mann-Whitney test was used to compare the differences in the number of feeding lesions caused by *N. tenuis*. All statistical procedures were performed using SPSS version 21.0 (SPSS Inc., Chicago, USA).

Results

The Y-tube olfactometer set-up used in this study worked well for both mirid predators: 76.8% of *M. pygmaeus* females chose one of the volatile sources whereas 23.2% did not make a choice within 10 minutes, and 93.1% of all *N. tenuis* females responded to the volatile sources whereas only 6.9% did not make a choice. No influence of experimental day was found on the response of both predators and this conclusion is valid for all treatments (GLM, $P > 0.05$).

Single infestation experiments

Neither naïve nor experienced *N. tenuis* females showed a preference for uninfested tomato plants over air from an empty container ($\chi^2_{\text{naïve}} = 0.84$; df = 1; $P = 0.359$; $\chi^2_{\text{experienced}} = 4.01$; df = 1; $P = 0.052$), whereas naïve and experienced *M. pygmaeus* females showed a significant preference for the volatile of uninfested tomato plants over clean air ($\chi^2_{\text{naïve}} = 5.46$; df = 1; $P = 0.019$; $\chi^2_{\text{experienced}} = 5.67$; df = 1; $P = 0.017$) (Fig. 1).

The responses of naïve and experienced predators of both species to volatiles emitted by plants infested with *T. absoluta* eggs or clean air were similar. Both predators showed a significant preference for volatiles from egg-infested plants over clean air (*N. tenuis*: $\chi^2_{\text{naïve}} = 5.68$; df = 1; $P = 0.017$; $\chi^2_{\text{experienced}} = 4.36$; df = 1; $P = 0.037$; *M. pygmaeus*: $\chi^2_{\text{naïve}} = 8.52$; df = 1; $P = 0.004$; $\chi^2_{\text{experienced}} = 5.8$; df = 1; $P = 0.016$), whereas they did not prefer the volatiles from egg-infested plants over volatiles from uninfested plants (*N. tenuis*: $\chi^2_{\text{naïve}} = 0.55$; df = 1; $P = 0.455$; $\chi^2_{\text{experienced}} = 1.12$; df =1; $P = 0.289$; *M. pygmaeus*: $\chi^2_{\text{naïve}} = 2.24$; df =1; $P = 0.134$; $\chi^2_{\text{experienced}} = 0.41$; df = 1; $P = 0.520$) (Fig. 1).

Naïve and experienced females of *N. tenuis* showed a significant preference for volatiles from plants infested with larvae of *T. absoluta,* both over volatiles from uninfested plants ($\chi^2_{\text{naïve}} = 10.95$; df = 1; $P = 0.001$; $\chi^2_{\text{experienced}} = 4.09$; df = 1; $P = 0.043$) and over clean air ($\chi^2_{\text{naïve}} = 6.46$; df = 1; $P = 0.011$; $\chi^2_{\text{experienced}} = 8.93$; df = 1; $P = 0.003$) (Fig. 1a). Naïve females of *M. pygmaeus* did not show preference for volatiles from plants infested with larvae over volatiles from uninfested plants ($\chi^2 = 0.62$; df = 1; $P = 0.43$), but after experience with the prey, females significantly preferred volatiles from infested plants ($\chi^2 = 7.86$; df = 1; $P = 0.05$) (Fig. 1b). Thus, *N. tenuis* showed an innate preference for plants infested by *T. absoluta* larvae, whereas *M. pygmaeus* acquired a preference as a result of experience.

Naïve and experienced *N. tenuis* females significantly preferred volatiles released by whitefly-infested plants over volatiles from uninfested plants ($\chi^2_{\text{naïve}} = 3.6$; df = 1; $P = 0.05$; $\chi^2_{\text{experienced}} = 5.39$; $P = 0.02$) and clean air ($\chi^2_{\text{naïve}} = 13.5$; df = 1;$P < 0.001$; $\chi^2_{\text{experienced}} = 7.71$; df = 1; $P = 0.005$) (Fig. 2a). Naïve *M. pygmaeus* females were not able to distinguish volatiles from whitefly-infested plants over volatiles from uninfested plants ($\chi^2 = 1.74$; df = 1; $P = 0.18$), whereas experienced females showed a significant preference for volatiles from whitefly -infested plants ($\chi^2 = 10.27$; df = 1; $P = 0.001$) (Fig. 2b).

