UNIVERSIDADE FEDERAL DE LAVRAS

DIEGO BASTOS SILVA

POPULATION GROWTH, PHYTOPHAGY AND OLFACTORY RESPONSE OF THREE BRAZILIAN MIRID PREDATORY BUGS (HEM.: MIRIDAE) AND HERBIVORE-INDUCED PLANT VOLATILES FROM EITHER *Tuta absoluta* OR *Bemisia tabaci* ON TOMATO

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

Orientadora

Dra. Vanda Helena Paes Bueno

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CRESCIMENTO POPULACIONAL, FITOFAGIA E RESPOSTA OLFATIVA DE TRÊS MIRIDEOS PREDADORES BRASILEIROS (HEM.: MIRIDAE) E VOLATEIS DE PLANTAS INDUZIDOS POR *Tuta absoluta* OU *Bemisia tabaci* EM TOMATE

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Orientadora

Lavras – MG

2016

“Nunca deixe que lhe digam que não vale a pena acreditar no sonho que se tem,
ou que seus planos nunca vão dar certo....
....Quem acredita sempre alcança”

Renato Russo.

A Deus, sempre me dando forças e iluminando meus caminhos.

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GENERAL ABSTRACT

Tomato borer *Tuta absoluta* (Meyrick) and silverleaf whitefly *Bemisia tabaci* (Gennadius) are key pests for 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. However, alternative control methods are needed. Mirid predatory bugs *Macrolophus basicornis* (Stal), *Engytatus varians* (Distant) and *Campyloneuropsis infumatus* (Carvalho) present high capacity for preying tomato pests such as *B. tabaci* and *T. absoluta*, and are now being evaluated as biological control agents. The objectives of this work were to evaluate the population growth of these three mirid species feeding on *T. absoluta* eggs and larvae, as well as to assess the type and risk of damage caused by nymphs and adults on tomato seedlings and fruits in the absence of prey and at high population density. Subsequently, the response of these three mirids to herbivory caused by *T. absoluta* and *B. tabaci* were investigated using Y-tube olfactometer experiments. Finally, a comparative analysis of volatile blends released by tomato plants infested with *T. absoluta*, with *B. tabaci* and with both pests was conducted. Nymphs and adults successfully used *T. absoluta* as prey, although significant differences in the biology of the species were observed. They were able to survive and their populations increased having *T. absoluta* as prey. Both nymphs and adults of the three mirid predators caused feeding rings on the leaflets of tomato seedlings. The injuries consisted of blemishes characterized by feeding punctures surrounded by a yellowish, bleached area. The nymphs of all three mirid species caused injuries to tomato fruit. The type of damage was similar to that occurred on tomato seedling, however, in less quantity. At the end of the evaluations the number of feeding rings on tomato seedling and fruit did not exceed one per individual, and tomato seedlings developed normally. Results of the Y-tube experiments revealed that *T. absoluta* egg deposition did not cause plants to be more attractive to the predators than uninfested plants. *Macrolophus basicornis* is attracted to plants infested with either *T. absoluta* larvae or *B. tabaci* nymphs. *Engytatus varians* and *C. infumatus* responded only to tomato plants infested with *T. absoluta* larvae over uninfested plants. In addition, plants simultaneously infested with both prey species were as attractive to the predators as plants infested with one only of the prey species. A total of 80 volatiles organic compounds (VOCs) was recorded. The difference in VOC composition among the treatments was largely influenced by herbivory, enhancing the emission rate of several compounds. Plants damaged by *T. absoluta* emitted higher levels of many compounds when compared to plants damaged by the phloem-feeder *B. tabaci* and intact plants. In conclusion, *M. basicornis*, *E. varians* and *C. infumatus* populations increased feeding on *T. absoluta*; they do not cause damage on tomato seedlings/fruits and respond to tomato infested with key pests. Thus, they are good candidates for the biological control of *T. absoluta* and other tomato crop pests.

Keywords: *Macrolophus basicornis. Engytatus varians. Campyloneuropsis infumatus*. Miridae. Biological control. Chemical ecology. HIPVs. Zoophytophagy. Tomato

RESUMO GERAL

A traça-do-tomateiro *Tuta absoluta* (Meyrick) e a mosca-branca *Bemisia tabaci* (Gennadius) são pragas-chave na cultura do tomate e podem causar perdas consideráveis quando medidas de controle não são adotadas. O uso de inseticidas é a principal estratégia de controle dessas pragas no Brasil; no entanto, outras medidas de controle devem ser adotadas. Os predadores generalistas *Macrolophus basicornis* (Stal), *Engytatus varians* (distante) e *Campyloneuropsis infumatus* (Carvalho), estão sendo avaliados como agentes de controle biológico de pragas do tomateiro. Estes apresentaram entre outras características, alta capacidade predatória tanto por *B. tabaci* quanto *T. absoluta*. Este trabalho teve por objetivos avaliar o crescimento populacional das três espécies de mirídeos quando alimentados de ovos e larvas de *T. absoluta*. Foi também determinado a caracterização do tipo e o risco de danos em plântulas e frutos de tomate na ausência de presas e em alta densidade de indivíduos. Também em experimentos com olfatômetro em Y, foram avaliadas as respostas comportamentais das três espécies de mirideos aos odores de plantas de tomate sadias, e aos voláteis induzidos por herbivoria em plantas infestadas com *T. absoluta* e *B. tabaci*. Por fim, foi realizada uma análise comparativa dos voláteis emitidos pela planta de tomate infestada com *T. absoluta*, *B. tabaci* e plantas infestadas com as duas pragas simultaneamente. Embora tenham sido observadas diferenças significativas na biologia das espécies, tanto ninfas como adultos predaram ovos e larvas de *T. absoluta*, apresentando altas taxas de sobrevivência e aumento populacional. Ninfas e adultos de *M. basicornis*, *E. varians* e *C. infumatus* causaram injurias em plântulas de tomate. Estas injúrias se caracterizam por puncturas rodeadas por uma área esbranquiçada. Injúrias no fruto de tomate foram causadas somente pelas ninfas dos predadores. O tipo de injúria foi semelhante às plântulas de tomate, porém em menor quantidade. Ao final das avaliações número de injúrias sobre plântulas e fruto de tomate não foram superiores a um por indivíduo e tanto plântulas quanto frutos desenvolveram normalmente. Os experimentos com olfatometro revelaram que a deposição de ovos por *T. absoluta* não tornam as plantas mais atrativas para os predadores comparadas as plantas sadias. *Macrolophus basicornis* é atraído por plantas infestadas tanto por larvas de *T. absoluta* quanto *B. tabaci*. No entanto, *E. varians* e *C. infumatus* responderam apenas para os tratamentos com *T. absoluta*. Plantas infestadas simultaneamente com duas espécies de presas foram atrativas para as três espécies de predadores. Análises químicas registraram um total de 80 compostos. A diferença entre os tratamentos foi influenciada pela herbivoria, a qual apresentou um aumento na taxa de emissão de vários compostos. Plantas danificadas por *T. absoluta* emitiram níveis mais elevados de compostos em comparação com plantas danificadas por *B. tabaci* e plantas sem infestação. Os resultados mostram que a população de *M. basicornis*, *E. varians* e *C. infumatus* aumentaram quando se alimentaram de *T. absoluta*; não causaram danos tanto em mudas quanto em frutos de tomate e responderam a voláteis de plantas de tomate infestadas por suas principais pragas (*T. absoluta* e *B. tabaci*). Assim estes mirídeos têm potenciais como bons candidatos a agente de controle biológico de *T. absoluta* e de outras pragas presentes na cultura do tomate.

Palavras-chave: *Macrolophus basicornis. Engytatus varians. Campyloneuropsis infumatus*. Miridae. Controle biológico. Ecologia química. Zoofitofagia. Tomate.

SUMMARY

FIRST PART

GENERAL INTRODUCTION

The tomato, *Lycopersicon esculentum* Mill, the most common vegetable worldwide, is cultivated outdoors as well as in greenhouses for both fresh market consumption and processing (LANGE; BRONSON, 1981). It is also one of the main vegetable crops in Brazil, which has a significant social and economic importance due large to production areas, mainly in the states of Goias, Sao Paulo and Minas Gerais, and also because involves a lot of labor (MARCHIORI et al., 2004; GAIN REPORT, 2009). Brazil occupies a prominent place in world production of this vegetable with a cultivated area of 65.255 hectares and production of 4.294.912 ton (AGRIANUAL, 2015).

Tomato crops are hosts for many herbivorous insects, and all parts or structures of the plant represent food, shelter and reproductive micro-habitats for insects (LANGE; BRONSON, 1981). Expansion of tomato cultivation in Brazil has led to the development of several pests, which cause considerable damage to the crop, as tomato leaf miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). Since its introduction in Brazil in the 1980s, from Chile, *T. absoluta* has rapidly spread in Brazil and become a serious problem to tomato production and chemical control has been the dominant control strategy for this pest (OLIVEIRA et al., 2008). Currently, many countries are facing *T. absoluta* problems. In Europe *T. absoluta* was accidentally imported and first detected in eastern Spain in 2006 and has since spread to several European, Middle Eastern, North and Midle African and Asian 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). Originated from the Middle East-Asia

Minor region (DE BARRO et al. 2011), it has become a cosmopolitan invasive pest species in subtropical and tropical areas (OLIVEIRA et al. 2001, DE BARRO et al. 2011). 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 (DE 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.

As a result of concern of fast development of resistance by insects to pesticides, human health problems, environmental pollution, and the interest to utilize sustainable agricultural methods, application of biological control is an essential component which provides long-term pest control and has several benefits in comparison to chemical control (BUENO; VAN LENTEREN 2012; MOLLA et al., 2014). It includes methods that combine pests with their natural enemies or which can lead to modifications to the environment (or to the natural enemy itself) that favor natural enemy population growth and its impact on pest dynamics. Its strategies have been used against arthropod pests in Solanaceous crops and have proven to be effective and reliable (VAN LENTEREN, 2012).

In Brazil there is not yet a strategy of biological control for *B. tabaci* (HAJI et al., 2005) or *T. absoluta*. Currently, releases of the egg parasitoid

Trichogramma pretiosum (Riley) (Hymenoptera: Trichogrammatidae) in limited scale can be done (PARRA; ZUCCHI, 2004) to control *T. absoluta*. 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).

Heteropteran predators are widely recognized and reported to significantly contribute to the biological control of pests (BUENO; VAN LENTEREN 2012), they are among the most commonly used agents in augmentative biological control, comprising about 8.3 % of all arthropod natural enemies used in pest management worldwide in many agricultural systems, including cotton, soybeans, corn, alfalfa, various vegetables and horticultural crops (NARANJO; GIBSON, 1996; VAN LENTEREN 2012). In Brazil and other South American countries limited research has been done on predators as biological control agents of *T. absoluta* and *B. tabaci* (DESNEUX et al., 2010; BUENO; LENTEREN, 2012)

In Europe, Africa and Asia, predators of the family Miridae are largely used in augmentative biocontrol of important pests in Solanaceous crops (VAN LENTEREN, 2012; LYKOURESSIS et al., 2014; MOLLA et al., 2014; ABBAS et al., 2014; PÉREZ- HEDO; URBANEJA, 2015). During the last decade, biological control strategies for various major pests have been successfully implemented in greenhouse crops in southern Europe by using mirid predators (CALVO et al. 2009, 2011; VAN LENTEREN 2012).

One of the two most recent and remarkable successes of biological control have occurred in tomato production, with two mirid predators *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) (both Hem.: Miridae) which are effective in controlling the key tomato pest, the South American tomato borer *T. absoluta* (URBANEJA et. al., 2012; GONTIJO et al. 2013; PEREZ-HEDO; URBANEJA, 2015). However, they are bugs which possess piercing-sucking mouthparts and show zoophytophagy: they feed on both animal prey and on plants (WHEELER, 2001, CASTAÑÉ et al., 2011; INGEGNO et. al., 2011).

Until recently there are no reports about the efficacy of mirid predators to control the tomato borer pest in Brazil. Therefore, Bueno et al. (2013) started to explore and evaluate natural enemies. They found, among others, the three mirid predators *Macrolophus basicornis* (Stal 1860), *Engytatus varians* (Distant 1884) and *Campyloneuropsis infumatus* (Carvalho 1947). These mirids are distributed throughout Latin America (CARVALHO; FERREIRA, 1972; CASTINEIRAS, 1995; FERREIRA; SILVA; COELHO, 2001; FERREIRA; HENRY, 2011; MELO et al., 2004; MARTINEZ et al., 2014). *E. varians* is reported preying *B. tabaci*, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) and tomato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) in several crops including tomato (CASTINEIRAS 1995; LUNA et al., 2006; MARTINEZ et al., 2014). According to Bueno et al. (2012) *C. infumatus*, *E. varians* and *M. basicornis* present characteristics which enable them to be promising candidates to control *T. absoluta*. They can walk and reproduce on tomato, prey on eggs and larvae of *T. absoluta*, have a fast development, a high fecundity and survival.

The successful use of these predators as biological control agents of tomato pests may depend of some criteria used for evaluation of natural enemies. Life table for example provide important information to the development of models that have shown useful in the study of insect population dynamics (GILBERT et al., 1976). In addition, life tables are simple and effective means of reducing the volume of data and present them in a quickly analyzable, and thus, evaluate features such as mortality, survival, longevity, reproduction and life span of the study population (COPPEL; MERTINS 1977). Such studies are important to the development of strategies for pest control, facilitating the integrated pest management (HIGLEY et al., 1986). After determining the life tables of *M. pygmaeus* and *N. tenuis*, Mollá et al. (2014) concluded that successfully adopted *T. absoluta* as prey. *Nesidiocoris tenuis* could both develop and reproduce on this prey. On the other hand, although the immature stages of *M. pygmaeus* consumed significantly more *T. absoluta* eggs than *N. tenuis* did, *M. pygmaeus* females are not able to reproduce when feeding only on *T. absoluta* eggs and tomato. Several biological parameters, like survival, nymph development and sex ratio, of *M. basicornis*, *E. varians* and *C. infumatus* when feeding on *Ephestia kuehniella* (Zeller) eggs were determined by Montes (2013). He found that the interval between 24 and 28°C is optimal for their development and survival. However, studies have not yet examined the role of these three mirids as predators of *T. absoluta*, or the influence of this prey on the predator development and reproduction.

Another important factor is the zoophytophagous behavior. As was previously reported, this group of predators is zoophytophagous, they can use food from more than one trophic level by feeding on both plants and prey during the same developmental stage. (PIMM AND LAWTON, 1978; COLL; GUERSHON, 2002). Crop damage caused by species of the family Miridae has been described for several predators' species (ARNÓ et al., 2010; CASTAÑE et al., 2011; CALVO et al., 2009; 2012). The feeding symptoms can be characterized by several types of lesions observed on the stems, leaves or flower petioles. Particularly on tomato, feeding behavior of some mirid species may reduce the vigor of the plant and/or cause flower abortion (ARNÓ et al., 2010; CALVO et al., 2011; 2012). On tomato fruit, injuries are well recognized by feeding punctures surrounded by whitish halos, blemishes, scars and fruit deformations (ALOMAR; ALBAJES, 1996; CALVO; URBANEJA 2003). However, injuries can be variable and depend on the mirid species and their developmental stage.

As a result, use of plant-feeding predators for biological pest control has traditionally been neglected, mainly due to the risk of their feeding on crop plants and causing significant economical damage (CASTAÑE et al., 2011). *Nesidiocoris tenuis* and *M. pygmaeus* may cause damage on tomato plants (SANCHEZ, 2008; CALVO et al., 2009; MOERKENS et al., 2015), but they also offer advantages for biological pest control. They are generalist predators that have an impact on several crop pests. They may also be able to establish on crops early in the growing season, when pests colonize them, and can remain on the target crop when prey is scarce. The damage capacity of *M. basicornis, E. varians* and *C. infumatus* has not been measured so far. Elucidating their risk of causing damage on tomato plants is of crucial importance to know if they can be integrated in biological control programs. I have studied the zoophytophagous behaviour of three Brazilian mirids.

Next to knowing the development of the mirids and their potential plant damage, it is interesting to know if they are attracted to infested tomato plants. Tomato plants have evolved numerous defense mechanisms that are effective against insect pests like glandular trichomes (SIMMONS et al., 2006) and also by regulating the biosynthesis of a wide array of proteins and metabolites that range from protease inhibitors (GREEN; RYAN, 1972) to volatile organic compounds (VOCs) (DICKE; VAN LOON; SOLER, 2009). VOCs may play multiple roles as communication signals and defense agents, mediating interactions with other plants, microorganisms and animals (HEIL, 2008; PANGESTI et al., 2013). It has long been known that plants attacked by herbivorous insects emit complex blends of VOCs that are used by predators and parasitoids in searching for their prey or hosts, commonly known as herbivore-induced plant volatiles (HIPVs) (DICKE; SABELIS, 1988; TURLINGS et al., 1990; GEERVLIET et al., 1997).

There is some, but rather limited, information about the response of mirid predators to HIPVs. It is known that *N. tenuis* and *M. pygmaeus* are attracted by prey-infested plants (MOAYERI et al., 2006a; MOAYERI et al., 2006b, INGEGNO et al., 2013; MOAYERI et al., 2007; LINS JR et al., 2015). Attractiveness of plant volatile blends induced by *T. absoluta* and *B. tabaci* have also been demonstrated for *Dicyphus errans* and *D. maroccanu* and *D. Hesperus* (MCGREGOR; GILLESPIE 2004; INGEGNO et al. 2013; PÉREZ-HEDO; URBANEJA 2014; ABBAS et al., 2014).

Differences in the attractiveness of a natural enemy may be explained by differences in quality and quantity of the emitted blend. It can vary according to plant variety and physiological condition, environmental conditions, identity and stage of the herbivore as well as duration and conditions of infestation prior to volatile collection (DUDAREVA et al. 2006; NIINEMETS et al., 2013).

Thus, in order to provide information about the possible use of the predatory mirid bugs *C. infumatus*, *E. varians* and *M. basicornis* as biological control agents of tomato pests, we evaluated (1) the population growth dynamics of the Brzilian mirids when feeding on *T. absoluta* eggs and larvae, (2) their capacity of causing damage on tomato seedling and fruit. Next, (3) the behavioral response of these three mirids to odors of clean tomato plants, and to volatiles induced by herbivory of infested plants with *T. absoluta* and *B. tabaci*, as well as, identification of volatiles blend released by tomato plants infested by either *T. absoluta* or *B. tabaci*. Finally, (4) a comparative analysis of volatiles blend attractive to European mirid predators released by tomato plant infested with *T. absoluta*, tomato plant infested with *B. tabaci* and tomato plants infested with both pests were determined.

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SECOND PART - ARTICLES

ARTICLE 1 Population growth of three mirid predatory bugs feeding on eggs and larvae of *Tuta absoluta* on tomato

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Population growth of three mirid predatory bugs feeding on eggs and larvae of *Tuta absoluta* on tomato

Abstract *Tuta absoluta* Meyrick quickly developed into a significant pest of tomatoes worldwide. While the mirid bugs *Macrolophus basicornis* (Stal), *Engytatus varians* (Distant) and *Campyloneuropsis infumatus* (Carvalho) prey on this tomato borer, their biologies have not been well characterized. Using a mixture of *T. absoluta* eggs and larvae as food on tomato, mirid life histories were studied. Nymphs and adults successfully used *T. absoluta* as prey, although significant differences in biology of the species were observed. *Campyloneuropsis infumatus* and *M. basicornis* were quickest and slowest to become adult at 16 and 19 days, respectively. Nymphal survival was high and varied from 70 to 75%. Female biased sex ratios were observed for *C. infumatus* (0.80) and *M. basicornis* (0.71), but not for *E. varians* (0.56). Intrinsic rates of increase for *C. infumatus*, *E. varians* and *M. basicornis* were 0.11, 0.10 and 0.11 respectively. Our results indicate rapid development and population increase of these three mirids, making them interesting candidates for biological control of *T. absoluta* on tomato.

Keywords: Mirid Predator; Life table; Reproduction; *Campyloneuropsis infumatus*, *Engytatus varians*, *Macrolophus basicornis*

Introduction

The tomato borer *Tuta absoluta* Meyrick (Lep.: Gelechiidae) is quickly developing into a serious, worldwide pest of tomato (Desneux et al. 2011; Pfeiffer et al. 2013; Mohamed et al. 2015; Silva et al. 2015). Its larvae form leaf mines, but also penetrate the stems and fruit, and, as a result, tomato yields can be completely lost if no control methods are used. Control of mining insects such as *T. absoluta* demands frequent application of chemical pesticides which results in rapid development of resistance to these pesticides, necessitating application of new active ingredients in pesticides and/or a search for non-chemical control methods such as biological control. Use of biological control agents requires a fundamental understanding of their behavior and life history.

The pest *T. absoluta* is able to colonize both protected and open-field tomato crops throughout the plant growth cycle, and proliferates in a wide range of environmental conditions (Miranda et al. 1998; Desneux et al. 2010). In South America, *T. absoluta* is still mainly controlled with chemical pesticides. However, use of frequently applied conventional pesticides is considered problematic due to environmental pollution, negative effects on human health and the development of pests that become resistant to these pesticides. As a result, design of sustainable pest management methods not resulting in the above mentioned negative effects is an important research issue. One of these sustainable methods is biological pest control. This strategy provides long-term pest control as well as early protection and prevents or minimizes the need for chemical pesticides. Commercial biological control has been effectively and reliably implemented for the use against many agricultural arthropod pests and recently, the use of generalist heteropteran predators for control of *T. absoluta* has become popular (van Lenteren 2012).

Generalist heteropteran predators are widely recognized to significantly contribute to biological control of pests (Bueno and van Lenteren 2012). They are among the most commonly used predators in augmentative biological control, comprising about 8% of all arthropod natural enemies used in pest management worldwide (van Lenteren 2012). Particularly, predators of the family Miridae are used in biological control of Solanaceous pests (Calvo et al. 2011; van Lenteren 2012; Mollá et al. 2013; Abbas et al. 2014; Lykouressis et al. 2014; Pérez-Hedo and Urbaneja 2014). During the last decade biological control strategies have been successfully implemented in tomato greenhouse crops in

Mediterranean Europe (Calvo et al. 2011; Calvo et al. 2012; van Lenteren 2012), with two mirid predators (*Nesidiocoris tenuis* (Reuter)) and *Macrolophus pygmaeus* (Rambur) (Hem.: Miridae) providing effective control against the accidentally introduced *T. absoluta* (Urbaneja et al. 2012; Gontijo et al. 2013; Pérez-Hedo and Urbaneja 2014).

