CLEIDE ROSA DIAS

PLANT DEFENCES, SPIDER MITES AND WEB AFFECTING ARTHROPOD RESPONSES TO TOMATO PLANTS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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BIOGRAFIA

Cleide Rosa Dias, filha de Ilda Rosa Dias e José Dias, nasceu em 13 de outubro de 1984 na cidade de Conselheiro Lafaiete - MG. Em dezembro de 1994 concluiu o ensino fundamental na Escola Estadual Deputado Elly Franco Ribeiro. Em dezembro de 2001, concluiu o ensino médio na Escola Estadual Professor Asthor Vianna. Em maio de 2006, iniciou o curso de Agronomia na Universidade Federal de Viçosa, graduando-se Engenheira Agrônoma em janeiro de 2011. Durante a graduação foi bolsista de Iniciação científica por três anos no Departamento de Entomologia/ laboratório de Acarologia sob a orientação do professor Angelo Pallini, e por um ano na EPAMIG sob a orientação da Dra. Madelaine Venzon. Em março de 2011, iniciou o curso de mestrado em Entomologia na Universidade Federal de Viçosa sob a orientação do professor Angelo Pallini e coorientação de Arne Janssen e Madelaine Venzon, defendendo sua dissertação em fevereiro de 2013. Em abril de 2013, iniciou o curso de doutorado em Entomologia na Universidade Federal de Viçosa sob a mesma orientação recebida no mestrado. De junho de 2015 a outubro de 2016 desenvolveu parte de sua pesquisa na Universidade de Amsterdam - The Netherlands sob orientação do Dr. Arne Janssen. Em julho de 2017, submeteu se à defesa da tese.

SUMÁRIO

ABSTRACT

DIAS, Cleide Rosa, D.Sc., Universidade Federal de Viçosa, July, 2017. **Plant defences, spider mites and web affecting arthropod responses to tomato plants.** Adviser: Angelo Pallini Filho. Co-advisers: Arnoldus Rudolf Maria Janssen and Madelaine Venzon.

Plants developed a wide range of defences in response to herbivore attack. These defences can affect herbivores directly, for example, through production of defensive compounds and proteins that reduce herbivore performance; or indirectly, by attracting the natural enemies of the herbivores. The spider mite *Tetranychus urticae*, for example, induces defences in tomato plants which decrease herbivorous mites performance and attract predatory mites. Moreover, some herbivores have adapted and are able to suppress plant defences, such as the spider mite *Tetranychus evansi*. Additionally, *T. evansi* cover the plant with a dense web, which among other functions, avoid competitors to profit from the suppressed defences. In this thesis, I studied the direct and indirect effects of changes in plant defence due infestation of the spider mites *T. urticae* and *T. evansi*, as well as their presence and their web, on other arthropods that occur in tomato plants. Studies that aim to investigate the effect of plant defences on herbivores, are commonly performed through evaluation of herbivores performance on treated plants, being done on whole plants or plant parts. Both methods have pros and cons, and the choice of which one to use depends on the system being studied: research question, organism and plant. Thus, in the first chapter, I investigated the effects of plants with defences induced and suppressed on the subsequent performance of an herbivore, on whole plants and on leaf discs. The method of evaluation and plant treatment affected the performance of *T. evansi*. However, there was no effect of the method on *T. evansi* response to plant defences: the oviposition rate of *T. evansi* was lower on plants infested with the inducer *T. urticae* than on plants infested with the suppressor *T. evansi* and uninfested plants on both methods, whole plants and leaf discs. In the second chapter, I investigated the attraction of arthropods to plants with defences suppressed by *T. evansi* and induced by *T. urticae* in an outdoor area, with infested plants covered or not with spider mite web. The plant defence interfered on the host choice of some herbivores, but not on the choice of natural enemies. The presence of web in combination with plant defences interfered on whiteflies host plant choice: plants with defences suppressed were more attractive to whiteflies in absence of web, but not when plants were covered by spider mite web. In the third chapter, I investigated the effect of indirect and direct plant defences and spider mite web on host plant choice and performance of the whitefly *B. tabaci*. Whiteflies preferred plants where they had higher survival of juvenile and recently emerged adults, that was uninfested and plants with defence suppressed without web. Finally, in the fourth chapter, we investigated whether the changes in tomato plant defences through infestation with the defence-inducer *T. urticae* and the defence-suppressor *T. evansi*, as well as the presence of the spider mites and their web, affect the plant host preference and performance of *Frankliniella occidentalis*. The presence of the spider mites and the induction of plant defences did not affect the survival and performance of juveniles of *F. occidentalis*. Nevertheless, the presence of spider mite web on plants infested with *T. evansi* made these plants more attractive to *F. occidentalis*, but did not affect the performance and survive of juveniles. In conclusion, the results showed that both whole plants and leaf discs can be used for studies that aim to evaluate effects of tomato defences on the spider mites. Also, tomato plants with defences suppressed or induced by the spider mites can affect the host plant preference and performance of whiteflies but not of thrips. Likewise, the combination of web with plant defences also interfered on thrips and whiteflies host choice.