Double infestation experiment

Naïve and experienced females of both predators showed a preference for volatiles from plants infested with whiteflies and larvae of *T. absoluta* over clean air (*N. tenuis*: $\chi^2_{\text{naïve}} = 9.63$; df = 1; $P = 0.002$; $\chi^2_{\text{experienced}} = 5.39$; df = 1; $P = 0.02$; *M. pygmaeus*: $\chi^2_{\text{naïve}} = 7.75$; df = 1; $P < 0.005$; $\chi^2_{\text{experienced}} = 9.17$; df = 1; $P = 0.002$) (Fig 3). Naïve and experienced females of *N. tenuis* did not show a preference for any of the volatile sources when exposed to volatiles from plants infested with both whiteflies and *T. absoluta* versus volatiles from plants infested with whiteflies only ($\chi^2_{\text{naïve}} = 0.06$; df = 1; $P = 0.795$; $\chi^2_{\text{experienced}} = 2.29$; df = 1; $P = 0.13$) or larvae of *T. absoluta* only ($\chi^2_{\text{naïve}} = 0.008$; df = 1; $P = 0.93$; $\chi^2_{\text{experienced}} = 0.009$; df = 1; $P =0.928$) (Fig. 3a). When offered a choice between volatiles from plants infested with whiteflies and volatiles from plants infested with both whiteflies and *T. absoluta* larvae, naïve females of *M. pygmaeus* had a significant preference for the latter ($\chi^2 = 12.01$; df = 1; $P = 0.001$), but lost this preference after experience ($\chi^2 = 3.49$; df = 1; $P = 0.061$) (Fig. 3b). Neither naïve nor experienced females of *M. pygmaeus* showed a preference when exposed to volatiles from plants infested with both whiteflies and *T. absoluta* over volatiles from plants infested with *T. absoluta* only ($\chi^2_{\text{naïve}} = 3.99$; df = 1; $P = 0.06$; $\chi^2_{\text{experienced}} = 1.14$; df = 1; $P = 0.28$).

Experiment with natural and synthetic sex pheromone of **T. absoluta**

Females of *N. tenuis* and *M. pygmaeus* were neither significantly attracted by the natural pheromone from calling virgin females of *T. absoluta* (*N. tenuis*: χ^2 = 0.15; df = 1; *P* = 0.69; *M. pygmaeus*: χ^2 = 0.04; df =1; *P* = 0.85), nor by its synthetic pheromone (*N. tenuis*: χ^2 = 0.28; df = 1; *P* = 0.59; *M. pygmaeus*: χ^2 = 1.69; df =1; *P* = 0.19) (Fig. 4).

Mirid plant feeding experiment

The number of feeding lesions, i.e. necrotic rings, caused by *N. tenuis* females was significantly higher on tomato plants exposed to 10 days (17.2 ± 1.3) of feeding than on plants exposed for 4 days (10.0 ± 3.93) (N = 5, Mann-Whitney test: *Z* = -2,562; *P* = 0.008). No visible feeding lesions were found on plants exposed to *M. pygmaeus*. *Macrolophus pygmaeus* females preferred the volatiles from plants previously exposed to feeding by conspecifics during four days over clean air (χ^2 = 6.22; df = 1; *P* = 0.013), but not when offered clean plants as alternative (χ^2 = 0.09; df = 1; *P* = 0.76). Also, they were no longer attracted after 10 days of plant feeding (χ^2 = 1.53; df = 1; *P* = 0.21) (Fig. 5), and these damaged plants even became repellent (χ^2 = 5.06; df = 1; *P* = 0.02). In contrast, females of *N. tenuis* were attracted by damaged plants after 4 days of damage (χ^2 = 11.81; df =1; *P* = 0.001), and attraction increased over time. After 10 days of feeding by conspecifics, volatiles from damaged plants were preferred over clean air (χ^2 = 13.18; df = 1; *P* < 0.001) and over volatiles from undamaged plants (χ^2 = 5.89; df = 1; *P* = 0.015) by *N. tenuis* (Fig. 5).

Discussion

Our results show that *M. pygmaeus* and *N. tenuis* are attracted to plants infested with either *T. absoluta* or *B. tabaci*. Volatile blends emitted by plants infested with both pests exerted a similar degree of attraction of the predators as plants infested with either the *T. absoluta* or *B. tabaci*. Moreover, our study provides the first evidence that the mirid predator *M. pygmaeus* can learn to respond to volatiles emitted by plants that are infested with prey, which might increase its efficiency as biocontrol agent. The two mirids do not respond to sex pheromone of *T. absoluta.* Furthermore, the volatiles produced by plants damaged by conspecifics attract *N. tenuis* but repel *M. pygmaeus*. These findings help understanding the prey searching behaviour and provide insight in how to use these natural enemies in controlling pests in tomato.

We found that plants simultaneously infested with both prey species were as attractive to the predators as plants infested with one prey species only, which is a promising result because both pests often simultaneously occur in tomato. Two species of herbivores sharing a common plant may alter the production of HIPVs with effects on tritrophic interactions (de Boer et al. 2008; Dicke et al. 2009; Moayeri et al. 2007b; Ponzio et al. 2013; Zhang et al. 2009). It is known that herbivores inducing the SA-pathway (e.g., *B. tabaci*) can modify or attenuate the defensive response of a plant subsequently attacked by a JA-pathway-inducer (e.g. *T. absoluta*), and vice-versa (Ponzio et al. 2013). Zhang et al. (2009) showed that Lima bean plants mix-infested with *B. tabaci* and the spider mite *Tetranychus urticae* Koch (Acarina: Tetranychidae) emitted lower amounts of (*E*)-β-ocimene than plants infested by spider mites only. As a result, mix-infested plants were less attractive to the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Tetranychidae) than single-infested plants. In contrast, a synergistic interaction between different defense pathways causing changes in HIPV blends and resulting in stronger attraction of predators also has been reported (de Boer et al. 2008; Moayeri et al. 2007b). For example, quantitative analyses of the volatile blends emitted by sweet pepper plants mix-infested by *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *T. urticae* emitted significantly higher amounts of α-zingiberene and dodecyl acetate than single-infested plants. Moayeri et al. (2007b) found that the predator *M. pygmaeus* responded stronger to volatiles emitted by mix-infested plants than those released from single-infested plants. Our results show that naïve females of *M. pygmaeus* preferred volatiles from mix-infested plants over volatiles from single-infested *B. tabaci* plants, but that experienced females did no longer show a preference, and that in *N. tenuis* both naive and experienced females showed no preference. We hypothesize that experience of *M. pygmaeus* with double infested *B. tabaci* plants resulted in increased attraction.