Although *T. absoluta* originates from South America and is since long known as an important pest, research on predators of this pest has only started recently. Bueno et al. (2013) reported the first promising results about the mirid predators *Macrolophus basicornis* (Stal), *Engytatus varians* (Distant) and *Campyloneuropsis infumatus* (Carvalho) (Hem.: Miridae) when exposed to *T. absoluta* on tomato. These predators are able to walk and reproduce on tomato plants (Bueno et al. 2012), prey on eggs and larvae of *T. absoluta*, and they show high fecundities and survival rates on tomato (Bueno et al. 2013). The objective of the study reported in this paper is to provide information about the life history traits of *C. infumatus*, *E. varians* and *M. basicornis* when feeding upon a mixture of eggs and 1st instar larvae of *T. absoluta*.

Material and Methods

Collection and rearing of *T. absoluta*

Eggs, larvae and pupae of *T. absoluta* were collected from tomato plants at the experimental area of the campus of the Federal University of Lavras. Recently emerged adults from the collections were released in a mesh cage (5 x 3 x 2.5 m) with tomato plants (cv. Santa Clara) inside a greenhouse. New tomato plants were introduced regularly to the cage to the stock colony of *T. absoluta*. Eggs and 1^{st} instar larvae used as prey in the experiments were collected from this stock colony.

Collection and rearing of the mirid predators

A survey for mirid predators was done on tobacco *Nicotiana tabacum* L. located in the municipalities of Ribeirão Vermelho and Lavras, Minas Gerais State, Brazil (21°08.596′S and 045°03.466′W, 808 m of altitude). Nymphs and adults were collected and identified on basis of visual morphological characters according to the key of mirid species of Ferreira and Henry (2011). Later, specimens were sent for confirmation to the mirid specialist P. S. F. Ferreira (Dept. of Animal

Biology, Federal University of Viçosa, Viçosa, Brazil). Three mirid species were identified: *C. infumatus*, *E. varians* and *M. basicornis.*

Stock colonies of these mirids were maintained in the laboratory according to the methodology described in Bueno et al. (2013). Adults of each species collected from the field were released individually in acrylic cages (60 x 30 x 30 cm) containing tobacco plants (*Nicotianna tabacum* L. cv. TNN) as an oviposition substrate and a water source. *Ephestia kuehniella* (Zeller) (Lep.: Pyralidae) eggs were offered *ad libitum* as food. Plants and adult predators remained in the cages for a period of seven days. Then, plants containing mirid eggs were transferred to new cages. After the nymphs emerged, *E. kuehniella* eggs were added twice weekly until adults emerged. For experiments, adult predators were transferred to new cages containing a tomato plant (*Solanum lycopersicum* cv. Santa Clara). After the nymphs emerged, *E. kuehniella* eggs were added twice per week as food. The stock colonies on tomato were kept in a climate room at 24 ± 1°C, RH 70 ± 10% and 12-h photophase. After two generations on tomato, individuals from these stock rearings were used in the experiments.

Developmental time and nymph survivorship of the mirid predators

Newly-emerged female predators were kept in jars with tomato seedlings (15 cm in height) in acrylic cages (60 x 30 x 30 cm) to lay eggs for 24h. Next, the seedlings were transferred to new acrylic cages and observed daily for emergence of nymphs. Forty newly-emerged nymphs of each mirid species were individually placed in a glass jar (1.7 l). The glass jars contained a plastic cup (8.5 cm diameter x 5.5 cm high) with a tomato seedling (cv Santa Clara) 20 days old after transplanting, with 3 leaves. The glass jar was covered with organza on the top to avoid escape of the nymphs. Nymphs were supplied with a mix of 70% *T. absoluta* eggs and 30% 1st instar larvae, *ad libidum* until the emergence of the adults. Prey eggs and larvae were carefully placed on the tomato leaves with the aid of a thin brush. Tomato seedlings were replaced three times a week. *Tuta absoluta* 1st instar larvae + eggs were carefully placed on the tomato leaves of the new seedlings with the aid of a thin brush (Molla et al., 2014). Daily, tomato seedlings were removed from the glass jars to determine the presence of nymphal exuviae by using a magnifier (10x). With these exuvial data we could determine nymphal development (Molla et al. 2014). The number and duration of each instar, total nymphal development time, nymphal survival and sex ratio for each species of mirid predator were determined. The study was carried out in a climate room at 24± 1°C, RH 70 ± 10% and 12h photophase.

Reproduction and longevity of the mirid predators

Twenty five pairs of 1 day old females and males originating from the nymphal development experiment were kept individually in glass jars (1.7 l) covered with organza. The glass jars contained a plastic cup (8.5 cm diameter x 5.5 cm high) with a tomato seedling (cv. Santa Clara) of 20 days old after transplanting and with 3 leaves, which was used as oviposition substrate. Every three days, a mixture 70% of eggs and30% of 1st instar larvae of *T. absoluta* were given as prey *ad libidum*.

It was very difficult to see the eggs of the mirids in the stems and veins of tomato because of the high number of trichomes and low cuticular transparency. We have done several tests where we counted eggs and nymphs, and always found more nymphs than eggs (Silva et al. unpublished). Therefore we decided to count nymphs instead of eggs as follows: we removed the seedlings with eggs every day and replaced them with fresh ones. Next, the seedlings on which oviposition occurred were placed in Petri dishes (20cm Ø) and observed daily for emergence of nymphs. We realize that these data about nymphal emergence might be underestimation of female fecundity. The study was carried out in climatic chamber, at 24± 1°C, RH 70 ± 10% and 12h photophase. Adult mortality, the pre-oviposition, oviposition and post-oviposition periods, and an estimate of fecundity based on nymphal emergence (daily and total) were recorded. Longevity was recorded separately for males and females. The data were used to construct a fertility life table.

Statistical analysis

The experimental design consisted of three treatments representing the three mirid species *C. infumatus, E. varians* and *M. basicornis*. Before analysis, the raw data were tested for normality and homogeneity of variances using the Shapiro-Wilk and Chi-squared analysis test, respectively and data were log transformed if necessary. Nymphal development, pre-oviposition, ovipositon and post-ovipositon periods, and longevity were submitted to one-way analysis of variance (ANOVA) and when significant differences among averages were found, Tukey’s HSD test at $p < 0.05$ level of significance was applied. Nymphal survival data were analyzed using Kaplan–Meier survivorship curves, and differences between mirid species were analyzed using a Gehan–Breslow–Wilcoxon test; differences were considered significant at $P<0.05$. A Chi-square analysis at $p < 0.05$ was used to compare the observed numbers of males and females with the number of each sex expected under sex ratio equality. For both tests, the statistic software R Development Core Team (2014) was used.

Life tables were determined using daily survival values and the number of progeny produced by the females, starting with nymphal emergence. Data about egg development time were obtained from Montes et al. (2013). The following demographic growth parameters were calculated: (1) the net reproductive rate where x is the pivotal age of individuals in days, lx is the age-specific survival as proportion of individuals still alive at age x and mx is the age-specific fertility; (2) the generation time (T), where T represents the average time interval separating female births of one generation from the next; (3) the intrinsic rate of increase (r_m), which is the innate capacity of a species to increase in numbers per unit of time (Birch 1948); the doubling time, where DT is the time required for a given population to grow exponentially, to double in size when increasing at a given r_m (Mackauer 1983). These parameters were estimated using the computing program of (Maia et al. 2000) in the software package SAS (SAS institute 2000). This program included both a Jackknife test to estimate confidence levels and a one-way analysis of variance (ANOVA). When significant differences among averages were found, Tukey's HSD test at $p < 0.05$ level of significance was applied to compare all parameters (Maia et al. 2000).

Results

Development and survival of nymphs, and adult sex ratio

All three mirid species showed five nymphal instars when developing on eggs + 1st instar larvae of *T. absoluta* (Table 1). The development of the 1st nymphal instar of *E. varians* was significantly slower (3.9 days) ($F = 13.44$; $df = 2$; $P = 0.001$) than that of *C. infumatus* and *M. basicornis* (Table 1). *Macrolophus basicornis* took more time ($F = 37.7$; $df = 2$; $P = 0.001$) to develop from the 3rd to the 4th nymphal instar than the other species (Table 1). The duration of the 5th nymphal instar of

C. infumatus was longer (4.5 days) (F = 11.56; df = 2; P = 0.001) than that of *E. varians* (3.7 days) and *M. basicornis* (3.8 days) (Table 1).

The total nymphal developmental time significantly differed amount the three species, (F = 24.57; df = 2; P = 0.001). *Campyloneuropsis infumatus* took 16.2 days to become adult, while *E. varians* took 17.4 and *M. basicornis* took 18.6 days.

Seventy five percent of *E. varians* and *C. infumatus*, and 70% of *M. basicornis* nymphs reached adulthood (Fig. 1). No significant differences in age-specific survival were found for the three mirids (P >0.05). However, the three species differed significantly in the proportion of emerged females and males (χ^2 = 115.38; df = 12; P = 0.001). The sex ratios were 0.80, 0.71, 0.56, respectively, for *C. infumatus*, *M. basicornis* and *E. varians* (Table 2).

Pre-oviposition, oviposition and post-oviposition periods, fecundity and longevity

The pre-oviposition periods of the three mirid species fed on eggs + 1st larvae instar of *T. absoluta* significantly differed (F = 5.65; df = 2; P = 0.004). *Campyloneuropsis infumatus* showed the shortest (3.5 days) and *E. varians* the longest (4 days) pre-oviposition period (Table 2).

When having eggs + 1st larvae instar of *T. absoluta* as food and tomato as the host plant, the three mirid species reproduced well. The oviposition period was longer than 20 days, but *C. infumatus* has a significantly shorter oviposition period (22 days) than *M. basicornis* (25 days) (F = 4.25; df = 2; P = 0.017). Egg numbers are estimated based on nymphal emergene data. Comparisons among the three mirids showed that *M. basicornis* females laid significantly more eggs (124 eggs) than *E. varians* (106 eggs), which in turn laid more eggs than *C. infumatus* (81 eggs) (Table 2). The mean number of eggs per female per day ranged from 4 to 5 eggs for the three mirids. *Macrolophus basicornis* laid the highest number of eggs/female/day (5 eggs, Table 2).

The highest amount of eggs laid by the three mirids was at the fifth day, so at the second day of their oviposition period (Fig. 1). During the first week of oviposition, the number of eggs laid remained high after which egg laying gradually decreased (Fig. 1). The post-oviposition period was similar for the three mirids (F = 2.42; df = 2; P = 0.094), and ranged from 3.4 days (*C. infumatus* and *M. basicornis*) to 3.6 days (*E. varians*).

Longevity of the mirid females was approximately 30 days, but *C. infumatus* lived significantly shorter (28.4 days) than *E. varians* (32.2 days) and *M. basiconis* (32.1 days) (F = 10.63; df = 2; P = 0.001) (Table 2). Mirid female survival was high for about 24 days and then sharply declined (Fig. 1). Male longevity significantly differed among the mirids (F = 11.75; df = 2; P = 0.001); it was longest for *M. basicornis* (29.5 days), and similar for *C. infumatus* and *E. varians* (24.3 and 26.1 days) (Table 2). Generally, for all three mirid species, females lived at least three days longer than males (Table 2).

Population growth parameters

Macrolophus basicornis had a much higher net reproduction rate ($R_0 = 86.67$) than *C. infumatus* and *E. varians* (64.97 and 59.53, respectively (Tukey test; F = 48.17; df = 2; P < 0.001). The mean generation time (T) was longest for *M. basicornis* (Tykeytest; F = 32.65; df = 2; P < 0.001), followed by *E. varians* (Tukey test; F = 37.77; df = 2; P < 0.003) and *C. infumatus* (Tukey test; F = 36.59; df = 2; P < 0.001) (Table 3). The r_m value was similar (0.11 – 0.12) for the three mirid species (Tukey test; F = 0.10; df = 2; P = 0.258). The doubling time (DT) of the mirids (about 4 days) was also similar (Table 3) (Tukey test; F = 6.84; df = 2; P = 0.158).

Discussion

Of the three mirid species we have studied, only *E. varians* was earlier found on tomato in Cuba (Castineiras 1995) and Mexico (Martinez et al. 2014). Initially, we tried to collect predators of the tomato borer on tomato but hardly found any individuals. We suppose that the frequent sprays – 3 to 5 times per week during the whole production cycle – with cocktails of pesticides eliminate natural enemy populations (Bueno et al. 2012). Next, we successfully collected predators including the three mirid species on a tobacco crop which was rarely sprayed (Bueno et al. 2013). Meanwhile we have studied the reaction of the three mirid species to volatiles of uninfested and *T. absoluta* infested tomato plants. We found that (1) they are attracted to uninfested tomato plants in a Y-tube olfactometer test with volatiles of tomato in one arm and clean air in the other arm of the olfactometer, and (2) that significantly more mirids went to the olfactometer arm with volatiles of *T. absoluta* infested tomato than to the arm with uninfested tomato (Silva et al. 2015 unpublished). These olfactometer results indicate that volatiles produced by both tomato and the tomato borer attract the mirid predators. Also, the three mirid species successfully developed and reproduced on tomato with *T. absoluta* eggs and larvae as a food source. The developmental times of *E. varians* and *C. infumatus* with *T. absoluta* as food were similar to those with *E. kuehniella* as food (Montes 2013). Developmental times were similar to that of the European predator *M. pygmaeus* with *E. kuehniella* as food (Vandekerkhove and De Clercq 2010). The mirid predator *Dicyphus errans* (Wolff) took six days more to reach adulthood than the three mirids we studied, when feeding on *T. absoluta* eggs (Ingegno et al. 2013). Differences in developmental time have also been observed in other Dicyphine species reared on different diets, such as artificial food, bee pollen, various other prey and plants, and some of these diets were not suitable for mirids predator, resulting in considerable nymphal mortality (Gillespie and McGregor 2000; Perdikis and Lykouressis 2000; Castañé et al. 2002).

The three mirids showed immature survival rates higher than 70%, suggesting that *T. absoluta* as prey and tomato as a host plant provide a suitable environment for development. Similar nymphal survival was found for the mirids *N. tenuis* and *M. pygmaeus* having *T. absoluta* and *E. kuehniella* as prey (Urbaneja et al. 2005; Mollá et al. 2013). High nymphal survival might be the result of a combination of suitable prey and nourishment from the tomato plant, which young Dicyphine nymphs need to complete their development (De Puysseleyr et al. 2012).

The percentage of females found for *E. varians* was 56%, but much higher percentages were found for *M. basicornis* (71%) and *C. infumatus* (80%). Little is known about the variation of sex ratios in mirid predators (Wheeler 2001) and we have no explanation for the female biased sex ratios in two of the three mirids. If such high sex ratios are a characteristic of these species, it is a positive aspect as their population growth will be faster than for species with equal proportion of females to males.

The numbers of eggs laid (in this study expressed as the number of emerged nymphs) by *M. basicornis* and *E. varians* were superior to that of *C. infumatus*. Different egg laying capacities may be an intrinsic characteristic of these species, because Montes (2013) recorded the same differences with *E. kuehniella* was as food. Differences in egg laying capacity can partially be related to the differing degree of zoophytophagy among mirid species (Mollá et al. 2013). In our study, *M. basicornis* and *E. varians* may have been able to profit optimally from the nutrition offered by a mix of *T. absoluta* and tomato. Mollá et al. (2013) reported that the egg laying capacity of *N. tenuis* is much higher than that of *M. pygmaeus* on tomato with *T. absoluta* as food, suggesting that this prey is not a suitable food source for the population development of this predator species. In addition, *N. tenuis* is a plant feeder and is able to compensates inadequate nutrition of prey by feeding on plants (Sanchez 2008).

Information about life table parameters of *C. infumatus*, *M. basicornis* and *E. varians* when preying upon tomato pests has been lacking, and the current study is the first to deal with this issue. Mollá et al. (2013) recorded the life table parameters of *M. pygmaeus* and *N. tenuis* when fed on *T. absoluta* eggs under similar laboratory conditions as we used. These predators are successfully used in Europe for control of whitefly, *T. absoluta* and other tomato pests (Bonato et al. 2006; Rasdi et al. 2009; Urbaneja et al. 2012). It is, therefore, interesting to compare population growth data of the Brazilian and European species. The intrinsic rates of increase of *C. infumatus*, *E. varians* and *M. basicornis* (all around 0.11) are considerably higher than that of *M. pygmaeus*

(0.005), and slightly higher than that of *N. tenuis* (0.089) (Molla et al. 2013). Another study showed a low r_m (0.04) for *M. caliginosus* when preying on greenhouse whitefly, *T. vaporariorum* (Hamdan 2006), and for *Pilophorus typicusis* (Distant) (r_m = 0.07) when preying on *A. kuehniella* (Nishikawa et al. 2010). We realize, however, that the intrinsic rate of increase provides information about the potential rate of population growth, and that for a more critical comparison of candidates for *T. absoluta* control, data about their prey kill rate needs to determined (Tommasini et al. 2004; van Lenteren 2010). Therefore, we are currently collecting life time predation data for the three mirids. However, the fact that the r_m data of the three Brazilian mirids compare very positively with those of two successful European mirid predators indicates that these Brazilian species might be interesting candidates for control of *T. absoluta.*

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Tables

Table 1 Developmental time of nymphal instars (days) (± SE) of *C. infumatus*, *E. varians* and *M. basicornis* preying on eggs + 1st instar larvae of *T. absoluta* on tomato at 24 ± 1°C, RH 70 ± 10% and 12h photophase.

Instar	***C. infumatus***	**N**	***E. varians***	**n**	***M. basicornis***	**n**
N_1	3.2 ± 0.10 A	40	3.9 ± 0.11 B	40	3.4 ± 0.08 A	40
N_2	2.5 ± 0.11 A	38	3.5 ± 0.11 B	40	3.6 ± 0.09 B	40
N_3	2.5 ± 0.09 A	38	2.8 ± 0.10 A	36	3.7 ± 0.08 B	35
N_4	3.3 ± 0.08 A	34	3.3 ± 0.11 A	36	4.0 ± 0.07 B	32
N_5	4.5 ± 0.11 B	32	3.7 ± 0.12 A	32	3.8 ± 0.12 A	29
N1-N5	16.1 ± 0.19 A	30	17.4 ± 0.31 B	31	18.6 ± 0.20 C	28

*Means followed by same letter in the lines do not significantly differ, Tukey test ($P < 0.05$).

n= number of individuals

Table 2 Sex ratio, pre–oviposition, oviposition and post-oviposition periods (days) , daily and total fecundity, female and male longevity (days) (mean ± SE) of *C. infumatus, M. basicornis* and *E. varians* when preying on eggs + 1^{st} instar larvae of *T. absoluta* on tomato at 24 ± 1°C, RH 70 ± 10% and 12h photophase

Parameters	**n**	***C. infumatus***	***E. varians***	***M. basicornis***
Sex ratio*	25	0.80 ± 0.4 a	0.56 ± 0.7 c	0.71 ± 0.9 b
Pre – oviposition	25	3.5 ± 0.09 b	4.0 ± 0.08 a	3.7 ± 0.13 ab
Oviposition	25	22 ± 0.92 b	24 ± 0.40 ab	25 ± 0.45 a
Post – oviposition	25	3.4 ± 0.13 a	3.6 ± 0.19 a	3.4 ± 0.15 a
Daily fecudity	25	4 ± 0.20 b	4.3 ± 0.09 b	5 ± 0.14 a
Total fecundity	25	81.3 ± 1.59 c	106.9 ± 1.30 b	124.1 ± 1.55 a
Female longevity**	25	28.4 ± 0.66 Ba	32.2 ± 0.42 Aa	32.1 ± 0.52 Aa
Male longevity**	25	24.3 ± 0.91 Bb	26.1 ± 0.93 Bb	29.5 ± 0.67 Aa

All data: means followed the same lower case letter in the lines do not differ significantly, Tukey test (P<0.05).

*Means followed by the same letter in the line for the sex ratio data do not differ significantly, Chi-square test (P < 0.05).

**Means followed by the same capital letter in the colums for the longevity data do not differ significantly, Tukey test (P<0.05).

Table 3 Intrinsic rate of increase (r_m), net reproductive rate (R_0) , generation time T (days), generation doubling time DT (days) and the finite rate of increase (λ) (all values ± SE) of *C. infumatus*, *M. basicornis* and *E. varians* when preying on eggs + 1st instar larvae of *T. absoluta* on tomato, at 24 ± 1°C, RH 70 ± 10% and 12h photophase.

Species	**R_0**	**T**	**r_m**	**DT**	**λ**
C. infumatus	64.97 ± 0.48a	34.99 ± 0.14a	0.1192 ± 0.001a	4.31 ± 0.05a	1.1266 ± 0.002a
E. varians	59.53 ± 0.52 a	37.89 ± 0.12b	0.1078 ± 0.001a	4.73 ± 0.05a	1.1138 ± 0.001a
M. basicornis	86.67 ± 1.02b	40.29 ± 0.14c	0.1107 ± 0.001a	4.59 ± 0.06a	1.1170 ± 0.001a

Means followed by the same letter in columns do not significantly differ, Tukey test ($P < 0.05$).