RESUMO

DIAS, Cleide Rosa, D.Sc., Universidade Federal de Viçosa, julho de 2017. **Defesa de planta, ácaros e teia afetando respostas de artrópodes ao tomateiro.** Orientador: Angelo Pallini Filho. Coorientadores: Arnoldus Rudolf Maria Janssen e Madelaine Venzon.

As plantas desenvolveram uma ampla gama de defesas em resposta à herbívoria. Essas defesas podem afetar os herbívoros de forma direta, como por exemplo, produzindo compostos tóxicos e proteínas defensivas que reduzem o desempenho de herbívoros; ou indireta, atraindo os inimigos naturais. O ácaro *Tetranychus urticae*, por exemplo, induz as defesas do tomateiro o que reduz a performance de ácaros herbívoros e atrai ácaros predadores. Entretanto, alguns herbívoros se adaptaram e são capazes de suprimir as defesas das plantas, como por exemplo o ácaro *Tetranychus evansi*. Além disso, *T. evansi* cobre sua planta hospedeira com uma densa teia, que dentre outras funções, evita que os competidores se beneficiem da sua supressão de defesa. Nesta tese, estudou-se os efeitos diretos e indiretos das repostas do tomateiro ao ataque por *T. urticae* e *T. evansi*, bem como da presença dos ácaros e sua teia, em outros artrópodes que ocorrem no tomateiro. Estudos que visam investigar a resistência das plantas aos herbívoros são comumente realizados através da avaliação do desempenho de herbívoros nas plantas tratadas, o que pode ser realizado em plantas inteiras ou partes da planta. Ambos métodos possuem prós e contras, e sua escolha depende da pergunta a ser respondida e do sistema a ser estudado: organismo e planta. Desse modo, no primeiro capítulo, investigou-se os efeitos da defesa induzida ou suprimida do tomateiro no desempenho de um herbívoro, quando avaliado em arenas delimitadas em plantas inteiras e discos de folha. O tipo de arena e tratamento das plantas afetou o desempenho de *T. evansi*. No entanto, o tipo de arena não afetou a resposta de *T. evansi* aos tratamentos: *T. evansi* ovipositou menos nas plantas infestadas com o indutor *T. urticae* do que nas plantas infestadas com o supressor *T. evansi* e plantas não infestadas, tanto quando avaliado em plantas inteiras quanto em discos foliares. No segundo capítulo, foi investigado a atração de artrópodes por plantas com defesas suprimidas por *T. evansi* e induzidas por *T. urticae* em campo, com as plantas infestadas cobertas ou não pela teia dos ácaros. A manipulação da defesa das plantas interferiu na escolha de alguns herbívoros, mas não na escolha dos inimigos naturais. A presença da teia em combinação com a defesa das plantas interferiu na escolha de moscas brancas: plantas com defesas suprimidas foram mais atraentes para as moscas brancas na ausência de teia, mas não quando as plantas estavam cobertas de teia. No terceiro capítulo, investigou-se o efeito das respostas indiretas e diretas do tomateiro ao ataque de *T. evansi* e *T. urticae*, bem como a teia dos mesmos, na escolha da planta hospedeira e no desempenho da mosca branca *B. tabaci*. As moscas brancas preferiam plantas onde apresentavam maior sobrevivência de juvenis e adultos recém emergidos: plantas não infestadas e plantas com defesas suprimidas e sem teia. No quarto capítulo, estudou-se o efeito das respostas do tomateiro ao ataque de *T. urticae* e *T. evansi*, bem como a presença dos ácaros e sua teia, na preferência e desempenho de *Frankliniella occidentalis*. A presença de ácaros nas plantas e sua interferência na defesa não afetou a sobrevivência e desempenho de jovens de *F. occidentalis*. A presença de teia nas plantas infestadas com *T. evansi* tornou-as mais atrativas para *F. occidentalis*; no entanto, não afetou o desempenho e sobrevivência de juvenis. Em conclusão, os resultados demonstram que tanto as plantas inteiras como os discos foliares podem ser utilizados para estudos que visam avaliar os efeitos da resistência tomateiro sobre *T. evansi*. Adicionalmente, plantas de tomate com defesas suprimidas ou induzidas pelos ácaros podem afetar a preferência e o desempenho de moscas brancas, mas não de tripes. Da mesma forma, a combinação da presença de teia com manipulação da defesa do tomateiro também interferiu na escolha da planta hospedeira por moscas brancas e tripes.