The use of a single species of natural enemy that feeds on more than one pest (e.g. *T. absoluta* and *B. tabaci*) reduces the complexity and costs of biological control (Calvo et al. 2012). In a greenhouse experiment, Bompard et al. (2013) showed that predator-mediated indirect interactions depend on the time scale considered. At a short time scale, presence of *T. absoluta* caused a disruption of the control of *B. tabaci* by *M. pygmaeus*, resulting in increased densities of *B. tabaci*. However, after ten weeks the density of *B. tabaci* was 7.3-fold lower in the presence *T. absoluta*. The authors argued that the strong reduction in *B. tabaci* populations in the presence of *T. absoluta* at a longer time scale could be due to an increased number of predators in response to higher prey availability. The learning ability of *M. pygmaeus*, as demonstrated by us, may provide another explanation for the results reported by Bompard et al. (2013), because learning shortens the predator's foraging time.

Learning ability may increase foraging efficiency whereas the innate use of infochemical cues by generalist predators prevents exclusive reliance on random search and is supposed to result in a higher prey finding efficiency than with random search (Steidle and Van Loon 2003). Therefore, this innate use of infochemicals might be a desirable feature of a good candidate for biological control. It might even be better to use predators with innate responses than those which have to learn to respond to volatile cues, because learning may have costs in terms of foraging time, as responses are not immediate (Vet and Dicke 1992). Foraging costs might be compensated by subjecting predators during mass rearing to essential HIPVs or to prey prior to release in the environment (Carvalho et al. 2011), but this might not be easy to realize. Drukker et al. (2000) found that orchard-caught females of *Anthocoris nemoralis* (F.) (Hemiptera: Anthocoridae) responded to volatiles from pear seedlings infested with pear psyllid nymphs [*Cacopsylla pyricola* (L.) (Hemiptera: Pysillidae)], and to methyl salicylate (MeSA, a component of the HIPVs of pear plants), whereas first generation laboratory-reared predators were unresponsive. However, after experiencing MeSA together with prey, the laboratory-reared predators showed a pronounced preference for this volatile. A similar procedure might be used to obtain experienced *M. pygmaeus* predators. As demonstrated in single infestation experiments, experienced *M. pygmaeus* learned to discriminate between volatiles from plants infested with *T. absoluta*-larvae or whitefly and uninfested plants, while naïve ones were not able to discriminate. According to Paré and Tumlinson (1999), chemicals to which entomophagous insects are exposed during interactions with their prey may familiarize them with particular cues associated with the location of prey and our results show that experience with prey increased the predator's responsiveness to prey-associated volatiles. Although olfactory learning has been documented well in parasitoids (Vet and Dicke 1992), olfactory learning is not well studied for arthropod predators (De Boer et al. 2005; Drukker et al. 2000; Glinwood et al. 2011), and our results provide the first evidence of olfactory learning in a predatory mirid bug.

Egg deposition by *T. absoluta* did not render plants more attractive to the predators than uninfested plants. Egg deposition by herbivores can modify the plant's surface chemistry with consequences for egg survival and can also change the emission of plant volatiles favouring the production of oviposition-induced plant volatile (OIPVs) which are used by parasitoids to locate their hosts (Fatouros et al. 2012). Naive and experienced *M. pygmaeus* and *N. tenuis* were not able to distinguish volatiles from egg-infested plants from uninfested plants. There is no information about the induction of OIPVs by *T. absoluta* eggs, but such OIVPs are induced in tomato plants, either the level of infestation was not high enough to induce these volatiles, the amount of OIPVs was too low to elicit a response in the predators, or these predators do not respond to OIPVs from tomato plants. The mirid bug *Dicyphus errans* Knight was also not able to distinguish between volatiles from tomato plants infested with eggs of *T. absoluta* and volatiles from uninfested tomato plants (Ingegno et al. 2013). However, in another olfactometer study, Mollá Hernández (2013) demonstrated that *N. tenuis* responds to volatiles from plants infested with eggs of *T. absoluta*, but in this case the level of infestation was four times higher than the level of infestation used in our experiments.