Figure

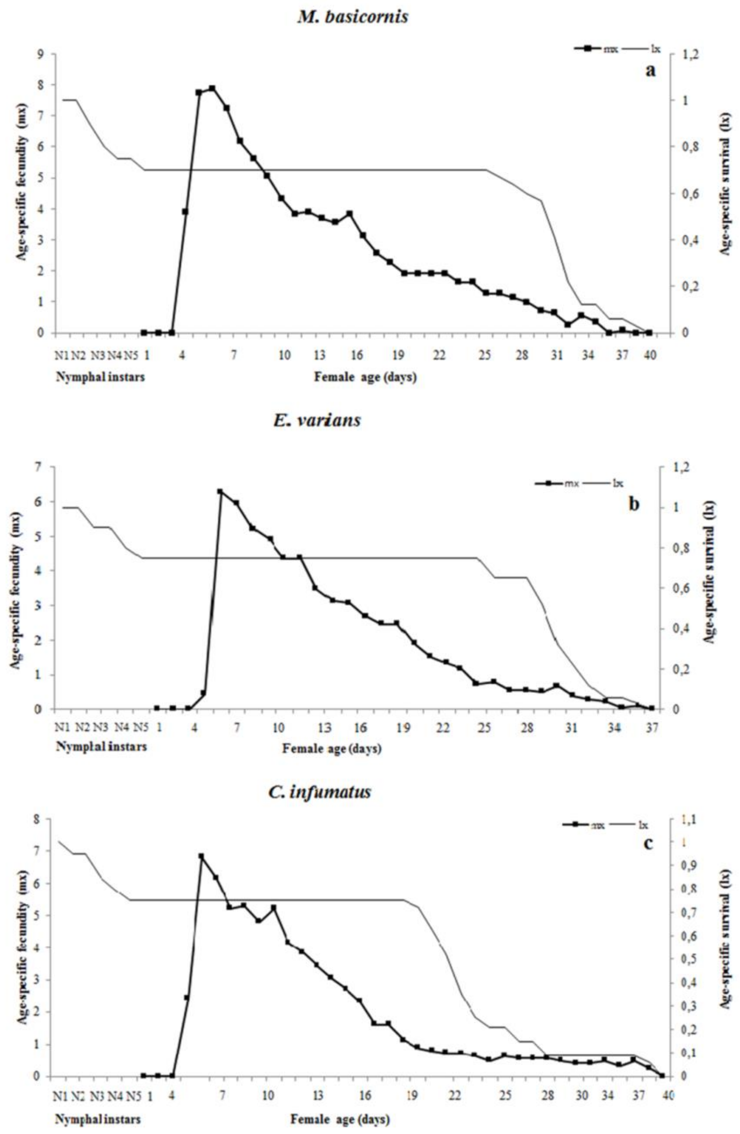

Fig. 1 Instar-specific nymphal survival (%) (±SE), age-specific survival rate (lx) and age-specific fecundity (mx) of *C. infumatus*, *M. basicornis* and *E. varians* preying on egg + 1st instar larvae of *T. absoluta* on tomato plants at 24 ± 1°C, RH 70±10% and 12h photophase.

ARTICLE 2 Do three Brazilian zoophytophagous predatory mirid bugs injury seedlings and fruits of tomato plants?

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Abstract The mirid predatory bugs *Macrolophus basicornis* (Stal), *Engytatus varians* (Distant) and *Campyloneuropsis infumatus* (Carvalho), are able to consume large numbers of tomato pests like *Bemisia tabaci* (Gennadius) and *Tuta absoluta* (Meyrick). However, they are zoophytophagous and feed on plants as well. The aim of this study was to evaluate the type and effect of injury by nymphs and adults of the three predators on tomato seedlings and fruit in the absence of prey. For each mirid species, twenty nymphs were kept inside PVC tubes with tomato seedling for 72 h, and four nymphs with tomato fruits for 48 h. In case of adults, twenty were kept inside the tubes with seedlings for 72 h and four adults for 72 h with tomato fruits The characterization and the number of feeding rings on the stem, petiole and each leaflet of tomato seedlings and on tomato fruits were recorded after the removal of the nymphs. Nymphs and adults of the three mirids caused feeding rings on the leaflets of the tomato seedlings. However, no feeding rings or scars on stem and petioles were observed. The feeding rings on leaflets consisted of blemishes, which were characterized by feeding punctures surrounded by a yellowish, bleached area. The number of feeding rings did not exceed one per individual predator and the tomato seedling developed normally. Nymphs also caused similar feeding rings on tomato fruit, however, in even lower numbers than on leaflets. After two weeks the tomato fruits were still fresh and no feeding rings were found. The mirid adults did not cause any injury on tomato fruit. The results indicate that nymphs and adults of these three zoophytophagous mirids cause little injury to tomato seedling and fruit, even when present in high density and in the absence of prey. Further studies should explore their potential to damage tomato plants after a longer interaction with the tomato plants in the field or greenhouse.

Keywords: *Macrolophus basicornis. Engytatus varians. Campyloneuropsis infumatus*. feeding rings.

Introduction

Tomato (*Lycopersicon esculentum*, Mill.) is the second most consumed vegetable crop worldwide, with a global production of over 160 million tons and cultivated area of 4.7 million hectares. (FAOSTAT, 2015). This large area renders tomatoes susceptible to a range of herbivore pests and relatively warm growing conditions are often well suited for rapid population growth of these pests (Silva et al. 2015).

Among important pests that attack tomato crops, some lepidopterans, as *Tuta absoluta* (Meyrick) and the whitefly *Bemisia tabaci* (Gennadius) are very harmful. The tomato borer *T. absoluta* has historically inflicted losses up to 100% in South America and more recently has become a significant tomato pest in Europe, Africa and Asia (Desneux et al. 2011; Mohamed et al. 2015; Pfeiffer et al. 2013), and it is in quarantine in the USA.

Pest control is a constant challenge for food producers. In the case of *T. absoluta* control, for example, the large amount of different sprays of pesticides are leading to a fast development of resistance and natural enemy populations are strongly reduced. Therefore, searching for alternative strategies,such as biological control, has become urgent (Bueno and van Lenteren 2012; van Lenteren 2012; Mollá et al. 2013), and for control of the tomato borer interest in using heteropteran predatory bugs has recently increased, based on positive experiences in Europe (Calvo et al., 2009; Calvo et al., 2012; Zappalà et al., 2013).

Heteropteran predators are reported as potential natural enemies of a number of pests throughout North America, Europe, Africa and Asia (van Lenteren 2012; Zappalà et al. 2013). Within Heteroptera, Miridae are among the most diverse group of zoophytophagous insects found in natural and agricultural ecosystems (Cassis and Schuh 2012). They are generalist predators and associated with several important pests, including whiteflies, aphids and various lepidopterans (Castañé et al. 2004; Alomar and Albajes, 2006; Urbaneja et al. 2009; Bueno et al. 2013). Various mirid species are being evaluated as biological control agents worldwide, and the species *Macrolophus pygmaeus* Rambur and *Nesidiocoris tenuis* (Reuter) are used in commercial greenhouses for tomato production in Europe against *T. absoluta* and *B. tabaci* (van Lenteren 2012; Moerkens et al. 2015). Hundred thousands to millions of individuals are sold per week (van Lenteren 2012).

However, the phytophagous behaviour of mirids may create unwanted side effects (Castañe et al. 2011). Zoophytophagy is a special case of omnivory in which the predators can feed on both plants and prey during the same developmental stage (Castañe et al. 2011). Plant-tissue (phloem and xylem) can be used as a source of water, as well as a source of nutrients (Gillespie and McGregor 2000). Plant feeding by predators may, however, cause injury to plant tissues and fruits. Serious crop damage caused by Miridae has been described for some species (Arnó et al. 2010; Castañe et al. 2011; Calvo et al. 2009; 2012). Particularly the species *N. tenuis* and *M. pygmaeus* are known to cause serious problems to tomato plants when they occur in high numbers (Arnó et al. 2010; Castañe et al. 2011; Moerkens et al. 2015). However, there are also mirids which seem to cause only little damage to plant tissue and fruits (Lucas and Alomar 2002; Calvo et al. 2012).

Feeding damage caused by zoophytophagous species can be characterized by several types of lesions observed on the stems, leaves, or flower petioles or fruits. Particularly in tomato plant, feeding behavior of some mirid species may reduce the vigor of the plant or cause flower abortion (Arnó et al., 2010; Calvo et al. 2011; 2012). On tomato fruit, injuries are well recognized by feeding punctures surrounded by whitish halos, blemishes, scars and fruit deformations (Alomar and Albajes, 1996; Calvo and Urbaneja 2003). But, the injuryies can be variable and depending of the mirid species, their developmental different stages and of the plant species.

In Brazil, three zoophytophagous mirid predators *Macrolophus basicornis* (Stal 1860), *Engytatus varians* (Distant 1884) and *Campyloneuropsis infumatus* (Carvalho 1947), are currently being evaluated in their biological and behavior characteristics as well as for in their potential as biological control agent to control mainly on pests in tomato crops (Bueno et al. 2012, 2013) and no information is yet available if these species cause damage to plants or fruit. However, no information regarding to their propensity of causing damage or injuries on tomato plant is available. This study was designed in order to answer the following questions: (1) Do nymphs and adults of *C. infumatus*, *E. varians* and *M. basicornis* cause injuriyes when feeding on tomato seedling and fruits in the absence of the prey? (2) How is the type of injury do that they cause? and, (3) what is the consequence for the development of the tomato seedlings and fruit? Depending on the degree of damage, serious or not, decisions will be taken to continue research and/or advise these predators for control of pests in tomato.The answers will be very important in the procedure of evaluation of them before take action in their introduction and or release as biological control agent on tomato field or greenhouse

Material and methods

Rearing of the mirid predators

Stock colonies from field collected material of three predatory mirid (*C. infumatus*, *E. varians* and *M. basicornis*) were kept at the Laboratory of Entomology, UFLA. For details about collecting and starting stock colonies, see Bueno et al. 2013). To obtain nymphs for the tests, adult females from the stock colony on tobacco plants (*Nicotiana tabacum* L. cv TNN) were transferred to new cages containing tomato seedlings (*Solanum lycopersicum*,cv. Santa Clara) in a climate room at 24 ± 1°C, RH 70 ± 10% and 12-h photophase. . After nymphs emerged they were observed daily until they developed to the 3rd or 4th instar

Determination of potential phytophagy on tomato seedlings and fruits by nymphs and adults of the mirid predators

Injury to seedlings was evaluated as follows. Twenty 3rd and 4th instar nymphs of each mirid species were placed in a PVC tube (20 x 5 cm) containing a small plastic cup (8.5 cm diameter x 5.5 cm heigh) with a tomato seedling (20 days old with 3 leaves, cv. Santa Clara). The plastic cup was sealed with parafilm avoiding the nymphs going down and getting water from the substrate. A Petri dish (Ø 9 cm) was placed at the bottom of the PVC tube as a support and on top of the tubea piece of voile tissue was added to allow ventilation. The nymphs were exposed to the tomato seedlings for three days. After the three days exposure, the injuries on tomato seedling were observed by checking the type and number of injuries on leaflets, stems and petioles during three sequential observations: (1) directly after removal of the mirids, (2) three days after removal of the mirids and (3) six days after mirid removal. Injury on leaflets, stems and petioles was observed with a stereomicroscope (magnification 40x, Leica 2000), according to the methodology described by Arnó et al. (2006; 2010). After the last evaluation of damage, the tomato seedlings were kept in a climate room at 24°C for two more weeks, after which tomato plant development was evaluated.

To test injury of nymphs to tomato fruit, a tomato leaflet containing eggs of its prey (*T. absoluta*) was inserted in an Eppendorf tube (2.0 ml) filled with water and sealed with a piece of cotton. Next, an Eppendorf tube was placed into a PVC cube (8.5 cm diameter x 5.5 cm heigh) having a Petri dish (Ø 15 cm) on the bottom as a support. Four nymphs,two 3rd and two 4th instars, of a mirid species were released in the PVC tube covered with a piece of voile tissue to allow ventilation. After 24h, the Eppendorf tube with the tomato leaflet was removed and a red tomato fruit (35-40 mm in diameter) was put inside the PVC tube. The nymphs were exposed to the tomato fruit for 48h and then removed. Their injury was observed by checking the type and the number of injuries on the tomato fruit (1) three days after removing the nymphs from the PVC tube, and (2) six days after removal. Damage to the tomato fruit was observed with a stereomicroscope (magnification 40x, Leica 2000), according to the methodology described by Lucas and Alomar (2002). After the last observation, the tomato fruits were kept in the PVC tube for 4 more days.

The seedling and fruit injury caused by female and male adults of each mirid species was evaluated usingthe same methodology as used for nymphs. . Males and females were evaluated separeted. Twenty individuals of both males and females of each mirid species were used in the test with seedlings and four individuals of both males and females of each species were used in the fruit test. All individuals were less than 7 days old.

Evaluation of damage by nymphs and female and male adults on tomato seedling and fruit tests included four treatments: (1) *M. basicornis*; (2) *E. varians*; (3) *C. infumatus* and (4) control. In the control treatment, tomato seedlings and fruit were placed into PVC tube without the nymphs or adults of three mirid species. All treatments were randomly distributed in a climatic chamber at 24 ± 1°C, RH 70 ± 10% and 12h photophase. Each treatment was replicate twenty five times considering the tomato seedling and fruit as the experimental unit.

Data analysis

Prior to analysis, the raw data were tested for normality and homogeneity of variances using the Shapiro-Wilk and Bartlett tests respectively. Data were log transformed if necessary. The numbers of feeding rings on leaflets and the number of feeding punctures on the fruit per day were submitted to a One-way analysis of variance (ANOVA) and when significant differences among averages were found, Tukey's HSD test at $p < 0.05$ level of significance were used to examine the differences detected. Differences between male and female were analyzed by a Chi-square test at $p < 0.05$ All tests were analyzed by statistic software R Development Core Team (2014).

Results

Tomato seedlings showed injury caused by nymphs and adults of three mirid species

The nymphs of three mirid predators *C. infumatus, E. varians* and *M. basicornis* only caused feeding rings on the leaflets of the tomato seedlings. Injury consisted of blemishes, which were characterized by feeding punctures surrounded by a yellowish, bleached area. No feeding rings or scars were observed on stem and petioles of tomato seedlings.

The percentages of nymphal survival feeding on tomato seedlings during three days without prey was similar for *M. basicornis, E. varians* and *C. infumatus* was 62.3, 65.5 to 65.9%, respectively. The nymphs of the three mirid species produced a variable number of lesions due to continuous feeding on the leaflets. The number of feeding rings after three days were higher compared to the control treatment (seedlings without mirid bug) (F= 30.34; df = 3; P < 0.0001). *M. basicornis*, *E. varians* and *C. infumatus* caused similar numbers of feeding rings (5.6; 5.9 and 5.0 feeding rings, respectively) (F = 0.68; df = 2; P = 0.505) (Figure 1).

On the 6th day (three days after the nymphs were removed), the number of feeding rings on the leaflets had increased. The leaflets exposed to *M. basicornis* presented 19.8 feeding rings, so more than 3 times higher than on the 3th day ($F = 2.41$; $df = 2$; $P < 0.0015$) (Figure 1). The number of feeding rings on leaflets exposed to *E. varians* and *C. infumatus* were 17.3 and 14.4, respectively, which is almost 2 times higher ($F = 1.81$; $df = 2$; $P < 0.0018$) and ($F = 3.52$; $df = 2$; $P < 0.0015$) than on the 3th day (Figure 1).Significant differences in number of feeding rings among the mirid species were observed: *M. basicornis* caused more feeding rings than *C. infumatus* ($F = 4.505$; $df = 2$; $P < 0.0143$).

On the 9th day (six days after the mirids were removed), the number of feeding rings on the leaflets caused by *M. basicornis, E. varians* and *C. infumatus* reached 26, 24.2 and 19.7 respectively, which means there was a significant increase compared to the 6th day ($F = 4.50$; $df = 2$; $P < 0.014$). Leaflets exposed to *M. basicornis* showed more feeding rings than leaflets exposed to *C. infumatus* ($F = 3.81$; $df = 2$; $P < 0.0266$). Furthermore, the increasing in number of feeding rings recorded on the interval from 6th to 9th days were less evident than the number recorded from the interval from 3rd to 6th day (Figure 1). So although visible feeding rings were recorded on leaflets of tomato seedlings, after two weeks no effects were observed on tomato plant growth and also no new feeding rings were found on the leaflets after the 9th day.

Adult females and males of three mirid caused similar feeding rings compared to nymphs on the leaflets of the tomato seedlings. Injury consisted of blemishes, which were characterized by feeding punctures surrounded by a yellowish, bleached area. Again, no feeding rings or scars on stem and petioles of tomato seedlings were observed.

The percentages of female survival feeding on tomato seedlings without prey during 3 days were 75.1, 79.0 and 81.1% for *M. basicornis*, *E. varians* and *C. infumarus*, respectively. Male survivals were 72.8, 77.3 and 78.5, respectively. Survival for males and females was similar ($P = 0.715$). Adult survival was significantly longer than that of nymphs (female: $F = 28.14$; $df = 2$; $P < 0.0365$), (male: $F = 14.69$; $df = 2$; $P < 0.0365$).

Females and males of all three mirid species produced a low number of lesions on the leaflets (i.e. between 3rd and 6th day during the 72h exposition period, and did not differ between the species . Females of *M. basicornis*, *E. varians* and *C. infumarus* caused 1.7; 1.4 and 1.4 feeding rings per tomato seedling, respectively ($F = 2.85$; $df = 2$; $P = 0.505$) and males, 1.4; 1.3 and 1.4 feeding rings per tomato seedling, respectively ($F = 4.20$; $df = 2$; $P = 0.538$) (Fig 2a, b). The number of feeding rings was significantly higher when mirids were present than in the control treatment ($F= 23.52$; $df = 3$; $P < 0.0001$) (female), ($F= 29.30$; $df = 3$; $P < 0.0001$) (male) (Fig. 2 a, b

On the 6th day (three days after the adults were removed), the number of feeding rings on the seedlings increased for both females and males (Fig 2 a, b), but was not significantly higher than at the 3rd day. *Macrolophus basicornis*, *E. varians* and *C. infumatus* females caused 3, 2.7 and 2.3 feeding rings/seedling respectively ($F = 2.71$; $df = 2$; $P = 0.510$) (Fig. 3a) and males 2.8 feeding rings/seedling, for all three species ($F = 4.25$; $df = 2$; $P = 0.505$) (Fig. 3a, b).

On the 9th day (six days after the mirids were removed), the number of feeding rings caused by females and males of *M. basicornis, E. varians* and *C. infumatus* reached 5.6; 4.8 and 4.7 feeding rings/ seedling, and 4.6; 4 and 4.2 feeding rings/seedling, respectively (Fig. 3 a, b). All three mirids (females and males) caused similar number of feeding rings compared to the 6th day ($F = 3.51$; $df = 2$; $P = 0.056$). But comparing to the 3th day, females and males of all three mirids species caused 3 times more feeding rings (female: $F = 3.81$; $df = 2$; $P < 0.0266$); (male: $F = 4.19$; $df = 2$; $P < 0.0347$) (Fig. 3 a, b). Therefore, although visible feeding rings were recorded on leaflets of tomato seedling, after two weeks no effects were observed on tomato plant growth and also no new feeding rings were found on the leaflets after the 9th day.

Females and males caused similar number of feeding rings/seedling during all observations ($P = 0.05$) (Figure 2). The numbers of feeding rings caused by nymphs were three times higher higher than those caused by adults on the 3rd day ($P < 0.0001$), 6.6 times on 6th day. On the 9th day the number of feeding rings by nymphs were 4.5 and 5.1 times higher than females and males, respectively.

Tomato fruit injuried by nymphs and adults of three mirid species

Nymphs were exposed to tomato fruit for 48 hours. The feeding rings were characterized by punctures surrounded by small whitish halos and only visible under a stereomicroscope at 40 X maginification). Nymphal survival on tomato fruit after two days without prey was 93.4, 95.5 and 98.2% for *M. basicornis*, *E.varians* and *C. infumatus*, respectively. Three days after nymphs had been removed, the number of feeding rings on tomato fruit were similar as directly after exposure.?? higher compared to the control treatment (tomato fruit without mirid bug) (F= 14.24; df = 3; P < 0.0001). Significant difference were found (F = 14.07; df = 2; P < 0.001) for the number of feeding rings caused by nymphs of the three mirid species, both after 48 hours and 5 days. Six days after the nymphs being removed, the number of feeding rings caused by *M. basicornis, E. varians* and *C. infumatus* increased (6.5; 5.0; 2.9 respectively), but no differences were found comparing to 3th day (F = 0.85; df = 2; P = 0.359). In adittion, regarding to the differences among the number of feeding rings among the nymphs of the three mirid species, *M. basicornis* and *E. varians* kept causing more feeding rings on tomato fruits than *C. infumatus* (F = 14.55; df = 2; P < 0.001) (Fig. 3). After 12 days of the first contact of the nymphs with the tomato fruits, the number of feeding rings were the same and the tomato fruit kept fresh and no wrinkle were recorded.

No feeding rings caused for adults males and females of three mirid species on tomato fruits were found. The adults survival when in contact with the tomato fruit were similar for all females of *M. basicornis E. varians* and *C. infumatus* (85.2, 88 and 86%) respectively. Similarly, no difference on survival for all three males mirids were observed, and were 70, 75 and 75% for *M. basicornis*, *C. infumatus* and *E. varians*, respectively. However, comparison between female and male of all three secies showed significative diference (P<0.05), being the survival of females higer than males survival when having tomato fruit as food.

Discussion

The injuries that individuals of *M. basicornis*, *E. varians* and *C. infumatus* can cause on tomato seedling when prey is absent is represented by the number of feeding rings. All three mirids species fed on tomato seedling and caused some injury in the form of feeding rings to leaflets. These feeding rings consist of a feeding puncture surrounded by a yellowish, bleached area, and is similar to those caused by other mirids like *N. tenuis*, *M. pygmaeus* and *Dicyphus hesperus* Knight (Mc Gregor et al. 2000; Shipp and Wang 2006; Arnó et al. 2010; Castañé et al. 2011; Calvo et al. 2009; 2012). Mirids can also cause injury on stems and leaf petioles which look like necrotic rings and scars. The necrotic rings can lead to withering of leaflets or even of the apex (Arnó et al. 2006; Castañe et al. 2011). However, injury to stems and petioles was not found on tomato seedling exposed to *M. basicornis*, *E. varians* and *C. infumatus* nymphs and adults.

The amount of injury is related to mirid abundance and availability of prey. With high numbers of mirids, injury can even lead to flower abortion in tomato plants, and downgrading of flowers in case of ornamental plants like gerbera (Castañé et al. 2003, 2011; Gillespie et al. 2007; Sánchez 2008). The number of nymphs and adults used in this study were four times higher than the densities found in commercial fields sampled by Arnó et al. (2006) where adults and nymphs of *N. tenuis* together ranged between 0.13 and 5.23 per plant.