We also found that *M. pygmaeus* respond to volatiles from uninfested plants whereas *N. tenuis* does not. Ingegno et al. (2011) concluded that olfactory attraction of *M. pygmaeus* by uninfested plants is important for host-plant selection and crop colonization, though we think this conclusion only holds if a polyphagous predator responds to volatiles of plants which are expected to be attacked by preferred prey species. As previously discussed, prey-infested plants were more attractive to both predators than the control treatments in all the trials, except when naïve *N. tenuis* females were exposed to volatiles from egg-infested plants against uninfested plants. These findings are in accordance with other Y-tube olfactometer studies in which mirid bugs preferred prey-infested plants over uninfested plants (Ingegno et al. 2013; Ingegno et al. 2011; McGregor and Gillespie 2004; Moayeri et al. 2006a, b; Moayeri et al. 2007b; Mollá Hernández 2013). It has been demonstrated that *M. pygmaeus* does not prefer volatiles emitted by prey themselves [e.g., *M. persicae*, *T. urticae* and *Trialeurodes* *vaporariorum* Westwood (Hemiptera: Aleyrodidae)] (Ingegno et al. 2011; Moayeri et al. 2006a). There is limited information about the olfactory response of *N. tenuis* to prey-infested plants. Based on the information about *M. pygmaeus,* we hypothesize that both mirid predators respond to HIPVs, following the concept of Steidle and Van Loon (2003) that generalist predators frequently use general cues to locate their prey such as plant volatiles emitted in response to herbivore feeding or oviposition.

We further found that *M. pygmaeus* and *N. tenuis* did not respond to specific cues – natural and synthetic sex pheromones – emitted by *T. absoluta,* but we consider our experiments as preliminary as we did not test a range of pheromone concentrations. However, we have some support for our conclusion that they do not react to pheromones, as both predators have never been found in *T. absoluta* pheromone traps in tomato crops*,* even when large-scale predator releases had been made (F. Griepink, 2013, personal communication). Although the response of parasitoids to host pheromones is well documented (Steidle and Van Loon 2003), there are only a few reports of predators reacting to prey sex pheromones. Mendel et al. (1995) demonstrated that the specialist predator *Elatophilus hebraicus* Péricart (Hemiptera: Anthocoridae) uses the sex pheromone of its prey *Matsucoccus josephi* Bodenheimer and Harpaz (Hemiptera: Matsuccocidae) as kairomone. Further, the generalist predators *Coleomegilla maculata* (Degeer) (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) respond to the sex pheromone of the aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) (Zhu et al. 1999).

Zoophytopagous predators eat mostly prey, but also use plant resources (Albajes and Alomar 2005; Coll and Guershon 2002). The plant feeding habit of some species can cause economical damage to the plant (Arnó et al. 2010). We found no visible feeding lesions caused by *M. pygmaeus* and according to

Castañé et al. (2011) this predator is considered safe for pest control because it does not cause significant damage to tomato. However, Sampson and Jacobson (1999) reported damage inflicted by *M. pygmaeus* to cherry tomatoes, but in this case the predator density was high (50-300 individuals/plant), while prey availability was very low. The *M. pygmaeus* lesions were described as distorted tomato leaf growth, necrotic spots on leaves, scars on fruit and fruit drop. *Nesidiocoris tenuis*, however, often causes visible feeding lesions on tomato stems and petioles, and damages plants in the reproductive stage by abortion of flowers and small fruits (Castañé et al. 2011). In our experiment, where we did not offer any prey, the number of necrotic brown rings was significantly higher in plants exposed to ten days of predator-feeding than in plants exposed for four days. In greenhouse and field experiments, it has been shown that the abundance of necrotic rings and flower abortion caused by *N. tenuis* is inversely proportional to prey availability (Calvo et al. 2009; Sanchez and Lacasa 2008; Sanchez 2009b) and, it was also confirmed, under laboratory conditions, that zoophytophagous mirids feed less on plants when ample prey is available (Hamdi et al. 2013).

Although *M. pygmaeus* did not cause visible feeding lesions on tomato in our experiment, the plant probably produced volatile compounds in response to predator feeding. Moayeri et al. (2007a) showed that feeding of *M. pygmaeus* for four days on green bean plants induced the production of 11 additional compounds as compared to the volatiles emitted by clean plants. However, *M. pygmaeus* females were not able to distinguish between volatiles from plants previously damaged by conspecifics and volatiles from clean plants (Moayeri et al. 2007a). We found similar results for *M. pygmaeu*s after four days of feeding by conspecifics. Surprisingly, after ten days of feeding, the majority of the predators preferred the volatiles released from undamaged plants and now seemed to avoid plants damaged by conspecifics. We hypothesize that *M.*

pygmaeus uses these specific volatiles to avoid habitats occupied by conspecifics in order to minimize intraspecific competition for feeding and oviposition sites. Further, the avoidance behaviour we found in *M. pygmaeus* may explain why this predator usually does not cause damage to tomato plants.

Repeated feeding by *N. tenuis* at the same site of the plant (Arnó et al. 2010) causes biochemical changes in wounded tissue leading to increased levels of oxidative enzymes and phenolic compounds (Raman et al. 1984). These changes may also influence the production of plant volatiles. After four days of feeding by conspecifics, we found that females of *N. tenuis* did not distinguish between volatiles from damaged and undamaged plants. However, after ten days of feeding, predators chose the volatiles from damaged plants. This suggests that production of volatiles increased with extended duration of plant feeding by the predator, and that the higher emission of volatiles attracted these predators, supporting the observation of Arnó et al. (2010).