In this study the number of feeding rings caused by 20 nymphs of *M. basicornis*, *E. varians* and *C. infumatus* did not exceed one per individual mirid. The number of feeding rings caused by females and males of the three mirid species was around 0.25 feeding rings/individual mirid. Under greenhouse conditions, 5 *N. tenuis* adults per plant caused 2.6 feeding rings/plant (Sanchez and Lacasa 2008). Twenty nymphs of this species enclosed per caged side shoot of a tomato plant for 48 h caused about 3 necrotic rings per shoot (Arnó et al. 2006). Although differences in number of injury among these studies might be due to differences in tomato varieties and experimental conditions (laboratory, greenhouse, microclimate), the mirid species *N. tenuis* seems to cause much more injury than *M. basicornis*, *E. varians* and *C. infumatus.* Presumably, *N. tenuis* presents more frequent stylet insertion in plant tissues, reflecting attempts to obtain more nutrients from the plant, consequently causing more injuries.

The frequency of stylet insertion in plant tissue for feeding and the resulting injury can also be influenced by prey availability (Sánchez and Lacasa 2008; Calvo et al. 2009; Sánchez 2008). According to Calvo et al. (2012), the number of feeding rings is higher when prey availability is lower The high mortality of on average of 40% of *M. basicornis*, *E. varians* and *C. infumatus* nymphs when exposed to leaflets of tomato seedling without availability of prey, indicates that young mirid predators need animal prey for optimal development (Urbaneja et al. 2005; Hamdi and Bonato 2013). Silva et al. (2015) have shown that when the prey *T. absoluta* is available, mortality during the whole nymphal period was less than 10% and feeding rings were not observed (Silva et al. 2015). *N. tenuis* also needs animal prey, is not able to complete its development on tomato plants alone, and only some 30% of the nymphs survived through the third instar (Urbaneja et al. 2005). Animal food is also essential in other omnivorous plant bugs, such as *M. pygmaeus* and *D. hesperus* to enhance adult reproduction (Sánchez et al. 2003; Moerkens et al. 2015).

Two weeks after exposure of leaflets to nymphs or adults we did not find any deformations. Even at the high preydensities we used, the three mirids only caused a low number of feeding rings, and, apparently tomato seedlings were capable of healing the wounds resulting in disappearance of feeding rings. Arnó et al. (2006) observed that 76% of the feeding rings caused by *N. tenuis* adults, released at same density as we did, had disappeared seven days after removal of the predators.

Miridae do not only cause injury to the leaf, but to the tomato fruit (Lucas and Alomar 2002; Moerkens et al. 2015). Feeding on tomato fruits appears to be species-specific and depends, among others, on the availability of prey prey (Lucas and Alomar 2002). Presence of *E. kuehniella* eggs did not prevent damage of *Dicyphus tamaninii* adults to tomato fruit (Lucas and Alomar 2002). However, in this study, even in the absence of prey and tomato leaves, nymphs only caused a limited number of feeding rings on fruits, and adults did not cause any injury at al.

In the field, injury to fruit caused by *M. pygmaeus* was observed when their density reached 1.6 per leaf (Moerkens et al. 2015). In this study, mirids at a density of 4 nymphs per fruit caused a limited number feeding rings, which were only visible at a magnification of 40x. . The feeding rings did not cause any damage to tomato fruit afterwards, Adults of several mirids, can also cause injury to tomato fruit (Lucas and Alomar 2002; Arnó et al. 2010; Moerkens et al. 2015), but the species we studied seem not to cause injury.

During the 48h exposure to tomato fruit, nymphal mortality was about 5% and adult mortality even 15%, but mortality on fruit was lower than on seedlings. Some mirid species show complete development on plant food only (give name of species here and ref) and tomato fruit may also result in better adult survival better than on leaves (Castañe et al. 2011; Hamdi and Bonato 2014), which we supposte to be linked to higher and different nutrient content. Lucas and Alomar (2002) showed that nymphs can complete their development while feeding on green and red tomato fruits, but not by feeding on tomato leaves. Tomato fruits are also used as a source of water by mirids (Gillespie and McGregor 2000).

The results of this study indicate for the first time that the 3rd and 4th instar nymphs and adults of the three Brazilian mirids only cause a few injuries to tomato seedlings and tomato fruits. The type of injury found was small and did not result in deformation of the fruit. This is a very positive finding and may make these species good candidates for biological control of tomato pests, as their predation capacities are impressive.

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Figures

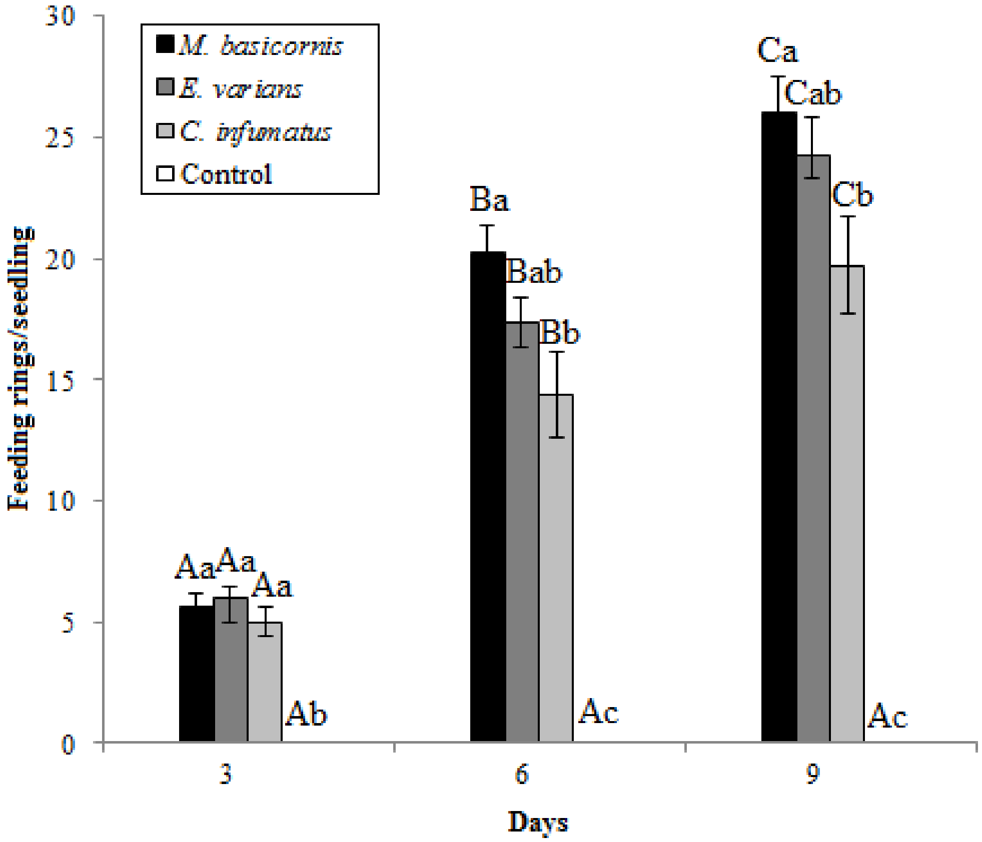

Figure 1 Number of feeding rings (mean ± SE) on leaflets of tomato seedlings caused by *C. infumatus*, *E. varians* and *M. basicornis* nymphs after 72 h exposure. Capital letters means difference between same species at days 3, 6 and 9 and lowercase letters means difference among the three species on the same day.

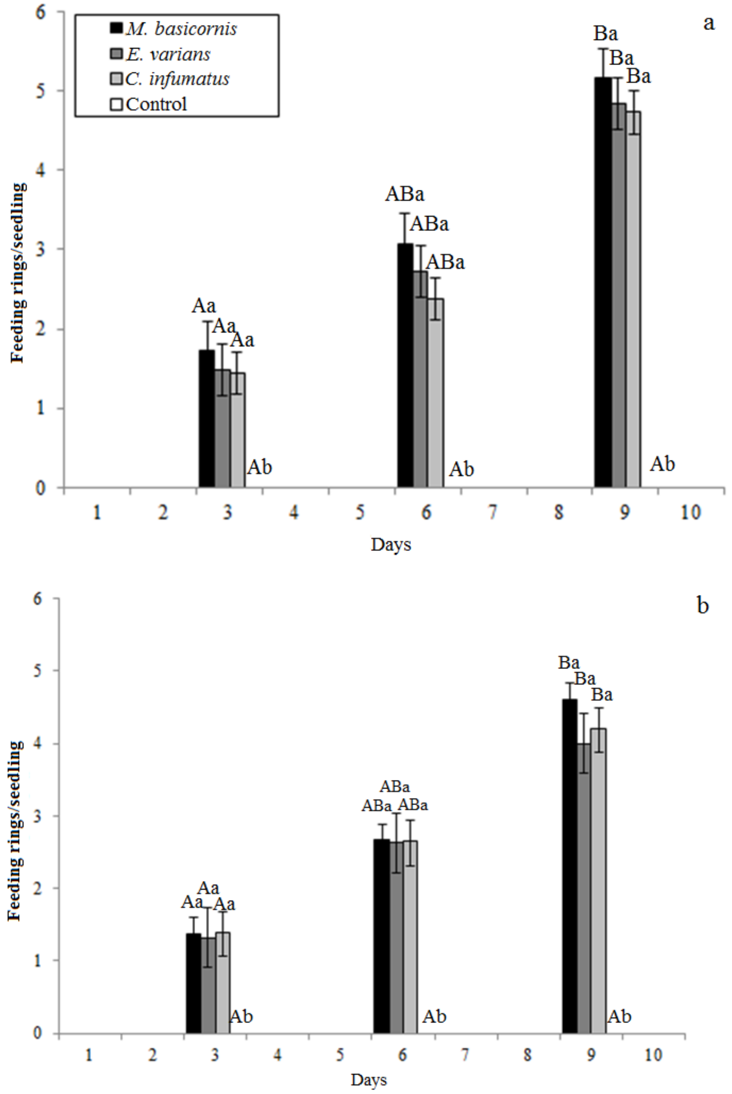

Figure 2 Number of feeding rings (mean ± SE) on the leaflet of tomato seedling caused by *C. infumatus*, *E. varians* and *M. basicornis* females (a) and males (b), after being tested on the plant for 72 h inside a climatic chamber at 24 ± 1°C, RH 70 ± 10% and 12h photophase. Capital letters means difference between same species at days 3, 6 and 9 and lowercase letters means difference among the three species on the same day.

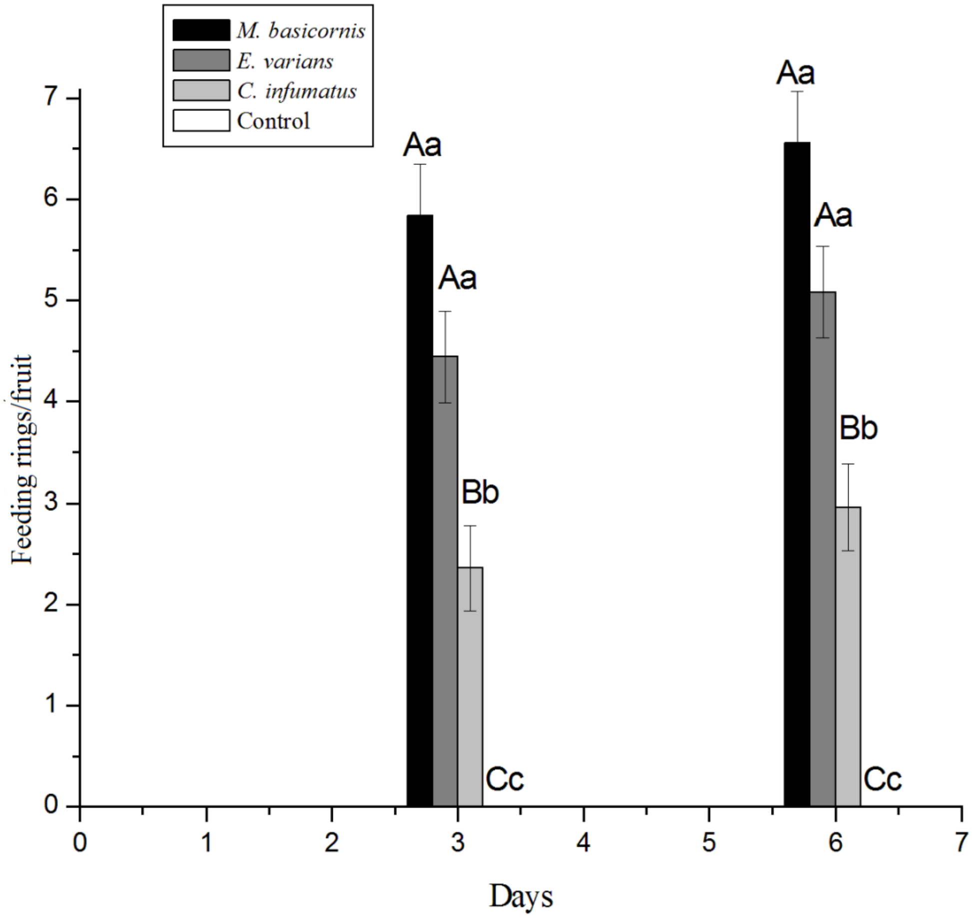

Figure 3 Number of feeding rings (mean ± SE) on tomato fruit caused by *C. infumatus*, *E. varians* and *M. basicornis*, after being exposed to the fruit for 48 h inside a climatic chamber at 24 ± 1°C, RH 70 ± 10% and 12h photophase. Capital letters means difference between same species at days 3 and 6 and lowercase letters means difference among three species on the same day.

ARTICLE 3 PHLOEM-FEEDER *Bemisia tabaci* DOES NOT INTERFERE WITH ATTRACTION OF MIRID PREDATORS to leaf feeder *Tuta absoluta* infested TOMATO

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Abstract. In response to insect herbivory, plants emit volatile compounds which may play multiple roles as communication signals and defense agents, mediating interactions with other plants, microorganisms and animals. The herbivore induced plant volatiles (HIPVs) may act as indirect plant defenses by attracting natural enemies of the attacking herbivore. Here, we report the first evidence of the attraction of three mirid predators (*Macrolophus basicornis*, *Engytatus varians* and *Campyloneuropsis infumatus*) toward plant volatiles induced by two tomato pests (*Tuta absoluta* and *Bemisia tabaci*) in olfactometer bioassays. We subsequently compared the volatile composition of different plant sources by collecting headspace samples and analyzing them with GC-MS. Egg deposition by *T. absoluta* did not make plants more attractive to the predators than uninfested plants. *Macrolophus basicornis* is attracted to plants infested with either *T. absoluta* larvae or *B. tabaci*. However, *E. varians* and *C. infumatus* responded only to tomato plants infested with *T. absoluta* larvae over uninfested plants. In addition, multiple herbivory by herbivores of two different feeding guilds (leaf chewers and phloem feeders) did not promote increased attraction of mirids. The results of the chemical analyses revealed a total of 15 different volatile compounds. Terpenoids represented the most important fraction of the total blend and there were significant differences between the volatile blends emitted by plants in response to attack by *T. absoluta* or *B. tabaci*, or to double infestation. In conclusion, *M. basicornis* performed better than the other two mirids. Further studies are needed to determine if and how this information about mirid semiochemical communication can be used in biological control programs

K**ey Words.** HIPVs; Multitrophic interactions; mirid; predator foraging behavior; tomato pests.

INTRODUCTION

It has long been known that plants under attack of herbivorous insects emit complex volatile blends, called herbivore-induced plant volatiles (HIPVs), which are exploited by predators and parasitoids for searching of their prey/host (Dicke and Sabelis 1988; Turlings et al. 1990; Geervliet et al. 1997). Understanding the role of HIPVs is essential since they may act to enhance the efficiency of predators and parasitoids by increasing prey or host habitat location (Pels and Sabelis 2000) and reducing searching time (Turlings et al. 1991; Vet and Dicke 1992; Ninkovic et al. 2001). However, the outcome of plant-mediated interactions between herbivores depends on a number of factors, including among others, herbivore species (De Moraes et al. 1998), developmental stage of herbivores (Takabayashi et al. 1995) and even the sequence of herbivore arrival (Erb et al. 2011; Wang et al. 2014).

Plants are usually colonized by more than one species of arthropod herbivore (Dicke et al. 2009; Ponzio et al. 2013). Despite of that, most of the literature on tritrophic interactions mediated by HIPVs is still based on the attack by a single herbivore species. However, under multiple herbivory, different feeding guilds can lead to changes in the composition of emitted plant volatiles, making them different than in case of single-species herbivory (Moayeri et al. 2007b; Zhang et al. 2009; Dicke et al. 2009). Recently researchers studied if multi-species herbivory affects attraction of natural enemies by analyzing the chemical composition of volatile blends upon single and multi-species infestation of plants (Shiojiri et al. 2001; Cardoza et al. 2002; Moayeri et al. 2007b;

Rasmann and Turlings 2007; de Borer et al. 2008; Gosset et al. 2009; Errard et al. 2015; Pangesti et al. 2015).

Studies have shown that the effect of multiple infestation on HIPV emissions is difficult to predict and highly variable. There is potential for crosstalk between the salicylic acid (SA) and jasmonic acid (JA) signaling pathways which infer in additive (Cardoza et al. 2003; Rodriguez-Saona et al. 2005; Cusumano et al. 2015) or negative response (Zhang et al. 2009; Schwartzberg et al. 2011) of natural enemy attraction. Until now, few studies have investigated the effects of the interaction between two herbivores of different feeding guilds on mirid predators (Moayeri et al. 2007b; Lins Jr., et al. 2014). Considering the cross-talk between the JA and SA signaling pathways, it is hypothesized that SA-inducing herbivores sharing the same host plant with JA-inducing herbivores will suppress the emission of volatiles (Zhang et al. 2009, 2013) influencing on mirids` attraction.

Tomato (*Lycopersicon esculentum* Mill.) is the second most consumed vegetable crop around the world (FAOSTAT 2015), cultivated outdoors as well as in greenhouses for both fresh market consumption and processing (Lange and Bronson 1981). It is also one of the main vegetable crops in Brazil, where it has a significant social and economic importance (Marchiori et al. 2004; Gain report 2009). Tomato yield is usually reduced by the attack of various pests, such as the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and many other pests (Errard et al. 2015).

Biological control strategies for various major pests of tomato have been successfully implemented in Europe (Calvo et al. 2009, 2011, 2012; van Lenteren 2012). Recent biological control successes have occurred due to the selection and implementation of native generalist predators, including several mirids. In tomato, the release of the predatory mirid bugs *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* Rambour (both Hemiptera: Miridae) proved very effective in controlling key tomato pests, such as *B. tabaci* and *T. absoluta* (Perdikis et al. 2008; Urbaneja et al. 2009, 2012; Calvo et al. 2012; Molla et al. 2014) and they are now commonly used in augmentative biological control programs (van Lenteren 2012).

Several mirid predators, like *Dicyphus* (McGregor and Gillespie 2004; Moayeri et al. 2006, 2007a, b; Ingegno et al. 2011, 2013; Pérez-Hedo and Urbaneja 2014; Abbas et al. 2014), *N. tenuis* and *M. pygmaeus* (Ingegno et al. 2013; Lins Jr. et al. 2014; de Backer et al. 2015), which are successfully used in Europe for controlling these pests (Urbaneja et al. 2012) use HIPVs emitted by tomato plants infested by either *T. absoluta* or *B. tabaci*.

We recently reported promising results on the efficiency of the mirid predators *Macrolophus basicornis* (Stal 1860), *Engytatus varians* (Distant 1884) and *Campyloneuropsis infumatus* (Carvalho 1947) (Bueno et al. 2013) as biological control agents. They are able to walk and reproduce on tomato plants (Bueno et al. 2012) and exhibit high predation rates, similar to the predators used in large-scale control of lepidopteran species and several other greenhouse pests in Europe (Urbaneja et al. 2012; Bueno et al. 2013). Despite their apparent potential as biological control agents, we still need to assess their capacity to localize the main tomato pests, *T. absoluta* and *B. tabaci*, mediated by HIPVs release by tomato plants.

Here, we investigate the olfactory response of the polyphagous mirid predators *M. basicornis*, *E. varians* and *C. infumatus* to HIPVs released by tomato plants infested with *T. absoluta* eggs and larvae, and *B. tabaci* (nymphs and adults). We also examine if double infestation by *B. tabaci* and *T. absoluta* affects reactions of the mirids to HIPVs released by tomato plants attacked by two herbivore species. We performed olfactometer assays and chemically characterized the volatile compounds emitted by single and double infested tomato plants.

MATERIAL AND METHODS

Plants and Insects

Tomato plants, *Solanum lycopersicon* L. cv. Santa Clara, were grown in a greenhouse in pots (3l)/ plant containing substrate mixed with 200 g NPK 4-14-8 complex fertilizer. Plants were grown from May to October 2015 under natural light, temperature and humidity (Piracicaba, SP, Brazil). All plants used for experiments were 30–35 days old, 20–25 cm high with 5–6 expanded leaves.

Mirid predators were collected in tobacco (*Nicotiana tabacum* L.) fields located in the municipalities of Ribeirão Vermelho and Lavras (MG, Brazil, 21°08.596′S and 045°03.466′W, 808 m of altitude). Nymphs and adults were identified based on the family- specific dichotomous key by Ferreira and Henry (2011). Identifications of the three mirids found in the field were confirmed with the taxonomist P. S. F. Ferreira (Federal University of Viçosa, Viçosa, Brazil) as *Camplyloneuropsis infumatus*, *Engytatus varians* and *Macrolophus basicornis.*

Mirid stock colonies were kept under laboratory conditions (24 ± 1°C, RH 70 ± 10% and 12L:12D) following the method described in Bueno et al. (2013). Female adults were individually maintained in acrylic cages (60 x 30 x 30 cm) containing eggs of *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) for feeding, and tobacco plants (*Nicotiana tabacum* L. cv. TNN) as oviposition substrate and water source. After seven days, tobacco plants containing eggs were transferred to new cages where nymphs hatched and fed on *E. kuehniella* eggs until reaching adult stage. From the second generation in the laboratory, 1-to-7 day old female mirids were used in behavioral assays.

Initial population of *T. absoluta* (eggs, larvae and pupae) was collected in a tomato field in an experimental area at Federal University of Lavras (Lavras, MG, Brazil, 21°14'S 45°00'W, 918 m of altitude). Collected and newly-formed pupae were sexed (Coelho and França 1987) and released in a cages (60 x 30 x 30 cm) with tomato plants (cv. Santa Clara) inside. Colonies of the three mirids and *T. absoluta* were maintained under laboratory conditions (25 ± 2°C, RH 70 ± 10% and 12L:12D) at the University of São Paulo/ ESALQ (Piracicaba, SP, Brazil). Larvae and adults were kept separately inside cages (60 x 30 x 30 cm) made from fine mesh and regularly supplied with tomato plants (cv. Santa Clara) for feeding or oviposition.