In summary, we found that HIPVs play an important role in prey finding of the two generalist mirid predators. *Nesidiocoris tenuis* responds innately to volatiles from prey-infested plants, whereas *M. pygmaeus* learns to detect the infochemical cues released by attacked plants in addition to an innate response. Our results also show that the blend of volatiles produced by plants simultaneously attacked by *T. absoluta* and *B. tabaci* does not negatively affect the search behaviour of both predators. Both predators seem not to respond to pheromones of *T. absoluta*. Furthermore, plant volatiles produced in response to feeding by conspecifics in absence of prey attracts *N. tenuis* but repels *M. pygmaeus*. Based on our findings and knowing that the predation capacity of the two predators is similar (Urbaneja et al. 2009), we speculate that *N. tenuis* might be the best natural enemy for control of tomato pests, because without needing experience, it responds faster to HIPVs and a substantially larger percentage *N.*

tenuis responded to volatiles than *M. pygmaeus* offered in the olfactometer. The only drawback of *N. tenuis* is its phytophagy during periods of low prey density.

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Table

Table 1. Volatile sources tested in Y-tube olfactometer experiments with *Macrolophus pygmaeus* and *Nesidiocoris tenuis*.

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*i – Single infestation**

Uninfested tomato **vs.** clean air
Tomato infested with *T. absoluta* eggs **vs.** clean air
Tomato infested with *T. absoluta* eggs **vs.** uninfested tomato
Tomato infested with *T. absoluta* larvae **vs.** clean air
Tomato infested with *T. absoluta* larvae **vs.** uninfested tomato
Tomato infested with *B. tabaci* **vs.** clean air
Tomato infested with *B. tabaci* **vs.** uninfested tomato

*ii – Double infestation**

Tomato infested with *T. absoluta* + *B. tabaci* **vs.** clean air
Tomato infested with *T. absoluta* + *B. tabaci* **vs.** tomato infested with *B. tabaci*
Tomato infested with *T. absoluta* + *B. tabaci* **vs.** tomato infested with *T. absoluta*

iii – Natural and synthetic sex pheromone of T. absoluta

Natural pheromone (calling virgin) **vs.** clean air
Synthetic pheromone **vs.** clean air
Pure hexane solution (control) **vs.** clean air

iv - Mirid plant feeding

Damaged tomato (4 days of feeding) **vs.** clean air
Damaged tomato (4 days of feeding) **vs.** undamaged plant
Damaged tomato (10 days of feeding) **vs.** clean air
Damaged tomato (10 days of feeding) **vs.** undamaged plant

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*In the single and double infestation experiments, both naïve and experienced predators were tested.

Figures

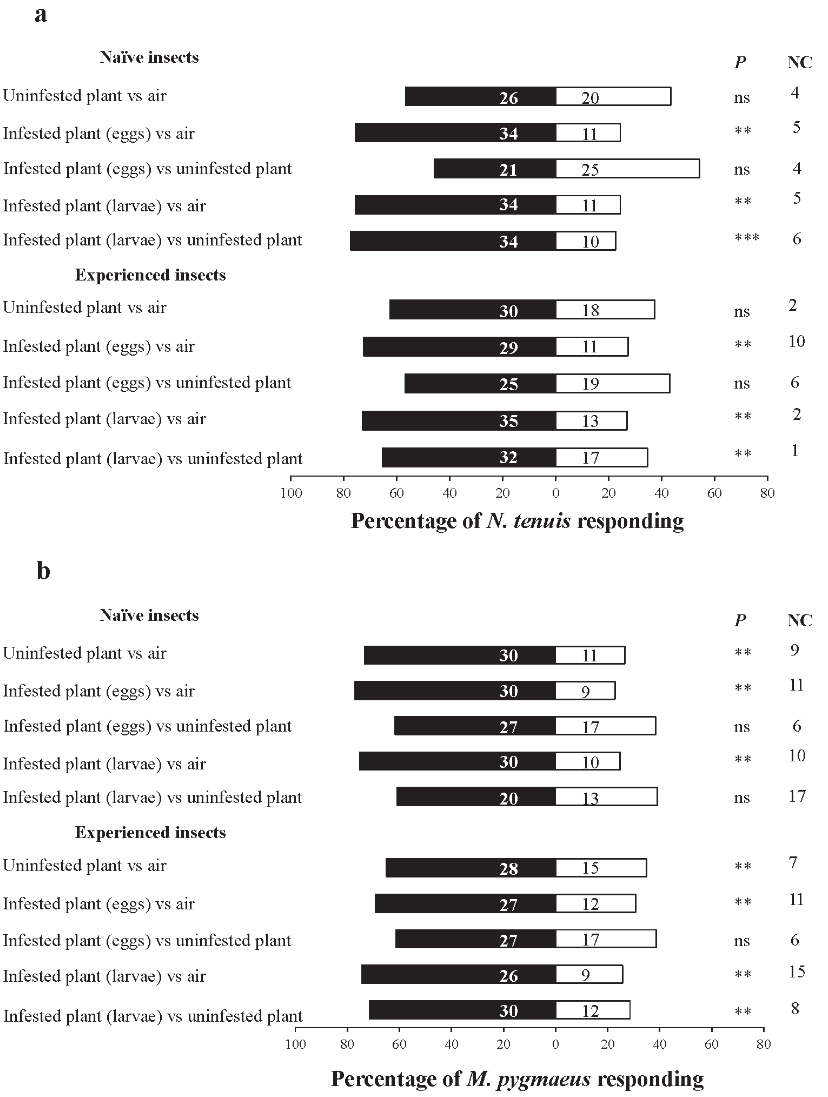

Figure 1. Responses of naïve and experienced females of *Nesidiocoris tenuis* (a) and *Macrolophus pygmaeus* (b) to volatiles from tomato plants infested with eggs or larvae of *Tuta absoluta* in a Y-tube olfactometer. Numbers in bars represent individual predators that moved towards the volatile sources indicated. NC indicates the number of tested individuals that did not make a choice. *** $P < 0.01$, ** $P \leq 0.05$, ns $P > 0.05$ (GLM, chi-square Wald test).

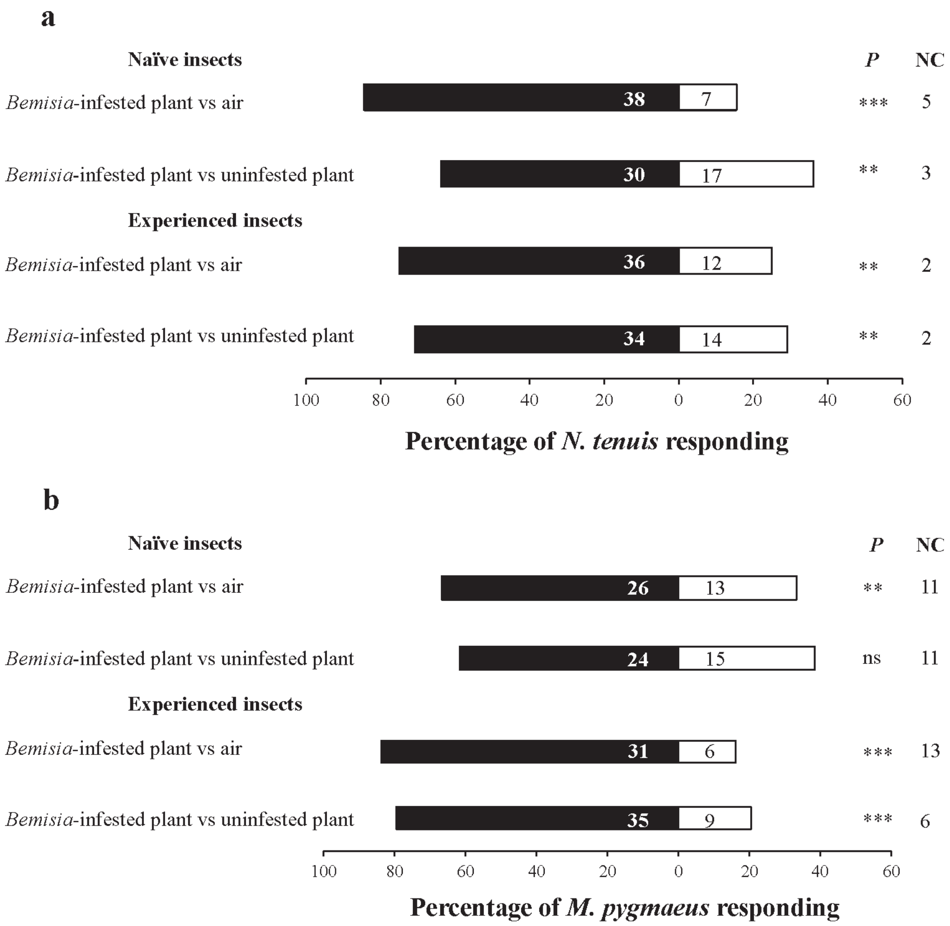

Figure 2. Responses of naïve and experienced females of *Nesidiocoris tenuis* (a) and *Macrolophus pygmaeus* (b) to volatiles from tomato plants infested with *Bemisia tabaci*. Numbers in bars represent individual predators that moved towards the volatile sources indicated. NC indicates the number of tested individuals that did not make a choice. *** $P < 0.01$, ** $P \leq 0.05$, ns $P > 0.05$ (GLM, chi-square Wald test).