Whiteflies, *B. tabaci* biotype B, were provided from the rearing maintained at the Agronomic Institute of Campinas- AIC (Campinas, SP, Brazil). Colonies were kept in fine mesh cages (60 x 40 x 40 cm) with cabbage (*Brassica oleracea* L. var. acephala DC. cv. Manteiga) plants for feeding and oviposition inside a greenhouse under natural light, temperature and humidity at the University of São Paulo/ ESALQ (Piracicaba, SP, Brazil).

Olfactometer Assays

Responses of predator females to volatiles were assessed in a glass Y-tube olfactometer (3.0 cm diameter, main arm: 20 cm long, side arms 23 cm long, 70° angle between the side arms). The olfactometer device was vertically positioned, like in other studies with mirid predators (Moayeri et al 2006a, b; Ingegno et al 2011, 2013; Lins Jr. et al 2014) and connected to an ARS Volatile Collection System (Analytical Research Systems, Gainesville, FL, USA). Each olfactometer side arm was connected to a 15-L glass chamber with a single plant. Plastic pots in which the plants were growing were wrapped with aluminum foil. Inlet air flow was adjusted to 0.8 $L.min^{-1}$ for each side arm. Treatment chambers were covered with a black card to prevent insects from visually detecting the plants.

A single naive female mirid was introduced in the main arm of the olfactometer and observed for up to 10 min. Mirids were considered to have made a choice when they crossed a line traced 13 cm from the center of the olfactometer. Females not choosing a side arm within 10 min were considered as non-responsive and were excluded from data analysis. Each female was tested only once to prevent associative learning. A total of 30 replicates were performed for each treatment and each mirid species, using at least three pairs of plants on three experimental days. After every two replicates, the olfactometer side arms were switched to minimize positional bias. After testing ten females, the Y-tube and glass chambers/pots were washed with neutral soap and alcohol (70%). Bioassays were carried out in an acclimatized room at 24 ± 1 °C and 70 ± 10 % RH between 10-12 am and 2-4 pm, photophase.

Olfactory reactions of mirid predators were assessed to volatiles of: (*i*) uninfested tomato plants ('uninfested'), (*ii*) *T. absoluta* egg-infested plants ('*T. absoluta* eggs'), (*iii*) *T. absoluta* larvae-infested plants ('*T. absoluta* caterpillars'), (*iv*) *B. tabaci* infested plants ('*B. tabaci*'), and (*v*) *T. absoluta* larvaer + *B. tabaci* (double infestation – '*T. absoluta* + *B. tabaci*'). Plants were transferred from the greenhouse to the room of olfactometer assays just before treatment. To obtain *T. absoluta* egg-infested plants, tomato plants were covered with organza bags containing five 1–3-day-old *T. absoluta* males and females and removed 48 h later. Tomato plants with eggs 24 h after oviposition were used for the olfactometer tests.The same procedure was used to obtain *T. absoluta* larvae-infested plants, but eggs were left on the plant until larvae hatched and fed on the plants for 72 h.

To infest plants with whiteflies, 50 *B. tabaci* adults were released in a cage (60 x 30 x 40 cm) with one tomato plant for 10 days. Thereafter, *B. tabaci*-infested plants containing mixed stages (i.e., eggs, nymphs and adults) were used in the tests (Lins Jr. et al. 2014).

Because injury by *T. absoluta* larvae on tomato is more severe than the one inflicted by whiteflies, we infested plants for treatments with both pests first with *B. tabaci* as described above and, after seven days, with 30 first and second instars *T. absoluta* larvae, which fed on plants for 72 h, totalizing 10 days of induction.

Headspace Collection and Analysis of Plant Volatiles

Volatiles of uninfested, infested tomato plants (treatments described above) and pots filled with soil (blank) were collected under laboratory conditions at 24 ± 1 °C and 70 ± 10 % RH between 10-12 am and 2-4 pm photophase, in a push-pull volatile collection system (ARS, Gainesville, FLA, USA).

Prior to volatile collection, plant pots were carefully wrapped with aluminum foil to avoid volatiles from plastic and soil and individually enclosed in a 15-L glass chamber. The moments of volatile collection were randomly distributed between treatments. Six plants per treatment were sampled for 2 h (flow rate 0.8 L min^{-1}) using a column filter with 30 mg of HayeSep® (Supelco, Bellefonte, PA, USA). Filters were immediately eluted with 150 µl dichloromethane (Merck, Kenilworth, NJ, USA) mixed with 30 µl of nonyl acetate solution (Sigma-Aldrich, Sto Louis, MO, USA) at 10 ng/µL used as internal standard. All extracts were stored at −80°C until analyses. Immediately after the collection of volatiles, above ground plant fresh weight was measured. Headspace samples were analyzed with a gas chromatograph (Agilent 6890 Series GC system G1530A) coupled to a mass spectrometer that operated in electron impact mode (Agilent 5973 Network Mass Selective Detector; transfer line 230°C, source 230°C, ionization potential 70 eV, scan range 33–280 amu). Briefly, a 2-µl aliquot of each sample was injected in the pulsed splitless mode into a HP-1 capillary column (Alltech Associates, Deerfield, IL, USA - 30 m, 0.25 mm ID, 0.25 μm film thickness). Helium (0.9 ml/min) was used as carrier gas. GC oven temperature was initially held at 40°C for 3 min, raised to 100°C at 8°C/min and subsequently to 200°C at 5°C/min followed by a postrun of 5 min at 250°C. Detected volatiles were identified by comparing their mass spectra with those of the NIST 11 library and with the retention times of analyses from previous studies (López et al. 2012; Anastasaki et al. 2015; De Backer et al. 2015).

Volatile quantification was based on peak areas of individual compounds relative to the internal standard and corrected by fresh shoot biomass (g) of each replicate.

Data Analysis

Mirid choices in olfactometer assays were analyzed using generalized linear models (glm) and assuming a binomial distribution. Difference in insect choice between treatments was tested using binominal test ($P < 0.05$ and $P < 0.01$).

Volatile emission data (relative amounts of individual compounds corrected by plant fresh weight) were tested for normality and homogeneity of variances using the Shapiro-Wilk and Bartlett tests, respectively. As distributions, even after log-transformation, did not meet the assumptions for parametric tests volatile emission data were analyzed by the non-parametric Kruskal-Wallis test followed by Bonferroni post hoc analysis ($P < 0.05$ and $P < 0.01$). The total emission of volatiles was submitted to one-way analysis of variance (ANOVA) and when significant differences among averages were found, Tukey's HSD test at

$p < 0.05$ level of significance was applied. Principal component analysis (PCA) was also applied. All statistical analyses were performed using R statistical software (R Core Team 2014).

RESULTS

Response of the Three Mirid Species to Tomato Volatiles

Macrolophus basicornis, *Engytatus varians* and *Campyloneuropsis infumatus* preferred volatiles from uninfested, *T. absoluta* egg-infested, *T. absoluta* larvae-infested, *B. tabaci* infested and *T. absoluta* larvae + *B. tabaci* infested tomato plants over clean air (Fig.1, binomial test, $P < 0.05$ and $P < 0.01$). Likewise, all three mirids oriented preferentially to *T. absoluta* larvae -infested over uninfested plants (Fig.1, binomial test, $P < 0.05$ and $P < 0.01$), but they did not discriminate volatiles *T. absoluta* egg-infested plants from uninfested plants (Fig.1, binomial test, $P > 0.05$).

Females of *M. basicornis* preferred odors of *B. tabaci*-infested plants over uninfested plants (binomial test, $P < 0.01$), but *E. varians* and *C. infumatus* did not discriminate between the treatments (Fig.1, binomial test, $P > 0.05$). None of the three mirid species were able to discriminate between volatiles of *T. absoluta* larvae + *B. tabaci* infested over *T. absoluta* larvae -infested (Fig.1, binomial test, $P > 0.05$). However, *M. basicornis*, *E. varians* and *C. infumatus* preferred volatiles emitted from double infestation (*T. absoluta* larvae + *B. tabaci*) over *B. tabaci* infested plant (Fig.1, binomial test, $P < 0.05$ and $P < 0.01$).

Chemical Composition of the Headspace Volatile Blend of Uninfested and Infested Tomato Plants

Egg deposition by *T. absoluta* on tomato plants induced up-regulation of six terpenes: α-pinene, α-terpinene, β-phellandrene, γ-terpinen, terpinolene and caryophyllene relative to undamaged plants (Table 1, Bonferroni, $P < 0.05$). Damage by *T. absoluta* larvae, in turn, promoted a general elevation in amounts of volatile emission, including green leaf volatiles and 12 terpenes, except for β-myrcene, when compared to the control (undamaged tomato) (Table 1, Bonferroni, $P < 0.05$). The monoterpenes carene, α-terpinene and β-phellandrene were the most abundant compounds in *T. absoluta* larvae -infested blend.

Infestation with the sucking insect *B. tabaci* on tomato plants induced a blend composed by higher concentrations of nine terpenes and the green leaf volatiles (*E*)-2-hexenal relative to the control (Table 1, Bonferroni, $P < 0.05$). Besides carene and β-phellandrene, which were also major compounds in volatile emission of *T. absoluta* larvae -infested plant, terpinolene was also among the most abundant compounds in *B. tabaci*-infested plant blend.

Double infestation (*T. absoluta* + *B. tabaci*) of tomato plants promoted augmented amounts of all terpenes found in the tomato volatile blend, but only one of the green leaf volatiles ((*Z*)-3-hexen-1-ol), relative to the uninfested tomato blend (Table 1, Bonferroni, $P < 0.05$). Blend of double infested plant contained higher concentrations of β-myrcene, limonene, γ-terpinen, terpinolene and β-elemene, but lower of α-pinene, compared to *T. absoluta* larvae-infested plant blend (Table 1, Bonferroni, $P < 0.05$). Besides β-myrcene, limonene, γ-terpinen and β-elemene, the

T. absoluta + *B. tabaci*-infested plant blend also contained higher amounts of α-terpinene, β-phellandrene, β-ocimene, δ-elemene and humulene than the *B. tabaci*-infested plant blend (Table 1, Bonferroni, $P < 0.05$). Similar to single infested plants, carene and β-phellandrene were abundant compounds in *T. absoluta* + *B. tabaci*-infested plant blend. However, *T. absoluta* + *B. tabaci*-infested plant had also limonene as a major compound.

Tuta absoluta larvae -infested and *T. absoluta* + *B. tabaci*-infested plants emitted higher total amounts of volatiles compared to either uninfested plants (up to 32-fold difference) or *T. absoluta* egg-infested plant (up to 6-fold difference) or *B. tabaci* (up to *ca.* 3-fold difference) (Figure 2, Tukey HSD, $P < 0.05$).

Composition analysis for plant volatile emission using PCA showed separation of uninfested and *T. absoluta* egg-infested plants from *T. absoluta* + *B. tabaci*-infested plants along PCA 1 (55% of the variance) and PCA 2 (13%) (Figure 3). All volatiles were positively correlated with PC1 (Fig. 3). Along PC2, PCA clearly showed that differences in ratios of similar blends contribute to the separations of the UP and TE over DI treatment (Fig. 3). Between these treatments, α- pinene, was correlated to UP and TE. Besides, this compound was found in higher quantity in TE than DI treatment. The same can be observed for β-myrcene, γ- terpinen, terpinolene δ-elemene and β-elemene, however in this case they were related to DI treatment both on the PCA analysis and on the amount of volatiles emitted (Table 1). We suggest these compounds mostly influenced to the separations to these treatments.

DISCUSSION

Our study shows that the three mirid predators, *M. basicornis*, *E. varians* and *C. infumatus*, are attracted to tomato plants infested with *T. absoluta* larvae. Although *B. tabaci* is a potential prey for the mirids (Lins, 2014) only *M. basicornis* oriented towards *B. tabaci*-infested over uninfested tomato plants. Mirids did not distinguish double infested (*T. absoluta* + *B. tabaci*) plants from *T. absoluta* larvae-infested plants, but preferred double infested over *B. tabaci*-infested plants.

Tomato plants infested with *B. tabaci* released augmented amounts of several terpenes relative to uninfested plants. However, compared to the blend emitted by *T. absoluta*-larvae infested plants, the *B.tabaci*-infested plant blend emitted lower concentrations of six terpenes and higher concentrations of terpinolene. Mirids likely benefit more by being attracted to the *T. absoluta*-larvae infested plant blend, than the blend emitted by *B. tabaci*-infested, because *T. absoluta* are more suitable prey than whiteflies (Lins, 2014) therefore enhancing their performance and reproduction. An alternative, explanation might be that *E. varians* and *C. infumatus* are not attracted to *B. tabaci*-infested plant volatiles because they had no previous experience with this prey, in a similar way as Lins et al. (2014) found for the mirid *N. tenuis*. To locate prey in a complex system that undergoes changes in plant-derived odour cues due to multiple or single pest infestation, mirid females could rely on learning abilities to enhance responses and motivate predator and/or parasitoid searching. Insect learning is a well-known and widely studied experience-based modification of behavior (Steidle and van Loon 2003; De Boer et al. 2005; Glinwood et al. 2011; Rim et al., 2015), in particular for parasitoids (Vet and Dicke 1992). It was only recently studied for predatory mirid bugs (Lins Jr. et al., 2014).

Some studies show that HIPVs released by double infested plants are more attractive than single infested plants (Rodriguez-Saona et al. 2005; Moayeri et al. 2007b; Cusumano et al. 2015). However, herbivores with different feeding modes (chewing and sucking like in this study) can negatively affect attraction of natural enemies (Zhang et al. 2009; Schwartzberg et al. 2011). Results shown here did not confirm our initial hypothesis that whiteflies sharing the same host with the tomato leaf miner could suppress volatile emission relative to *T. absoluta* larvae-infested plants and therefore interfere in mirid attraction. Indeed, infestation with *T. absoluta* + *B. tabaci* in tomato triggered an overall increase of terpenes compared to infestation by either of the two herbivores, suggesting that JA and SA biosynthetic pathways may not interact antagonistically. Despite this, mirids did not distinguish between *T. absoluta* + *B. tabaci*-infested and *T. absoluta*-infested plant volatile blends, demonstrating that double infestation by *T. absoluta* + *B. tabaci* does not increase attraction to mirid predators compared to the singly infested plant with *T. absoluta*. Similarly, two other mirid predators (*M. pygmaeus* and *N. tenuis*) do not distinguish double infested from single infested tomatoes and this behavioral pattern did not change after experience (Lins Jr. et al. 2014).

Oviposition on tomato plants by *T. absoluta* triggered increased emission of some terpenes, but the composition of the overall blend was similar to the constitutive volatile profile according to PCA (Figure 3).

Although discrete differences between volatile blends of egg-infested and uninfested plants can be perceived by egg parasitoids (Wei et al., 2007 Fatouros et al., 2012; Hilker and Fatouros, 2015), the generalist mirid predators tested here did not distinguish *T. absoluta* egg-infested from uninfested plants. Other species of mirids, *N. tenuis, M. pygmaeus* and *Dicyphus errans* Knight, were also not attracted to *T. absoluta* egg infested tomato plants (Ingegno et al. 2013; Lins Jr. et al., 2014). However, our results and those from Lins Jr. et al. (2014) showing the lack of attraction of *T. absoluta* egg-infested to mirids might depend on the level of infestation. Mollá et al. (2013) found *N. tenuis* attraction towards volatiles from *T. absoluta* egg-infested tomatoes, but egg infestation was about four times higher than in our experiments and those of Lins Jr. et al. (2014).

Because of the complexity of volatile blends emitted by single and double infested tomatoes, it is difficult to select volatile compounds that potentially play a role in mirid attraction. Based on the attraction of mirids to *T. absoluta* larvae-infested and *T. absoluta* + *B. tabaci*-infested plants, and in contrast to the non-preference of *T. absoluta* eggs-infested and *B. tabaci*-infested over uninfested plants, we suggest that carene, α-terpinene, limonene, β-phellandrene and δ-elemene are important compounds for the attraction of mirids. However, this question should be addressed in future studies by manipulating the concentration of single terpenes in a non-attractive plant volatile blend in assays with mirids, and/or testing different mixtures and concentrations of single synthetic compounds on behavioral and electroantennogram responses.

In summary, our study shows that multiple herbivory by herbivores of two different feeding guilds (chewing and sap-sucking insects) did neither increase, nor decrease attraction of mirids. Although mirids capture and feed on whitefly nymphs and adults, *E. varians* and *C. infumatus* did not respond innately to *B. tabaci*-infested tomatoes. Unless these two mirids quickly learn to associate plant olfactory cues with *B. tabaci* injury, we would not recommend *E. varians* and *C. infumatus* as biological control agents in tomato crops where only whiteflies are a serious problem.

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Table

Table 1. Relative amounts of volatile emission (mean ± SE ng.g^{-1} shoot fresh weight) emitted by uninfested (UP), *Tuta absoluta* egg-infested (TE), *Tuta absoluta* larvae-infested (TL), *Bemisia tabaci* infested (BT) and *T. absoluta* larvae + *B. tabaci* infested (double infestation - DI) tomato plants. Quantification was based on peak area relative to the internal standard.

N	Compound	Chemical Class	Treatments*				
			UP (*N* = 6)	TE (*N* = 6)	TC (*N* = 6)	BT (*N* = 6)	DI (*N* = 6)
1	(*E*)-2-hexenal	GLV**	0.4 ± 0.1^{b}	0.7 ± 0.1^{b}	5.2 ± 1.1^{a}	2.1 ± 0.9^{b}	4.1 ± 1.5^{ab}
2	(Z)-3-hexen-1-ol	GLV	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	2.5 ± 0.9^{a}	1.7 ± 0.8^{a}	3.3 ± 1.2^{a}
3	α-pinene	Monoterpene	1.3 ± 0.4^{c}	49.6 ± 12.0^{a}	51.9 ± 26.9^{a}	13.8 ± 4.9^{b}	11.1 ± 1.8^{b}
4	β-myrcene	Monoterpene	0.3 ± 0.1^{b}	1.2 ± 0.3^{b}	4.5 ± 2.2^{b}	2.4 ± 0.4^{b}	10.7 ± 3.0^{a}
5	Carene	Monoterpene	13.3 ± 5.3^{b}	92.8 ± 19.4^{ab}	621.5 ± 333.0^{a}	128.9 ± 56.1^{ab}	519.4 ±145.6^{a}
6	α-terpinene	Monoterpene	4.6 ± 1.8^{c}	15.4 ± 1.9^{b}	125.7 ± 58.6^{a}	12.5 ± 4.8^{b}	85.2 ± 22.5^{a}
7	Limonene	Monoterpene	0.7 ± 0.3^{c}	2.9 ± 0.4^{c}	53.6 ± 18.4^{b}	3.7 ± 0.9^{c}	94.7 ± 23.9^{a}
8	β-phellandrene	Monoterpene	13.3 ± 5.3^{c}	61.1 ± 7.7^{b}	175.9 ± 69.1^{a}	68.0 ± 23.5^{b}	199.5 ± 19.5^{a}
9	β-ocimene	Monoterpene	0.0 ± 0.0^{c}	0.5 ± 0.3^{c}	6.6 ± 2.6^{a}	2.1 ± 0.8^{b}	6.6 ± 1.7^{a}
10	γ-terpinen	Monoterpene	1.4 ± 0.5^{c}	3.1 ± 0.5^{b}	4.2 ± 0.8^{b}	5.0 ± 1.2^{b}	20.2 ± 5.0^{a}
11	Terpinolene	Monoterpene	1.8 ± 0.5^{c}	5.6 ± 0.5^{b}	9.2 ± 2.1^{b}	72.9 ± 40.4^{a}	75.4 ± 40.6^{a}
12	δ-elemene	Sesquiterpene	1.8 ± 0.4^{b}	0.0 ± 0.0^{b}	4.4 ± 1.3^{a}	0.0 ± 0.0^{b}	5.0 ± 1.6^{a}
13	β-elemene	Sesquiterpene	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	2.8 ± 1.2^{b}	3.1 ± 0.9^{b}	8.3 ± 1.0^{a}

14	Caryophyllene	Sesquiterpene	1.2 ± 0.3^{c}	6.8 ± 1.4^{b}	34.5 ± 19.6^{a}	26.2 ± 7.2^{a}	24.6 ± 6.7^{a}
15	Humulene	Sesquiterpene	0.0 ± 0.0^{c}	1.1 ± 0.3^{c}	10.2 ± 3.8^{a}	3.8 ± 1.4^{b}	8.2 ± 1.3^{a}

*Means followed by different letters indicate a significant difference between treatments according to Kruskal Wallis non-parametric test

** GLV = green leaf volatile

Figures

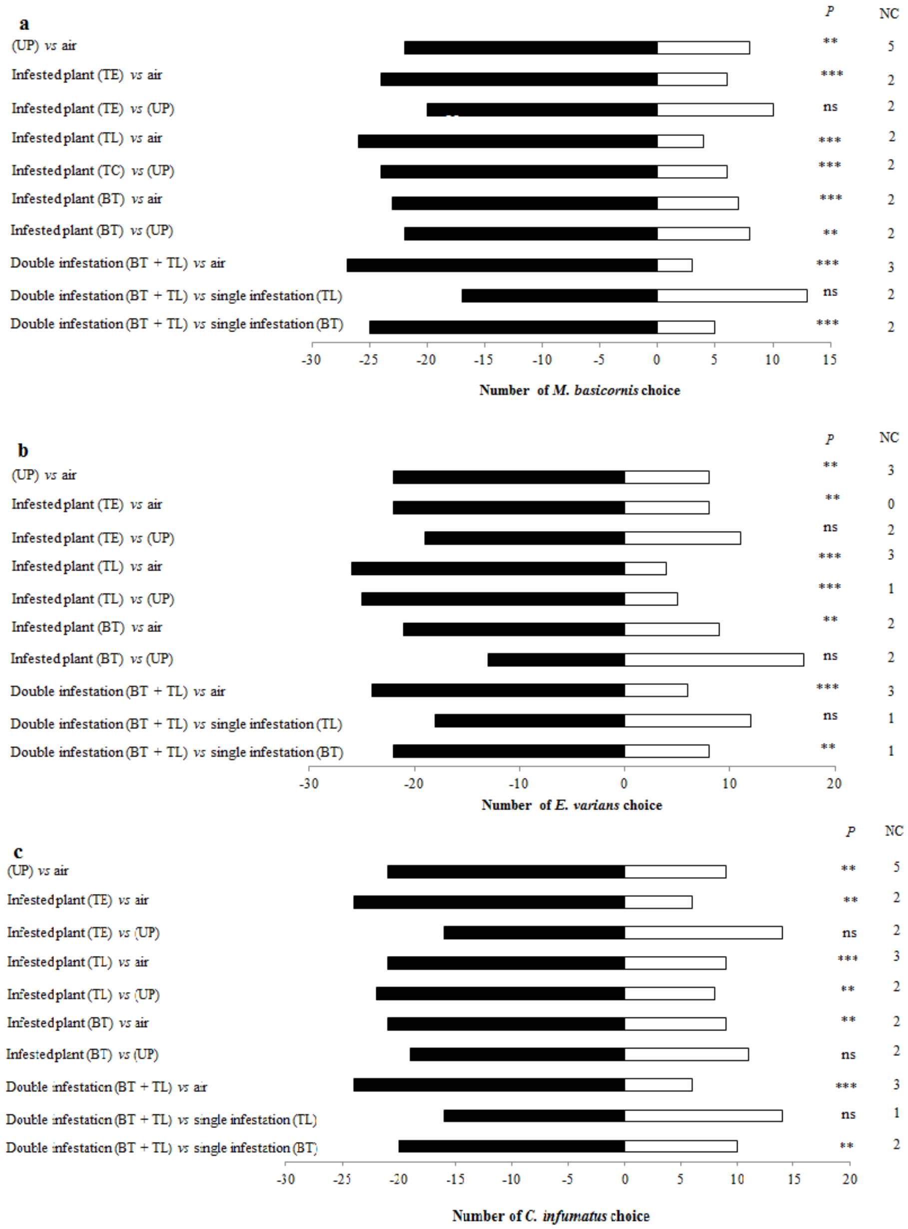

Figure 1 Responses of *M. basicornis* (a), *E. varians* (b) and *C. infumatus* (c) females to volatiles from tomato plants infested with eggs (TE) or larvae (TL) of *T. absoluta* or *B. tabaci* (BT) or double infestation (*T. absoluta* larvae + *B. tabaci*) in a Y-tube olfactometer. Numbers in bars represent 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<0.05, ns P = 0.05 (GLM, chi-square test).

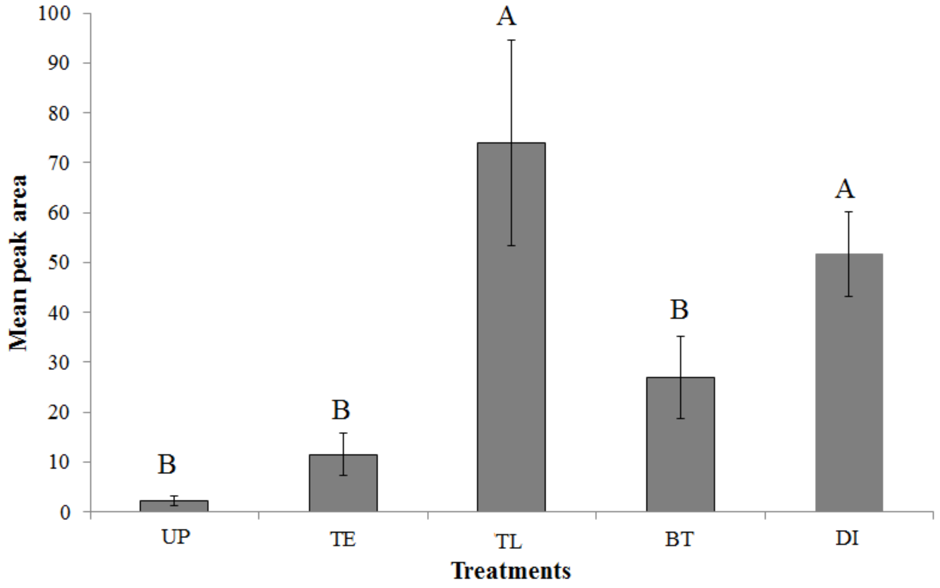

Figure 2. Average emission of volatiles for 6 replicates for uninfested tomato plants (UP), tomato plants infested with *Tuta absoluta* eggs (TE), tomato plants infested with *Tuta absoluta* larvae (TL), tomato plants infested with *Bemisia tabaci* (BT) and double infestation (*T. absoluta* larvae + *B. tabaci*) (DI). The values correspond to the mean and the error bars to the standard errors (±SE).

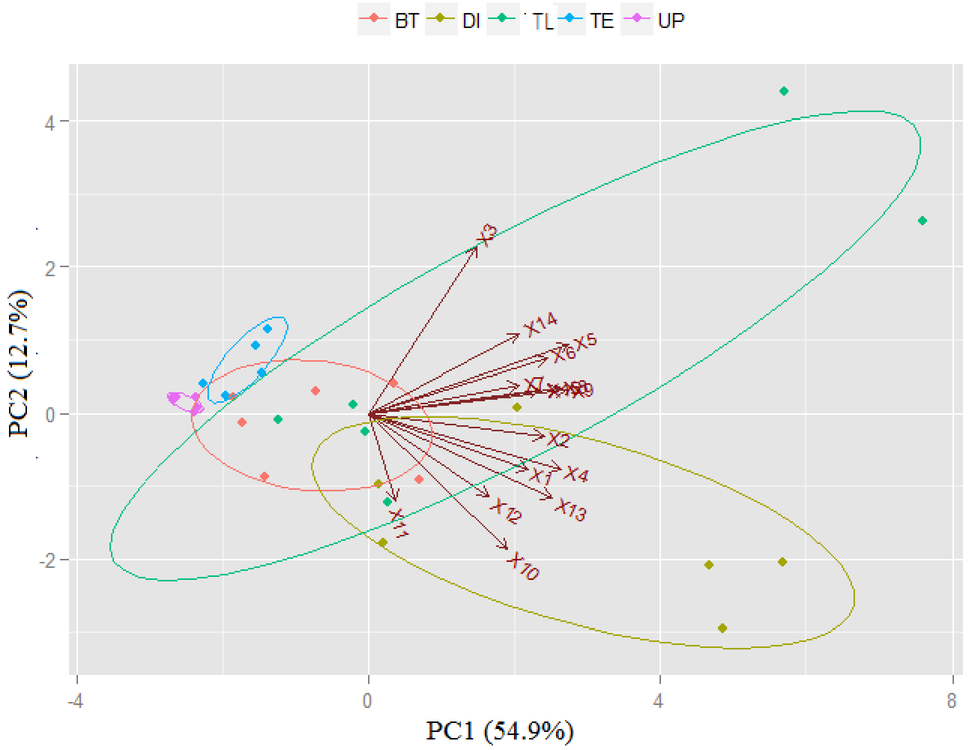

Figure 3. Principal component analysis (PCA) after tomato plants infested with *T. absoluta* eggs (TE, *n* = 6) or *T. absoluta* larvae (TL, *n* = 6) or *B. tabaci* (BT, *n* = 6) or double infestation (DI, *n* = 6) with no infestation as a control (UP, *n* = 6). Score plot visualizing the grouping pattern of the samples according to the first two principal components (PCs) with the explained variance in parenthesis. For compound identity in relation to the numbering, please refer to Table 1.

ARTICLE 4 COMPARATIVE ANALYSIS OF HERBIVORE-INDUCED PLANT VOLATILES FROM TOMATO PLANTS INFESTED BY EITHER *TUTA ABSOLUTA* OR *BEMISIA TABACI*

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Abstract. Plants release a variety of volatile organic compounds (VOCs) that play multiple roles in the interactions with other plants and animals. Natural enemies of plant-feeding insects use these volatiles as cues to find their prey/host. Here we report differences between the volatile blends of tomato plants infested with whitefly *Bemisia tabaci* or the tomato borer *Tuta absoluta*. We compared the volatile emission of (1) clean tomato plants; (2) tomato plants infested with *T. absoluta* larvae and (3) tomato plants infested with *B. tabaci* adults, nymphs and eggs by headspace sampling and analysis by gas chromatography coupled to mass spectrometry (GC-MS). A total of 80 VOCs were recorded of which 10 compounds only occurred consistently in headspace samples from TA-infested plants. Many of the 70 VOCs detected in the headspace of the two herbivory treatments were emitted at very different rates. Plants damaged by the tomato borer *T. absoluta* emitted at least 10 times higher levels of many compounds compared to plants damaged by the phloem-feeder *B. tabaci* and intact plants. The separation of *T. absoluta*-infested plants from those infested with *B. tabaci* was largely due to the C_{18}-fatty acid- and chorismate-derived compounds that had higher emission rates from *T. absoluta*-infested plants, whereas the sesquiterpenes α- and β-copaene, valencene and aristolochene were emitted at higher levels from *B. tabaci*-infested plants. Our findings imply that feeding by *T. absoluta* induced quantitative and qualitative changes in the emission of volatile organic compounds compared to *B. tabaci*, providing a chemical basis for the recently documented behavioral discrimination by two generalist predatory mirid species, natural enemies of *T. absoluta* and *B. tabaci* employed in biological control.

Key Words. Tomato; *Tuta absoluta; Bemisia tabaci;* HIPVs; GC-MS.

INTRODUCTION

The defense of plants against insect herbivores involves different strategies. Plants can defend themselves directly through the production of morphological structures on the leaf surface e.g. trichomes and by producing toxic compounds that deleteriously affect the behavior or development of the herbivores (Schoonhoven et al. 2005). Plant defense can also involve indirect mechanisms, including the production and release of plant volatile compounds as a response to herbivore feeding, commonly known as herbivore-induced plant volatiles (HIPVs) that provide important foraging cues for natural enemies of the herbivores (Turlings et al. 1990; Dicke 2009).

HIPVs can be comprised of hundreds of compounds (Dudareva et al. 2006), varying quantitatively and qualitatively depending on both abiotic and biotic factors and are specific to each plant – herbivore association (Ingegno et al. 2011; Benelli et al. 2013). When a plant is attacked by a leaf-chewer, a phloem feeder (Maffei 2010; Raghava et al. 2010; Verheggen et al. 2013), or when attacked by more than one organism, it reacts differently (Gosset et al. 2009; Zhang et al. 2013). For instance, chewing insects, such as caterpillars, predominantly activate the jasmonic acid (JA)-mediated defense signaling pathway, whereas feeding by phloem-sucking herbivores frequently activate the salicylic acid (SA) signaling pathway (Walling 2000), each resulting in the synthesis of specific blends of HIPVs that attract herbivore natural enemies (Heil 2014; Wei et al. 2014; Zhang et al. 2013).

Tomato (*Solanum lycopersicon* L.) is an important fruit crop with high susceptibility to insect herbivory. It is a host plant for two important pests worldwide, belonging to two different feeding guilds, the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), and the phloem-sucking whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). In the absence of any measure of control, infestation by these insect herbivores can lead up to 100 % production loss (Desneaux et al. 2010), and pest management demands the application of insecticides (Zalom 2003). The large scale use of insecticides causes environmental concerns and is harmful for natural enemies. Therefore more sustainable pest management strategies are needed. Being an annual plant with a short life cycle, tomato would benefit from recruiting natural enemies even more than perennial plants (Hilker and Meiners 2006). Elucidating the chemical ecology of tritrophic systems of natural enemies, herbivores and host plants is important in the development of effective and successful pest control strategies, by which abundance and distribution of natural enemies could be manipulated by semiochemicals (Hilker and Fatouros 2015).

HIPV blends released by tomato plants under herbivore infestation attract carnivorous natural enemies such as predators and parasitoids (Rodriguez-Saona et al. 2005; Moayeri et al. 2007a; Abbas et al. 2014). HIPV blends produced in response to chewing and phloem-sucking herbivores increase the attraction of mirid predators (Moayeri et al. 2007b; Pérez-Hedo et al. 2015; De Backer et al. 2015). Differences in

HIPV blend composition enable carnivores to make choices between available plant-herbivore combinations.

It was recently shown that the mirid predators *Macrolophus pygmaeus* Rambour and *Nesidiocoris tenuis* (Reuter) (both Hemiptera: Miridae) preferred the HIPV blends of tomato plants infested with *B. tabaci* or *T. absoluta* over the volatile blend emitted by uninfested tomato plants (Lins Jr. et al. 2014). In the current study, we aimed to identify differences in HIPV blends from tomato plants infested with whitefly *B. tabaci* or the tomato borer *T. absoluta*, which may allow the predators to discriminate among the herbivore-infested and uninfested tomato plants.

MATERIAL AND METHODS

Plants and Insects

Tomato plants *Solanum lycopersicon* L. cv. Moneymaker were grown 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.

Adult *T. absoluta* were kept in mesh cages (60 x 40 x 40 cm) with a potted tomato plant in a controlled room (25 ± 2ºC, 60 ± 10% R.H., L16:D8) to allow oviposition until larvae hatched; uninfested tomato leaves were introduced into the cages when necessary to ensure *ad libitum* feeding.

Bemisia tabaci was reared under the same greenhouse conditions, however, in another compartment. Adults were kept in mesh cages on potted tomato plants. Once per week a new cohort of adults was started on uninfested plants.

Nymphs and adults of *M. pygmaeus* Rambour and *N. tenuis* Reuter were supplied by Koppert Biosystems (Berkel en Rodenrijs, The Netherlands and Almeria, Spain respectively), kept in climate cabinets (25 ± 1ºC, 70 ± 5% R.H., L16:D8) in cages (60 x 40 x 40 cm) containing a potted tomato plant and eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as food.

Plant Treatments

To characterize the differences in plant volatiles released in response to attack by *T. absoluta* and *B. tabaci*, we collected headspace volatiles of tomato plants subjected to different herbivore treatments. All tomato plants for the experiment were treated in a controlled room (25 ± 2 °C, 70 % R.H., L16:D8). The plants were subjected to three treatments: (1) control, i.e. without herbivory, (2) *T. absoluta* infestation, (3) *B. tabaci* infestation.

Herbivore-infested and control plants were kept in separate mesh cages (60 x 40 x 40 cm) and separate climate-controlled rooms (25 ± 2ºC, 60 ± 10% R.H., L16:D8).

Tomato plants of 30 - 35 days-old were covered with organza bags and five couples of *T. absoluta* of up to 3 days old were released into each bag. Females were allowed to lay eggs for 48 h, and then the adults were removed. Larvae hatched 4-5 days after oviposition, and were allowed to feed for 72 h (Lins Jr. et al. 2014).

Fifty adults of *B. tabaci* were released in a cage (60 x 40 x 40 cm) with tomato plants. Ten days after infestation, the plants with adults, eggs and nymphs were used in the tests (Lins Jr. et al. 2014).

Headspace Collection of Plant Volatiles

Prior to volatile collection, the pots in which the plants were growing were carefully wrapped with aluminum foil. Plant volatiles were collected for 2 h by drawing compressed air that was filtered by passing through charcoal before reaching the glass jar (30 l) with the plant at a flow rate of 200 ml min^{-1} generated by an external pump. Volatiles were collected by passing the air stream through a stainless steel cartridge filled with 200 mg Tenax TA (20/35 mesh; CAMSCO, Houston, TX, USA) (Weldegergis et al. 2015). Immediately after the collection of volatiles, aboveground plant fresh weight was measured and the Tenax TA cartridges with volatiles were dry-purged for 15 min under a stream of nitrogen (N_2, 50 ml min^{-1}) at room temperature (21 ± 2 °C) to remove moisture and then stored at ambient temperature until analysis. For each treatment, 10 replicate plants were sampled. In order to correct for any non-plant volatile contribution, volatiles were collected from aluminum wrapped pots filled with soil only.

Analysis of Plant Volatiles

Headspace samples were analyzed with a Thermo Trace Ultra gas chromatograph (GC) coupled to a Thermo Trace DSQ quadrupole mass spectrometer (MS), both from Thermo Fisher Scientific (Waltham, MA, USA) using a protocol described by Cusumano et al. (2015). The collected volatiles were released from the Tenax TA thermally on Ultra 50:50 thermal desorption unit (Markes, Llantrisant, UK) at 250 °C for 10 min under a helium flow of 20 ml min^{-1}, while re-collecting the volatiles at 0 ^{o}C on an electronically cooled sorbent trap (Unity, Markes) and then transferred in splitless mode to the analytical column (ZB-5MSi, 30 m × 0.25 mm I.D. × 0.25 µm film thickness with a 5 m built-in guard column; Phenomenex, Torrence, CA, USA) situated in the GC oven for further separation by rapid heating of the cold trap at a rate of 40 ^{o}C s^{-1} to 280 °C, where it was kept for 10 min. The GC oven temperature was initially held at 40 °C for 2 min and was then raised at 6 ^{o}C min^{-1} to a final temperature of 280 °C, which was maintained for 4 min under a column flow of 1 ml min^{-1} in a constant flow mode. The column effluent was ionized by electron impact ionization at 70 eV. Mass spectra were acquired by scanning from *m*/*z* 35 to 400 at a scan rate of 4.70 scans s^{-1}. The MS transfer line and ion source were set to 275 and 250 °C, respectively. Tentative identification of compounds was based on comparison of mass spectra with those in the NIST 2005 and Wageningen Mass Spectral Database of Natural Products MS libraries. Experimentally calculated linear retention indices (LRI) were also used as an additional criterion to identify the compounds. We quantified the importance of each VOC in the separation between treatment groups by using Partial Least Squares - Discriminant Analysis (PLS-DA) (Barker and Rayens 2003). Relative quantification by peak areas of individual compounds was done using the integrated absolute signal of a quantifier ion in the selected ion monitoring (SIM) mode. The individual peak areas of each compound were computed into peak area per gram shoot biomass to correct for differences in size of individual plants and were further used in the statistical analysis. Volatiles from the compressed air, glass jars, pots and soils as well as clean Tenax TA adsorbents and the analytical system itself were treated as blank samples and used to correct for artefacts during analysis.

Data Analysis

Prior to analysis, the raw data of corrected peak areas were tested for normality and homogeneity of variances using the Shapiro-Wilk and Bartlett tests respectively. To test for significant differences among treatments, a non-parametric test (Kruskal-Wallis) was used since their distribution did not meet the assumptions for standard parametric ANOVA. The statistical analyses were performed by R statistical software (R Core Team 2014). For the volatile emission patterns, the corrected peak areas divided by the plant fresh weight were log-transformed, mean-centred and scaled to unit variance prior to analysis using a multivariate data analysis approach: projection to latent structures discriminant analysis (PLS-DA) using SIMCAP +12.0 software (Umetrics AB, Umeå, Sweden). PLS-DA is a method commonly used for pattern recognition and group separation among samples of different treatments based on available qualitative and quantitative information (Wold et al., 2001). PLS-DA provides score plots displaying visually recognized sample structure separating treatment groups according to model components, and complementary loading plots, displaying the contribution of each variable (in this case volatile compound) to these components separating the treatment groups as well as the relationships among the variables themselves.

RESULTS

The headspace volatile blend of tomato plants exposed to herbivory by *T. absoluta* (TA), *B. tabaci* (BT) or no herbivory (control, C) revealed a total of 80 volatile organic compounds (VOCs), of which 68 compounds were present in all treatment groups, whereas 75 compounds were detected in at least one of the herbivory treatments (Table 1). Control plants emitted 70 VOCs, BT-infested plants 75 VOCs and TA-infested plants 80 VOCs. Qualitative differences were found for three VOCs that only occurred in headspace samples from TA-infested plants. Taking into account that seven compounds occurred in only one or two of 10 headspace samples from C- or BT-infested plants, 10 compounds only occurred consistently in samples from TA-infested plants, among which most are C_{18}-fatty acid derived compounds. Apart from these qualitative differences, quantitative differences of at least factor 10 were found for many VOCs among plants exposed to one of the two herbivory treatments (Table 1). More than half of the listed volatiles were emitted at significantly higher levels in the samples treated with the tomato borer *T. absoluta* when compared to either intact undamaged plants or those treated with *B. tabaci* whiteflies (Kruskal Wallis Test; $P < 0.001$). These compounds comprise all the C_{18}-fatty acid derived volatiles (C_5- and C_6-compounds including green leaf volatiles (GLVs) and jasmone), aromatic volatiles derived from chorismate such as benzyl alcohol, methyl salicylate, methyl anthranilate, benzyl butanoate, eugenol; terpenes: norisoprenes (β-cyclocitral, (*E*)-α- and β-ionene), acyclic monoterpenes: ((*E*)-β-ocimene, linalool, allo-ocimene, (*E*,*E*)-cosmene), an acyclic sesquiterpene, (*E*,*E*)-α-farnesene, and the homoterpene (*E*)-DMNT. On the contrary, some cyclic sesquiterpenes such as α- and β-copaene, valencene and aristolochene were released at significantly higher levels from the plants infested with the phloem-sucking whitefly *B. tabaci*. No significant differences in levels of cyclic monoterpenes were found between the treatments. Furthermore, the average total amount of volatiles released was significantly higher in the TA treatment when compared with the BT treatment (*ca.* 10-fold difference) and the undamaged control plants (*ca*. 6-fold difference) (Kruskal Wallis; $F = 8.57$; $df = 6.59$; $P = 0.0014$).

Projection to latent structures discriminant analysis (PLS-DA) of all treatments together presented three major clusters of samples, where the two herbivory treatments are separated from the undamaged control plants and from each other (Figure 1a). The separation was mainly influenced by the presence of herbivores, where the C_5 and C_6-compounds, chorismate-derived aromatic compounds, terpenoids: norisoprenes, acyclic mono- and sesquiterpenes as well as homoterpenes were highly correlated with *T. absoluta* infestation, whereas cyclic sesquiterpenes were highly correlated with *B. tabaci*-infested plants. Among the 80 headspace volatiles used for this analysis, 38 contributed most to the separation between the treatments, with variable importance for the projection (VIP) values > 1. These compounds included C_{18}-fatty acid-derivatives: **42, 5, 32, 2, 51, 9, 23, 41, 11, 1, 7, 30, 50, 42, 8, 6, 36** & **10**; aromatic volatiles: **47, 34, 45** & **46**; norisoprenes: **35** & **56**; sesquiterpenes: **49, 62, 59, 57, 52, 64, 65** & **63**; a monoterpene: **29**; a homoterpene: **68** and an amino acid-derived alcohol: **3**. The correlation between the contributions of these compounds with at least one of the three treatments is clearly visible from the loading plot in Figure 1b.