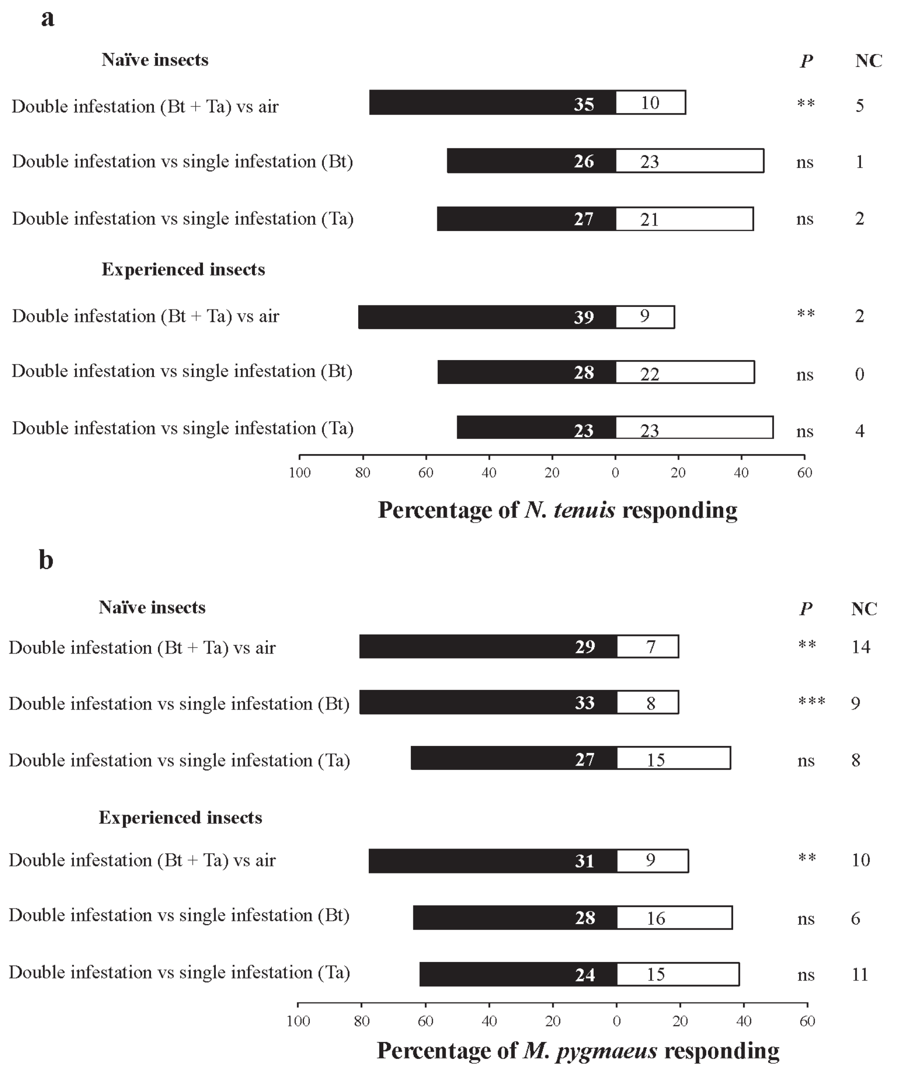

Figure 3. Responses of naïve and experienced females of *Nesidiocoris tenuis* (a) and *Macrolophus pygmaeus* (b)to volatiles from tomato plants double-infested with *Bemisia tabaci* (Bt) and *Tuta absoluta* (Ta) over odors from plants single-infested with *B. tabaci* or *T. absoluta*. Numbers in bars represent individual predators that moved towards the volatile sources indicated. NC indicates the number of tested individuals that did not make a choice. *** $P < 0.01$, ** $P \leq 0.05$, ns $P > 0.05$ (GLM, chi-square Wald test).

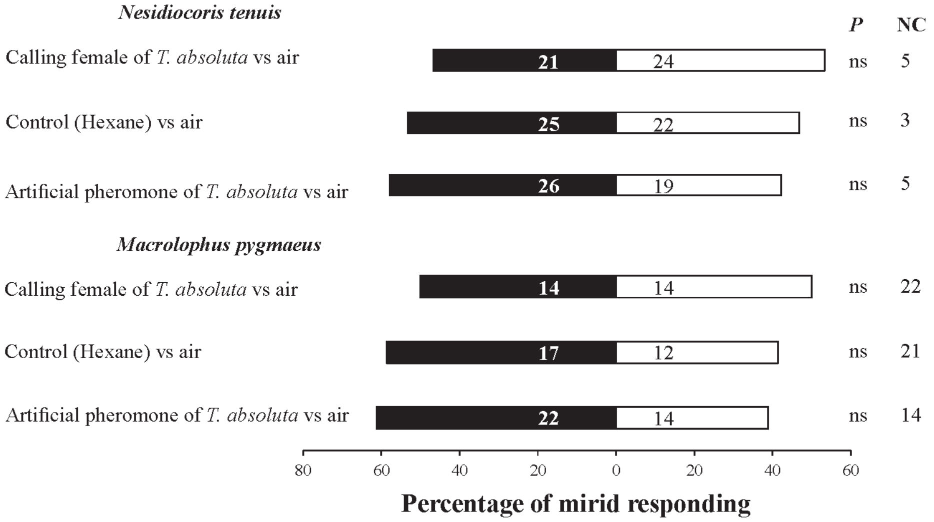

Figure 4. Responses of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* females to virgin caling females and to synthetic sex pheromone of *Tuta absoluta*. Numbers in bars represent individual predators that moved towards the volatile sources indicated. NC indicates the number of tested individuals that did not make a choice. ns $P > 0.05$ (GLM, chi-square Wald test).