A detailed analysis of the compositional differences between the HIPV-blends emitted by plants infested by either herbivore and the control plants was carried out. PLS-DA analysis yielded a clear separation between BT-infested and control plants (Figure 2a). In total 24 compounds contributed most to the separation (Figure 2b) based on VIP values higher than 1. These compounds listed with numbers in the order of decreasing VIP-value are: **49, 57, 62, 59, 52, 5, 3, 35, 34, 64, 2, 68, 21, 65, 10, 50, 1, 11, 7, 63** & **4** (Table 1 & 2, Figure 2b). All these compounds were positively correlated to the *B. tabaci* infested tomato plants (Figure 2b), and were emitted in elevated amounts when compared to uninfested plants.

A similar pairwise PLS-DA analysis between *T. absoluta*-infested and uninfested plants likewise showed a clear separation of the treatment groups based on their headspace volatile compositions (Figure 3a). The PLS-DA analysis identified 38 compounds with a VIP value higher than 1. These compounds are mainly dominated by the C_{18}-fatty acid-derivatives (in Table 1 and Figure 3b; compound numbers: **1**, **2**, **4** – **11**, **13**, **23**, **25**, **30** – **33**, **36**, **37**, **41**, **42**, **44**, **51** & **53**), and chorismate-derivatives (in Table 1 and Figure 3b; compound numbers: **24**, **34**, **43**, **45**, **46** & **47**), as well as the amino acid-derived alcohol: 3-methylbutan-1-ol and terpenes (in Table 1 and Figure 3b; compound numbers: **27**, **29**, **35**, **61**, **65** & **68**). In addition, several of the C_{18}-fatty acid derived volatiles such as (*Z*)-2-penten-1-ol, (*Z*)-2-penten-1-ol, acetate, (*Z*)-3-hexen-1-ol, formate, (*Z*)-butanoic acid, 2-pentenyl ester, (*Z*)-3-hexenyl (*E*)-2-butenoate, (*Z*)-3-hexen-1-ol, 2-methyl-2-butenoate and an amino acid derived alcohol: 3-methylbutan-1-ol all with VIP values higher than 1 were only detected in the headspace of *T. absoluta* treated plants (Table 1).

DISCUSSION

Plants are capable of synthesizing and releasing an array of volatile organic compounds derived from a diverse set of primary metabolites that include amino acids, fatty acids, and sugars (Schwab et al., 2008). These volatiles have a range of functions in intra- and inter-kingdom interactions, including those among plants and insects (Dicke and Baldwin, 2010). Insects respond according to the blend of volatiles perceived (Dicke et al., 2009; Maoyeri et al., 2007b; Lins Jr. et al., 2014). From the predator's point of view, beyond the time and energy costs of searching and the increased vulnerability of itself being preyed, it has to deal with variation in the availability of chemical cues emitted by the food plants of their prey. *Macrolophus pygmaeus* preferred volatile blends released by tomato plants over volatiles from other host plants like *Capsicum officinalis* L., *C. annuum* L. and *Salvia officinalis* L. (Ingegno et al., 2011).

Knowledge about orientation mechanisms of mirid predators is limited and deserves to be studied as they play an important role in biological control. Our previous behavioral study (Lins Jr. et al., 2014), have proved that *N. tenuis* and *M. pygmaeus* were attracted to volatile blends released by tomato plants infested by the two important tomato pests TA and BT. As a follow-up, we here present volatile emissions of this plant after exposure to these two main herbivores in order to evaluate the role of HIPVs in enhancing the efficiency of these biological control agents.

The lack or presence of particular compounds can make the plant unrecognizable for naive predators, and learning can be necessary to enhance responses and motivate predator and/or parasitoid searching. It was therefore evident that learning by *M. pygmaeus* improved their capacity to find their prey (Lins Jr. et al., 2014). Insect learning is a well-known and widely studied experience-based modification of behavior (Steidle and Van Loon 2003; De Boer et al. 2005; Glinwood et al. 2011; Rim et al., 2015), however, it was only recently studied for predatory mirid bugs (Lins Jr. et al., 2014).

Chemical analysis of volatile blends emitted by herbivore-infested plants and intact plants demonstrated qualitative and/or quantitative changes in the emission of infochemicals by the plant-herbivore complexes (Dicke et al, 2009; Fatouros et al., 2012; Poelman et al., 2012; Weldegergis et al., 2015). Immediately upon damage by herbivores, tomato plants enhanced the emission of fatty acid-derived volatile compounds, which are the result of the breakdown of lipids through the lipoxygenase pathway (Shen et al., 2014). Breakdown of plant cell membranes gives rise to free linoleic and/or linolenic acid, both of which are acted upon by lipoxygenase to form C_5 volatile compounds and the C_6 green leaf volatiles (Croft et al., 1993; McCormick et al., 2012; Shen et al., 2014). When released from the plant, these compounds can trigger responses in neighboring plants, including phytoalexin accumulation in cotton (Zeringue, 1992) and also lower insect feeding rates in tomato (Hildebrand et al., 1993).

Our chemical analysis of the headspace of uninfested and infested tomato plants provided similar results to previous studies (Degenhardt et al. 2010; Proffit et al. 2011; De Backer et al., 2015). However, we also detected 65 volatile compounds that have not been found by these authors, including compound numbers: **1-6, 8-11, 13, 17-19, 21, 23-25, 29-33, 35-48, 50, 51, 53, 54, 56-67 & 69-80** (Table 1). Differences in the emitted blend may be explained by plant variety and physiological condition, environmental conditions as well as duration and conditions of infestation prior to volatile collection (Dudareva et al. 2006; Niinemets et al., 2013). Our results also highlight the differential induction of plant volatiles on the basis of insect feeding styles, where the biting-chewing tomato borer induced the amount and number of compounds released from tomato plants to higher levels than the phloem sucking whitefly (Stout et al., 1994).

Volatiles that have been detected only in both herbivore-infested plants may be regarded as universal signs of herbivore damage and quantitative differences might play a role for predators/parasitoids locating their prey/host (Kessler and Baldwin, 2001). It is also notable in our findings that there were qualitative differences among the treatments.

Thirteen compounds were only consistently detected in the headspace of TA-infested plants compared to that of control plants and 10 compounds were not consistently found in the HIPV blend of BT-infested plants compared to TA-infested plants, most of which were fatty acid-derived and aromatic compounds. Presence or absence of compounds in volatile blends could have been important for mirid females to discriminate between odors of infested and uninfested tomato plants (Lins Jr.et al., 2014). According to De Boer et al. (2004), qualitative and quantitative differences in the odor blends of plants may enable natural enemies to discriminate between odor sources, while searching for their prey or hosts.

Differences between the different plant treatments were found for C_{18} fatty acid and chorismate-derived volatiles and some acyclic mono-, homo- and sesquiterpenes were significantly higher in the TA-infested plants. Strikingly, cyclic sesquiterpenes were the only group of volatiles that were strongly associated with BT-infested plants, and contributed strongly to separating them from the TA-infested and intact control samples. Insect feeding on tomato induced increased tissue levels of three oxidative enzymes: polyphenol oxidase (PPO), peroxidase (POD), and lipoxygenase (LOX) (Stout et al., 1994). Upon herbivore damage LOX is activated giving rise to the immediate release of volatiles and induction of the jasmonic acid (JA) signalling pathway (McCormick et al., 2012). The JA pathway is activated when a plant is attacked by chewing/mining herbivores and elicitors in their oral secretion activate multiple defense-related volatiles including GLVs and JA derivatives (Zebelo et al., 2014). This was evident by the level and number of volatiles derived from the

C_{18} fatty acids in the headspace of the TA-infested plants. The qualitative difference between control and tomato plants infested with *T. absoluta*, i.e. the release of C_5 and C_6 fatty acid-derived volatiles is the result of the plant's wound response during attack by the herbivore. Moreover, some of the C_{18} derived compounds including GLVs, (*Z*)-3-hexenyl (*E*)-2-butenoate, (*Z*)-3-hexen-1-ol, valerate, (*Z*)-3-hexen-1-ol, 2-methyl-2-butenoate, and (*Z*)-3-hexenyl (*Z*)-3-hexenoate were not found in the headspace of *B. tabaci*-infested plants. After chewing damage by TA, sequential activation of lipoxygenase and hydroperoxide lyase can result in the production of C_6 compounds (McCormick et al., 2012). Nevertheless, although these compounds were not found in the headspace of BT-infested plants, *N. tenuis* and *M. pygmaeus* discriminated the HIPV-blend of BT-infested plants over those of clean plants (Lins Jr. et al., 2014). The majority of predatory species are generalist natural enemies and they can learn to respond to the HIPV blends released by different herbivore-plant interactions (Moayeri et al., 2006; 2007b; Dicke et al 2009). In addition cyclic sesquiterpenes were found in high quantity in headspace samples from BT infested plants which could also have contributed for the BT-infested plant being more attractive for the predators over clean plants.

The differences in the amounts of volatiles emitted contributed also to the separation between the herbivore infestation treatments. TA-infested plants presented compounds which tend to be released in higher amounts than from BT-infested plants. These compounds include fatty acid derivatives like C_5 compounds, the C_6 green leaf volatiles and terpenes: **20, 26-29, 65 & 68** (Table 1); JA derivative: (*Z*)-jasmone and methyl *cis*-dihydrojasmonate. They were also increased when other plants are damaged by other biting-chewing insects (Poelman et al. 2012; Zhang et al., 2013; Ponzio et al. 2013; Weldegergis et al., 2015; Vuorinen et al., 2014; War et al. 2011) or when mechanically wounded leaves were treated with oral secretions of herbivores (Zebelo et al., 2014). Moreover, these compounds play an important role in the attraction of natural enemies such as parasitoids, predatory mites and lacewings (Dicke et al. 1990; Smid et al., 2002; Bukovinsky et al., 2005; War et al. 2011).

Another class of importance in revealing the difference between treatments worth looking at is that of the aromatic volatiles, the role of which in insect-plant interactions is often overlooked. In our study, their release was much influenced by the presence of the tomato borer TA. These compounds were those directly formed from chorismate or phenylalanine via multiple biosynthetic steps (Dudareva et al., 2006). Some of the volatiles that belong to the aromatic classes are: methyl benzoate, methyl salicylate, indole, methyl anthranilate, benzyl butanoate and eugenol, and they were found to occur at significantly higher levels in the emissions of TA-infested plants, with the latter three were mainly detected in the headspace of TA-infested plants. The emission of most of these volatiles is often associated with flowers and to a lesser extent with leaves (Dudareva et al. 2004) and they are known as defense chemicals. For example, Turlings et al. (1998) have reported emission of methyl anthranilate after exogenic application of caterpillar (*Spodoptera littoralis* Boisduval) regurgitant to mechanically damaged leaves. Herbivore-induced plant volatile emission is known to be mainly regulated by the octadecanoid or JA signal-transduction pathway (Ament et al., 2004).

Herbivores from different feeding guilds, however, have been shown to elicit distinct defense pathways in plants (Kempema et al., 2007; Zhang et al., 2009). Methyl salicylate, a volatile derivative of the SA signaling pathway is activated by piercing-sucking insects such as whiteflies and aphids (Kempema et al., 2007; Zarate et al., 2007). However, in the present study, the level of methyl salicylate was higher when tomato plants were infested by TA. Methyl salicylate might also be elicited downstream the cascade of JA-induced responses after chewing herbivore attack (Cardoza et al., 2002; Dicke et al., 1999; Rodriguez-Saona et al., 2001; Ament et al., 2004). In addition, chewing insects cause severe damage to plant tissues and are likely to induce stronger reactions in plants than sucking herbivores (Magalhães et al., 2012). For instance, the amount of HIPV induced by larvae was higher compared to plants infested with silver leaf whiteflies or pea aphids (Rodriguez-Saona et al., 2003; Schwartzberg et al., 2011). Furthermore, plants simultaneously infested by spider mites and whiteflies emitted lower amounts of HIPVs (Zhang et al., 2009).

Analyses of the HIPV blends of plants damaged by TA and BT showed significant variation in the proportional concentration of several components as well as qualitative differences between the HIPV-blends. The difference might be caused by the difference in feeding styles of these two herbivores, and the separation between BT- and TA-induced volatile blends could be attributed to the cyclic sesquiterpenes that were emitted at higher level when a plant was infested by the phloem-sucking herbivore BT. Gosset et al. (2009) have reported higher level of sesquiterpenes from potato plants (*Solanum tuberosum* L.) when infested by the Green Peach Aphid (*Myzus persicae* Sulzer), a piercing-sucking insect, compared to plants infested by the chewing Colorado Potato Beetle (*Leptinotarsa decemlineata* Say). When given choices between plants damaged by both herbivorous species (doubly infested by both BT and TA) compared to damage by one prey alone (single infestation), *N. tenuis* and *M. pygmaeus* did not discriminate (Lins Jr. et al., 2014). When the two herbivores induce the same plant there might be an antagonistic interaction between JA and SA pathways which may reduce the amount of volatiles emitted (Zhang et al., 2009). Generalist predators were predicted to utilize general cues released by all of their different host/prey complexes (Steidle and Van Loon, 2003).

The chemical data support the findings of our previous behavioral studies in the system tomato – herbivore - mirid predator. It was demonstrated that volatile profiles of tomato plants infested by two herbivore species differed both qualitatively and quantitatively. Investigation of the chemosensory response, *e.g.* electroantennography, of the mirid predators to each compound identified in the HIPV blends emitted from tomato infested by the herbivores may be used for identification of the HIPVs that contribute to attraction of mirids. Such information may provide a baseline to further studies aiming to develop semiochemical strategies to improve existing pest control approaches of these tomato pests.

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Tables

Table 1. Volatile compounds detected in the headspace of tomato plants without herbivore infestation (C), tomato plants infested with *Bemisia tabaci* (BT) and tomato plants infested with *Tuta absoluta* (TA) according to their elution order in a chromatographic window.

No	Compound	Class	LRIexp.	LRIlit.	Amount of volatile emitted[&] (Mean ±SE)		
					C (n=10)	BT (n=10)	TA (n=10)
1	1-Penten-3-ol	Alcohol	659	672	81.94 ± 23.78^{c}	550.46 ± 163.39^{b}	8111.89 ± 2737.95^{a}
2	3-Pentanol	Alcohol	673	690	308.54 ± 130^{c}	947.71 ± 298.06^{b}	9857.23 ± 2822.9^{a}
3	3-Methylbutan-1-ol	Alcohol	713	726	0 ± 0^{c}	51.13 ± 30.34^{b}	412.87 ± 121.43^{a}
4	(*E*) -2-Pentenal	Aldehyde	736	745	3.47 ± 1.54^{c}	19.35 ± 6.44^{b}	516.98 ± 184.34^{a}
5	(*Z*)-2-Penten-1-ol	Alcohol	760	767	0 ± 0^{c}	12.43 ± 4.64^{b}	505.93 ± 205.89^{a}
6	(*E*)-2-Hexenal	Aldehyde	850	850	2.14 ± 0.94^{c}	13.51 ± 4.52^{b}	1567.24 ± 634.63^{a}
7	(*Z*) -3-Hexen-1-ol	Alcohol	860	860	152.12 ± 46.94^{c}	1363.18 ± 461.23^{b}	18494.28 ± 6161.94^{a}
8	(*E*,*E*)-2,4-Hexadienal	Aldehyde	912	912	4.3 ± 2.54^{c}	22.64 ± 8.29^{b}	576.28 ± 189.73^{a}
9	(*Z*)-2-Penten-1-ol, acetate	Ester	915	909	0 ± 0^{b}	$*1.19 \pm 1.19^{b}$	312.47 ± 133.17^{a}
10	(*Z*)-3-Hexen-1-ol, formate	Ester	922	920	0 ± 0^{b}	6.23 ± 5.96^{b}	36.77 ± 14.54^{a}
11	(*E*)-4-Oxo-2-hexenal	Aldehyde	961	976[†]	13.39 ± 6.09^{c}	179.16 ± 63.59^{b}	11276.54 ± 4314.56^{a}
12	β-Myrcene	Monoterpene	991	991	806.8 ± 665.78^{a}	300.9 ± 149.57^{a}	821.53 ± 654.77^{a}
13	(*Z*)-3-Hexen-1-ol, acetate	Ester	1008	1008	18.28 ± 8.49^{b}	35.01 ± 16.7^{b}	5055.68 ± 2544.35^{a}
14	α-Phellandrene	Monoterpene	1010	1010	2857.38 ± 2579.48^{a}	814.26 ± 745.77^{a}	3962.29 ± 2580.66^{a}
15	α-Terpinene	Monoterpene	1021	1021	10891.34 ± 9711.93^{a}	2780.76 ± 2499.93^{a}	$39937.18 \pm 32945.11^{a}$
16	Limonene	Monoterpene	1030	1029	$17414.13 \pm 15480.37^{a}$	4711.07 ± 4184.55^{a}	34953.7 ± 25328.57^{a}
17	1,8-Cineole	Monoterpene	1032	1032	23.78 ± 11.55^{a}	21.27 ± 6.24^{a}	22.05 ± 10.68^{a}
18	Benzyl alcohol	Alcohol	1039	1039	52.69 ± 22.1^{b}	44.46 ± 11.95^{b}	1370.22 ± 616.3^{a}
19	Benzeneacetaldehyde	Aldehyde	1045	1045	11.74 ± 2.21^{b}	$18.64 \pm 3.55^{a,b}$	37.88 ± 5.97^{a}
20	(*E*)-β-Ocimene	Monoterpene	1049	1049	177.06 ± 121.07^{b}	295.16 ± 204.1^{b}	8875.36 ± 3070.76^{a}

21	Conophtorin	Ether	1058	1054	34.29 ± 5.45^{b}	71.89 ± 15.86^{a,b}	255.92 ± 61.2^{a}
22	Terpinolene	Monoterpene	1090	1090	229.48 ± 208.94^{a}	42.58 ± 27.49^{a}	3329.66 ± 3202.9^{a}
23	(*Z*) -Butanoic acid, 2-pentenyl ester	Ester	1091	1091	0 ± 0^{b}	*1.14 ± 1.14^{b}	518.54 ± 294.62^{a}
24	Methyl benzoate	Ester	1097	1097	7.09 ± 4.41^{b}	10.99 ± 6.33^{b}	469.09 ± 136.74^{a}
25	(*Z*) -3-Hexen-1-ol, propanoate	Ester	1100	1100	7.99 ± 4.5^{c}	11.49 ± 6.05^{b,c}	2054.65 ± 1020.32^{a}
26	Linalool	Monoterpene	1102	1102	18.39 ± 8.22^{b}	14.14 ± 7.63^{b}	937.43 ± 329.92^{a}
27	(*E*)–DMNT	Homoterpene	1117	1119 †	27.33 ± 10.77^{b}	43.03 ± 20.57^{b}	1286.67 ± 695.97^{a}
28	Allo-ocimene	Monoterpene	1131	1131	29.14 ± 23.93^{b}	17.56 ± 11.26^{b,c}	1145.33 ± 1065.68^{a}
29	(*E*,*E*)-Cosmene	Monoterpene	1132	1134	*49.84 ± 49.7^{b}	2.6 ± 1.96^{b}	104.75 ± 35.88^{a}
30	(*Z*) -3-Hexen-1-ol, isobutyrate	Ester	1145	1147	4.9 ± 2.6^{b}	6.67 ± 4.3^{b}	1564.66 ± 815.32^{a}
31	(*Z*)-3-hexenyl (*E*)-2-butenoate	Ester	1172	NF	0 ± 0^{b}	0 ± 0^{b}	875.46 ± 307.81^{a}
32	(*Z*)-3-Hexenyl butyrate	Ester	1186	1186	128.06 ± 61.32^{b}	106.82 ± 33.89^{b}	16872.08 ± 6969.29^{a}
33	Hexyl butanoate	Ester	1192	1192	13.01 ± 5.38^{b}	10.27 ± 2.39^{b}	853.94 ± 384.73^{a}
34	Methyl salicylate	Ester	1198	1198	83.76 ± 42.78^{c}	775.95 ± 518.59^{b}	7545.89 ± 2651.47^{a}
35	β-Cyclocitral	Norisoprene	1224	1224	0.82 ± 0.53^{b}	4.47 ± 1.13^{b}	95.65 ± 21.43^{a}
36	(*Z*)-3-Hexenyl isovalerate	Ester	1233	1230	9.13 ± 4.98^{b}	13.17 ± 7.41^{b}	1983.95 ± 718.4^{a}
37	(*Z*)-3-Hexen-1-yl 2-methylbutanoate	Ester	1237	1237	4.07 ± 2.3^{b}	3.56 ± 1.59^{b}	564.45 ± 185.1^{a}
38	Linalyl acetate	Ester	1257	1257	25.48 ± 6.82^{a,b}	14.15 ± 7.39^{b}	106.43 ± 68.77^{a}
39	Pipertone	Monoterpene	1258	1258	53.41 ± 45.8^{a}	6.32 ± 3.22^{b}	28.45 ± 13.29^{a}
40	Unknown	NA	NA	NA	82.22 ± 17.24^{a,b}	56.63 ± 13.34^{b}	164.39 ± 33.12^{a}
41	(*Z*)-3-Hexen-1-ol, valerate	Ester	1285	1279	*0.45 ± 0.45^{b}	0 ± 0^{b}	129.94 ± 58.11^{a}
42	(*Z*)-3-Hexen-1-ol, 2-methyl-2-butenoate	Ester	1288	1315	0 ± 0^{b}	0 ± 0^{b}	112.99 ± 56.14^{a}
43	Indole	Heterocyclic	1299	1300	65.49 ± 25.05^{c}	428.94 ± 285.97^{b}	11180.01 ± 3527.63^{a}
44	(*Z*)-3-Hexen-1-yl tiglate	Ester	1326	1322	23.62 ± 10.28^{b}	20.83 ± 9.94^{b}	1672.61 ± 446.16^{a}
45	Methyl anthranilate	Ester	1346	1343	*1.19 ± 0.91^{b}	*1.71 ± 1.71^{b}	109.48 ± 43.42^{a}
46	Benzyl butanoate	Ester	1347	1347	2.95 ± 1.89^{b}	1.26 ± 0.76^{b}	155.15 ± 75.18^{a}
47	Eugenol	Alcohol	1361	1361	*0.97 ± 0.97^{b}	*0.39 ± 0.38^{b}	139.85 ± 55.87^{a}

48	2-Acetoxypulegone	Ketone	1373	NF	59.53 ± 21.43^{a,b}	38.71 ± 8.78^{b}	128.31 ± 38.35^{a}
49	α-Copaene	Sesquiterpene	1381	1382	95.67 ± 31.6^{c}	1681.38 ± 617.71^{a}	125.28 ± 78.25^{b}
50	(*Z*)-3-Hexen-1-yl hexanoate	Ester	1382	1382	9.55 ± 5.11^{b}	33.01 ± 11.5^{b}	269.32 ± 119.31^{a}
51	(*Z*)-3-Hexenyl (*Z*)-3-hexenoate	Ester	1386	1383	*2.7 ± 1.88^{b}	0 ± 0^{b}	120.81 ± 50.87^{a}
52	β-Elemene	Sesquiterpene	1396	1397	16.97 ± 15.06^{b}	58.46 ± 24.34^{a,b}	71.65 ± 59.36^{a}
53	(*Z*)-Jasmone	Ketone	1402	1403	64.28 ± 36.81^{b}	14.15 ± 6.96^{b}	421.42 ± 124.56^{a}
54	Unknown	NA	NA	NA	0 ± 0^{b}	0 ± 0^{b}	28.33 ± 11.48^{a}
55	(*E*) -β-Caryophyllene	Sesquiterpene	1428	1428	592.77 ± 568.16^{a,b}	249.65 ± 223.2^{b}	2569.85 ± 2401.01^{a}
56	(*E*)-α-Ionone	Norisoprene	1432	1432	2.15 ± 0.92^{b}	*1.09 ± 0.74^{b}	13.15 ± 5.25^{a}
57	β-Copaene	Sesquiterpene	1435	1435	9.26 ± 3.16^{b}	113.23 ± 43.18^{a}	12.42 ± 5.13^{b}
58	α-Caryophyllene	Sesquiterpene	1461	1461	301.89 ± 290.46^{a}	116.6 ± 105.07^{a}	1522.66 ± 1429.05^{a}
59	Valencene	Sesquiterpene	1484	1484	17.84 ± 8.73^{b}	72.64 ± 22.97^{a}	30.33 ± 8.93^{b}
60	Bicyclosesquiphellandrene	Sesquiterpene	1488	1487	15.85 ± 13.58^{b}	19.43 ± 12.05^{b}	81.46 ± 75.06^{a}
61	(*E*)-β-Ionone	Norisoprene	1490	1490	28.28 ± 10.24^{b}	37.63 ± 8^{b}	576.25 ± 93.82^{a}
62	Aristolochene	Sesquiterpene	1494	1486	2.97 ± 2.03^{b}	332.87 ± 222.65^{a}	2.56 ± 0.77^{b}
63	β-Chamigrene	Sesquiterpene	1502	1503	5.8 ± 5.43^{a}	8.87 ± 2.81^{a}	5.75 ± 3.91^{a}
64	Patchoulene	Sesquiterpene	1506	1484	4.23 ± 1.93^{b}	17.67 ± 6.08^{a}	7.41 ± 2.94^{ab}
65	(*E*,*E*)-α-Farnesene	Sesquiterpene	1509	1509	2.72 ± 2.12^{b}	11.78 ± 4.65^{b}	84.06 ± 23.47^{a}
66	Unknown	NA	NA	NA	4.09 ± 2^{b}	26.89 ± 13.05^{a,b}	53.19 ± 16.11^{a}
67	(*Z*)-3-Hexen-1-ol, benzoate	Ester	1574	1575	94.48 ± 48.53^{b}	48.59 ± 11.41^{b}	942.74 ± 360.51^{a}
68	(*E*,*E*)-TMTT	Homoterpene	1582	1584 ↑	965.38 ± 267.09^{b}	4286.68 ± 1887.88^{a,b}	9157.15 ± 2776.07^{a}
69	methyl *cis*-dihydrojasmonate	Ester	1657	1656	90.42 ± 26.17^{a}	82.31 ± 13.8^{a}	149.24 ± 35.69^{a}
70	Unknown	NA	NA	NA	581.84 ± 206.18^{a}	402.81 ± 162.87^{a}	981.6 ± 231.46^{a}
71	IPDMOHM	Sesquiterpene	1679	1659	348.57 ± 120.43^{a}	242.89 ± 93.41^{a}	607.5 ± 126.71^{a}
72	Unknown	NA	NA	NA	52.02 ± 17.21^{a,b}	37.58 ± 13.85^{b}	91.67 ± 16.47^{a}
73	Unknown	NA	NA	NA	152.73 ± 51.65^{a}	104.92 ± 42.65^{a}	242.8 ± 54.18^{a}
74	Unknown	NA	NA	NA	4.05 ± 0.88^{a}	9.41 ± 2.78^{a}	12.89 ± 6.55^{a}
75	Unknown	NA	NA	NA	4.01 ± 0.88^{a}	8.09 ± 2.05^{a}	12.38 ± 6.54^{a}

76	Unknown	NA	NA	NA	2.96 ± 0.59^{a}	6.93 ± 1.89^{a}	11.54 ± 6.56^{a}
77	4-Acetyl-α-cedrene	Ketone	1779	1776	297.11 ± 105.52^{a}	268.11 ± 66.43^{a}	417.32 ± 105.83^{a}
78	Unknown	NA	NA	NA	0 ± 0^{b}	3.83 ± 1.84^{a}	5.53 ± 4.27^{a}
79	Unknown	NA	NA	NA	0 ± 0^{b}	4.29 ± 1.99^{a}	3.88 ± 3.87^{a}
80	Unknown	NA	NA	NA	2.12 ± 1.23^{a}	4.92 ± 1.92^{a}	6.55 ± 3.87^{a}
	Average total peak area	-	-	-	480.82 ± 250.16^{b}	284.58 ± 87.01^{b}	2804.43 ± 732.94^{a}

Significant differences in the volatile emissions among plants exposed to three treatments based on the Kruskal Wallis non-parametric test exist when means have no superscript letters in common.

& Amount of volatile compound emitted from control plants (C), plants infested with *B. tabaci* (BT) or *T. absoluta* (TA) are given as mean peak area ± SE per gram fresh weight of foliage divided by 10^{3}. The number of replicates for each treatment in parentheses.

LRI$_{Exp.}$: Llinear retention indices experimentally obtained on a ZB-5MSi analytical column

LRI$_{Lit.}$: Llinear retention indices obtained from literature [NIST 2005, Wageningen University Mass Spectral library, and The Pherobase (http://www.pherobase.com/database/kovats/kovats-index.php) on a column with (5 %-Phenyl)-methylpolysiloxane stationary phase or equivalent.

†: LRI$_{Lit.}$ obtained from Marques et al. (2007) and Xavier et al. (2011).

NA: Not Applicable

NF: LRI$_{Lit.}$ Not Found

*: detected in 1 or 2 samples

(*E*)–DMNT: (*E*)-4,8-dimethylnona-1,3,7-triene

(*E*, *E*)–TMTT: (*E*, *E*) –4,8,12-trimethyltrideca-1,3,7,11-tetraene

IPDMOHM: (7a-Isopropenyl-4, 5-dimethyloctahydroinden-4-yl)methanol

Table 2. Values of Variable Importance to the Projection (VIP) of volatile compounds for the corresponding PLS-DA plots (Fig. 1, 2 & 3) based on the headspace composition of tomato plants subjected to: *T. absoluta* infestation (TA, *n* = 10) or *B. tabaci* infestation (BT, *n* = 10) or no infestation as a control (C, *n* = 10) of tomato plants. Compounds are listed according their elution order in a chromatographic window.

[a]No	Compound	[b]PLS-DA (C, TA & TB)	[c]PLS-DA (C vs BT)	[d]PLS-DA (C vs TA)
1	1-Penten-3-ol	**1.16**	**1.20**	**1.15**
2	3-Pentanol	**1.21**	**1.43**	**1.41**
3	3-Methylbutan-1-ol	**1.40**	**1.63**	**1.65**
4	(*E*) -2-Pentenal	0.81	**1.06**	**1.00**
5	(*Z*)-2-Penten-1-ol	**1.37**	**1.63**	**1.64**
6	(*E*)-2-Hexenal	**1.01**	0.95	**1.34**
7	(*Z*) -3-Hexen-1-ol	**1.15**	**1.16**	**1.16**
8	(*E*,*E*)-2,4-Hexadienal	**1.03**	0.91	**1.39**
9	(*Z*)-2-Penten-1-ol, acetate	**1.17**	-	**1.43**
10	(*Z*)-3-Hexen-1-ol, formate	**1.00**	**1.25**	**1.16**
11	(*E*)-4-Oxo-2-hexenal	**1.16**	**1.17**	**1.35**
12	β-Myrcene	0.23	0.38	0.24
13	(*Z*)-3-Hexen-1-ol, acetate	0.85	0.96	**1.07**
14	α-Phellandrene	0.39	0.63	0.10
15	α-Terpinene	0.26	0.60	0.05
16	Limonene	0.18	0.37	0.30
17	1,8-Cineole	0.55	0.34	0.58
18	Benzyl alcohol	0.55	0.52	0.51
19	Benzeneacetaldehyde	0.21	0.28	0.28
20	(*E*)-β-Ocimene	0.62	0.70	0.71
21	Conophtorin	0.32	**1.32**	0.20
22	Terpinolene	0.35	0.41	0.33
23	(*Z*) -Butanoic acid, 2-pentenyl ester	**1.17**	-	**1.46**
24	Methyl benzoate	0.77	0.62	**1.03**
25	(*Z*) -3-Hexen-1-ol, propanoate	0.77	0.84	**1.00**
26	Linalool	0.72	0.23	0.87
27	(*E*)–DMNT	0.98	0.71	**1.19**
28	Allo-ocimene	0.83	0.33	0.99

29	(*E*,*E*)-Cosmene	**1.05**	0.56	**1.36**
30	(*Z*) -3-Hexen-1-ol, isobutyrate	**1.12**	0.84	**1.29**
31	(*Z*)-3-hexenyl (*E*)-2-butenoate	**1.29**	-	**1.45**
32	(*Z*)-3-Hexenyl butyrate	0.99	0.85	**1.12**
33	Hexyl butanoate	0.83	0.56	**1.06**
34	Methyl salicylate	**1.22**	**1.54**	**1.36**
35	β-Cyclocitral	**1.43**	**1.62**	**1.37**
36	(*Z*)-3-Hexenyl isovalerate	**1.01**	0.84	**1.30**
37	(*Z*)-3-Hexen-1-yl 2-methylbutanoate	**1.07**	0.91	**1.27**
38	Linalyl acetate	0.49	0.37	0.40
39	Pipertone	0.26	0.32	0.53
40	Unknown	0.87	0.56	0.72
41	(*Z*)-3-Hexen-1-ol, valerate	**1.16**	-	**1.15**
42	(*Z*)-3-Hexen-1-ol, 2-methyl-2-butenoate	**1.37**	-	**1.59**
43	Indole	0.88	0.66	**1.12**
44	(*Z*)-3-Hexen-1-yl tiglate	0.88	0.68	**1.13**
45	Methyl anthranilate	**1.14**	0.84	**1.22**
46	Benzyl butanoate	**1.08**	0.89	**1.24**
47	Eugenol	**1.27**	-	**1.48**
48	2-Acetoxypulegone	0.29	0.30	0.53
49	α-Copaene	**2.20**	**2.21**	0.42
50	(*Z*)-3-Hexen-1-yl hexanoate	**1.09**	**1.23**	0.98
51	(*Z*)-3-Hexenyl (*Z*)-3-hexenoate	**1.19**	-	**1.11**
52	β-Elemene	**1.50**	**1.67**	0.39
53	(*Z*)-Jasmone	0.84	0.58	**1.03**
54	Unknown	**1.19**	-	**1.31**
55	(*E*) -β-Caryophyllene	0.20	0.34	0.33
56	(*E*)-α-Ionone	0.92	0.66	0.74
57	β-Copaene	**1.51**	**2.11**	**0.21**
58	α-Caryophyllene	0.16	0.32	0.35
59	Valencene	**1.81**	**1.85**	0.61
60	Bicyclosesquiphellandrene	0.85	0.92	0.21
61	(*E*)-β-Ionone	**1.22**	0.85	**1.48**
62	Aristolochene	**2.02**	**2.10**	0.93

63	β-Chamigrene	**1.06**	**1.07**	0.21
64	Patchoulene	**1.20**	**1.47**	0.51
65	(*E*,*E*)-α-Farnesene	**1.14**	**1.27**	**1.22**
66	Unknown	0.91	**1.00**	0.93
67	(*Z*)-3-Hexen-1-ol, benzoate	0.70	0.77	0.97
68	(*E*,*E*)-TMTT	**1.09**	**1.33**	**1.21**
69	methyl *cis*-dihydrojasmonate	0.35	0.46	0.66
70	Unknown	0.56	0.14	0.79
71	IPDMOHM	0.66	0.17	0.81
72	Unknown	0.65	0.04	0.83
73	Unknown	0.59	0.23	0.78
74	Unknown	0.59	0.48	0.73
75	Unknown	0.75	0.74	0.50
76	Unknown	0.25	0.32	0.59
77	4-Acetyl-α-cedrene	0.38	0.47	0.79
78	Unknown	**1.04**	**1.25**	0.80
79	Unknown	**1.24**	**1.25**	-
80	Unknown	0.49	0.64	0.54

[a]: Compound numbering corresponds with the loading plots in Figures 1, 2 & 3.
[b]: VIP values obtained during PLS-DA analysis of all treatments together (Fig. 1).
[c]: VIP values obtained during PLS-DA analysis of BT infested and control plants (Fig. 2 a & b).
[d]: VIP values obtained during PLS-DA analysis of TA infested and control plants (Fig. 3 a & b).
Bold face type scores are higher than 1 and are most influential for separation of the treatments in a given PLS-DA model.

Figures

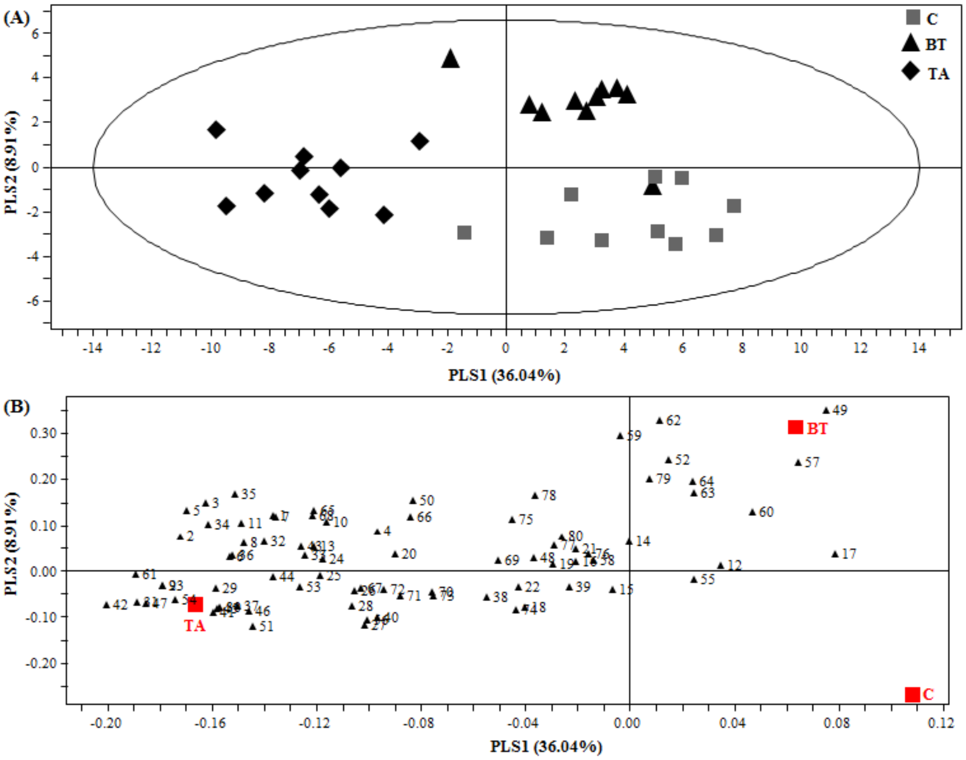

Figure 1. Separation of headspace composition of plants using projection to latent structures-discriminant analysis (PLS-DA) after tomato plants infested with *T. absoluta* (TA, $n = 10$) or *B. tabaci* (BT, $n = 10$) or with no infestation as a control (C, $n = 10$). **(A)** Score plot visualizing the grouping pattern of the samples according to the first two principal components (PCs) with the explained variance in parenthesis. The contribution of each volatile compound to the group separation is displayed in the loading plot (**B**). For compound identity in relation to the numbering in the loading plot, please refer to Table 1.

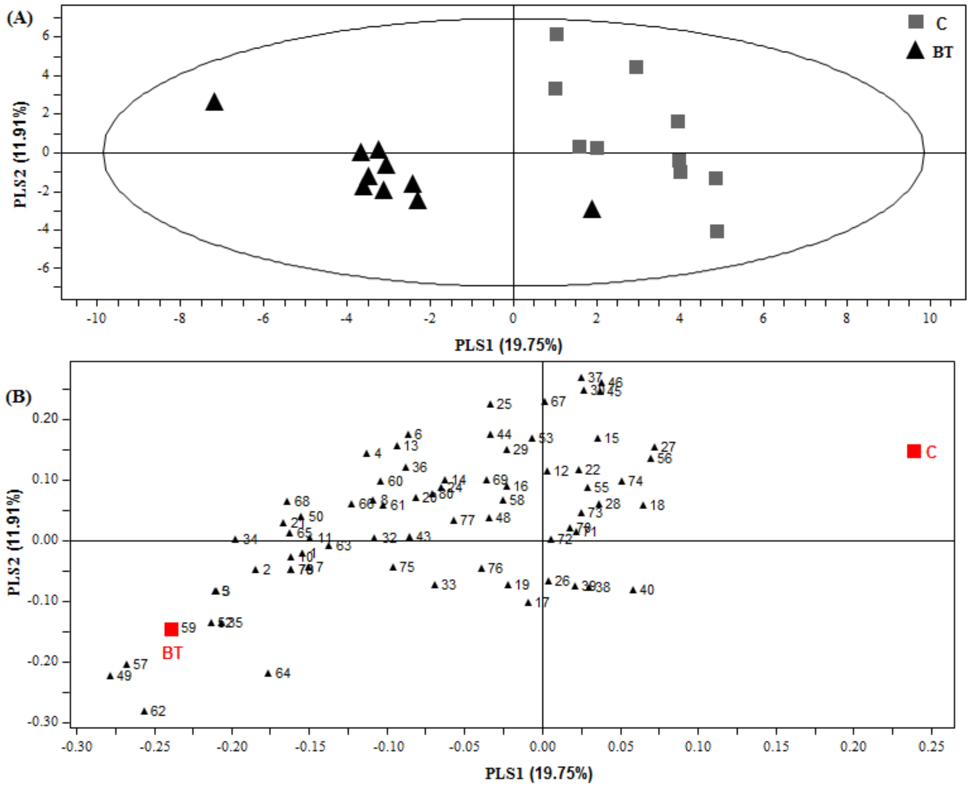

Figure 2. Comparison of headspace plant volatiles through pairwise comparison of treatments using Projection to latent structures discriminant analysis (PLSDA) between control (*C*, *n* = 10) and tomato plants infested with *B. tabaci* (BT, *n* = 10) based on the quantitative results of the volatiles in a two-dimensional score plot (A). The contribution of each volatile to the group separation is displayed in their corresponding loading plots (B). For compound identity in relation to the numbering in the loading plots, please refer to Table 1.

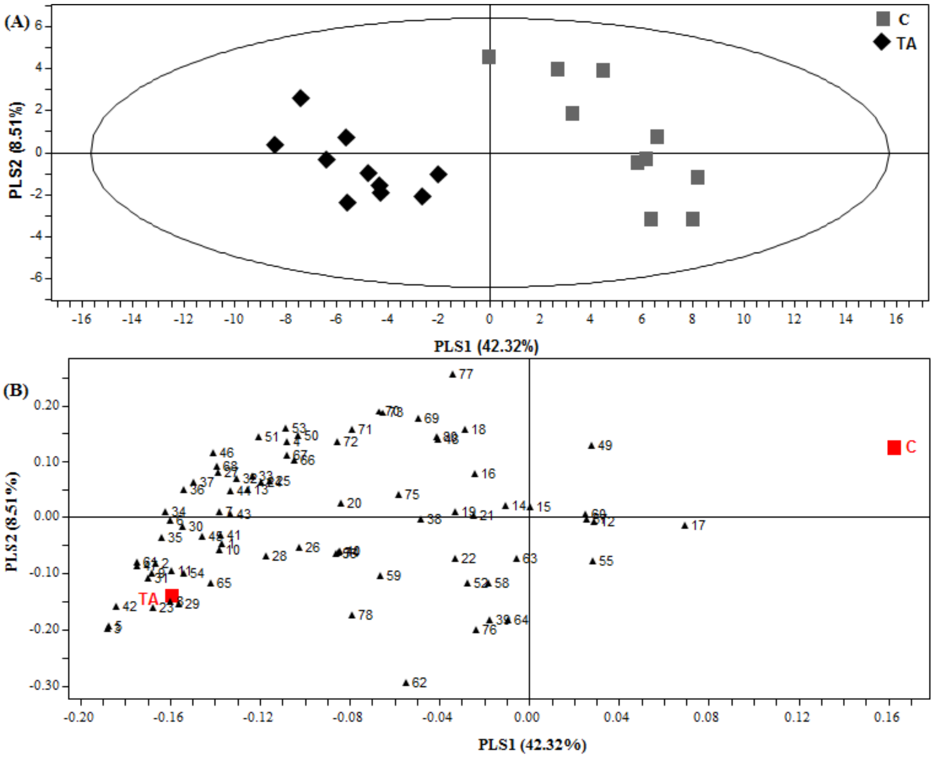

Figure 3. Comparison of headspace plant volatiles through pairwise comparison of treatments using Projection to latent structures discriminant analysis (PLSDA) between control (*C*, *n* = 10) and tomato plants infested with *T. absoluta* (TA, *n* = 10) based on the quantitative results of the volatiles in a two-dimensional score plot (A). The contribution of each volatile to the group separation is displayed in their corresponding loading plots (B). For compound identity in relation to the numbering in the loading plots, please refer to Table 1.