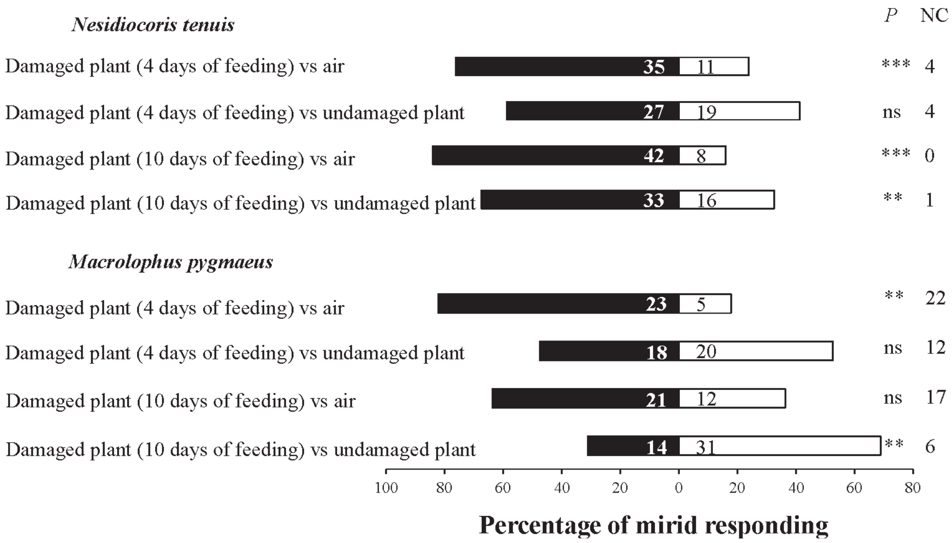

Figure 5. Responses of females of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* to odors from tomato plants previously exposed to 4 and 10 days of feeding by conspecifics in absence of any prey. Numbers in bars represent individual predators that moved towards the volatile sources indicated. NC indicates the number of tested individuals that did not make a choice. *** $P < 0.01$, ** $P \leq 0.05$, ns $P > 0.05$ (GLM, chi-square Wald test).

CLOSING REMARKS

In the process of evaluation of biological control agents many attributes of the candidates have to be taken into account such as internal synchronization with prey/host; climatic adaptation; no negative effects; good culture method; high kill-rate potential and good search efficiency (see general introduction section for a brief description of these evaluation criteria).

In the specific case of biological control of pests in tomato, one of the first steps into the selection process is related to the capacity of the natural enemies to walk and search for the prey on surfaces with glandular trichomes. Glandular trichomes and their exudates play an important role in plant resistance against pests but may also directly affect the efficiency of natural enemies. Results in this study show that, glandular trichomes and their exudates on two tomato cultivars do not affect the walking activities and prey searching efficiency of the mirid predatory bugs *Campyloneuropsis infumatus, Engytatus varians* and *Macrolophus basicornis* for the prey *Tuta absoluta*. All three predators also could climb the stems (higher quantity of glandular trichomes) of both tomato lines and find the eggs of *T. absoluta* on the top of the plant.

Prey preference and predation rates are other important attributes of a good candidate as biological control agents. In this sense, the results show that both *T. absoluta* and *Bemisia tabaci* are suitable prey for *C. infumatus*, *E. varians* and *M. basicornis* and that prey type affects the preferences of these predators. Prey size affect prey preference of *C. infumatus* whereas *M. basicornis* apparently prefers to feed on prey with highest nutritional value. *Engytatus varians* has no preference between *T. absoluta* eggs and *B. tabaci* nymphs. As both pests occur frequently on tomato and these generalist predators may attack more than one pest in the crop, we suggest that the presence of alternative food (not preferred prey) for them may enhance control of both pests.

Predation rates at different densities of the prey reveal the type of functional response of a predator and these data provides an indication of the predator performance in the field. The results suggest that these predators could be more efficient at regulating high densities of *T. absoluta* eggs. However, it is necessary to consider the results with caution because all experiments were done under simplified laboratorial conditions which may not reflect real situations in the field. Such studies are, however, useful in providing the first step for comparing the efficiency of species of predators, and also provide a valid means of comparing prey finding abilities of candidate predator species as biological control agents.

Also, the herbivore-induced plant volatiles play an important role in prey finding by predators. We found that *Macrolophus pygmaeus* and *Nesidiocoris tenuis* are attracted to plants infested with either *T. absoluta* or *B. tabaci*. We provide the first evidence that a mirid predator (*M. pygmaeus*) can learn to respond to volatiles emitted by prey infested plants, and that a mirid predator (*N. tenuis*) can be attracted by plants damaged by conspecifics. We speculate that *N. tenuis* might be the best natural enemy for control of tomato pests because it does not need experience, it responds faster to HIPVs and a substantially larger percentage *N. tenuis* responded to volatiles than *M. pygmaeus*. The only drawback of *N. tenuis* might be its phytophagy during periods of low prey density.

The data presented here have improved the knowledge of how generalist predators interact with their prey and host plant, and also provide important information about the potential use of the predators *C. infumatus*, *E. varians* and *M. basicornis* recently found in Brazil as biological control agents of *T. absoluta* and other pests of tomato as *B. tabaci*. But, of course, other biological characteristics of these predators should be evaluated as well as their efficiency in controlling pests in field and semi-field conditions.