GENERAL INTRODUCTION

Plants suffer constantly from herbivore attacks; they thereby defend themselves with chemical and physical defences that affect herbivores directly and indirectly (Price et al. 1980; Takabayashi and Dicke 1996; Karban and Baldwin 1997; Sabelis et al. 2001; Chen 2008). Direct defences are expressed through direct damage on herbivores, that can be through physical plant structures, as thick leaf cuticles or trichomes, or through increasing in the production of defensive compounds (Walling 2000; Howe and Jander 2008). Indirect defences affect herbivores indirectly by recruiting their natural enemies (Dicke and Sabelis 1988; Sabelis et al. 1998; Kessler and Baldwin 2001). Plant defences can be activated continuously, which are called "constitutive defences", or produced after herbivore attack, so-called "induced defences" (Karban and Baldwin 1997; Sabelis et al. 2001, 2007). Together, constitutive and induced defences play an important role on plant resistance which may result in negative effects on herbivores such as reduced feeding, growth and reproduction (Karban and Baldwin 1997; Walling 2000).

While attacking plants, herbivores release elicitors (Hahn 1996) that in contact with the plant effectors will activate plant defences. This results in accumulation of signalling phytohormones responsible for regulation of plant defences, such as Jasmonic acid (JA) and Salicylic acid (SA). The accumulation of signalling phytohormones results in increased levels of secondary metabolites or defence associate proteins (Ament et al. 2004; McCloud and Baldwin 1997; Walling 2000), which can disturb the performance of the attacker and later arriving herbivores (Karban and Baldwin 1997; Walling 2000; Kessler and Baldwin 2002; Howe and Jander 2008; Alba et al. 2011, 2015). Accumulation of JA, for example, can increase the expression of defensive proteins, as protease inhibitor (PI) and polyphenol oxidases (PPOs) (Ryan 1990; Howe et al. 1996; Glawe et al. 2003), which disrupt insect feeding, growth and reproduction (Green and Ryan 1972; Broadway and Duffey 1986; Howe et al. 1996; Lawrence and Koundal 2002; Pompermayer et al. 2003; Kant et al. 2004).

Attacked plants can also produce volatile cues that are attractive to natural enemies of herbivores (Dicke and Sabelis 1988; Sabelis et al. 1998; Kessler and Baldwin 2001), which may reduce the number of herbivores on plants and, consequently, reduce herbivory (Drukker et al. 1995; Janssen 1999). Although natural enemies often use these cues to locate plants infested with suitable prey (Sabelis and van de Baan 1983; Dicke et al. 1988, 1990, Lesna and Sabelis 1999), occasionally they can also fail and be attracted to plants attacked by low-quality prey or non-prey/host (Shimoda and Dicke 2000; van Poecke et al. 2003) which can negatively affect their development and reproduction (Sabelis and van de Baan 1983; Vet and Dicke 1992; Venzon et al. 2002). Likewise, these volatile cues can also be perceived and used by herbivores, which are either attracted or repelled to the plants (Visser 1986; Dicke et al. 1993; Poelman et al. 2008). By perceiving these cues, herbivores can avoid host plants where they would have reduced performance (Bleeker et al. 2009; Nombela et al. 2009; Saad et al. 2015), such as plants with competitors or with defences already induced by previous herbivore infestation (Karban and Carey 1984; Karban and Baldwin 1997; Pallini et al. 1997; Poelman et al. 2008; Nombela et al. 2009; Saad et al. 2015).

Nevertheless, there are herbivores that do not induce plant defences; and some are even able to suppress them (Musser et al. 2002; Lawrence et al. 2008; Sarmento et al. 2011a). On tomato plants, the spider mite *Tetranychus evansi* interferes with plant defences in a different manner from other herbivores. Unlike most of the herbivores including the co-related spider mite *Tetranychus urticae*, *T. evansi* suppresses defences in tomato plant. Plants attacked by *T. evansi* have lower levels of defensive compounds that than uninfested plants, which increase the performance of conspecifics (Sarmento et al. 2011a; Oliveira et al. 2015). In addition, it has been shown that other spider mites, such as *T. urticae* (Sarmento et al, 2011b) and *T. ludeni* (Godinho et al. 2015) had also increased performance on tomato plants previously infested by *T. evansi*. Hence, it is clear that the suppression of plant defences by *T. evansi* benefits conspecifics and also competitors. Furthermore, plants attacked by *T. evansi* produce cues that are attractive to conspecifics compared to cues produced by undamaged plants (Sarmento et al. 2011a; Dias et al. 2016). However, at this level of knowledge we still do not know whether other herbivore insects also pests of tomato crops can locate and profit from the down regulated defences by *T. evansi*.

Thereby, *T. evansi* have other strategies to prevent competitors to profit from its plant down-regulated defences, like production of web. Mites from the family Tetranychidae, called spider mites, are well known by covering their colonies on plants with web (Saito 1983). *T. evansi*, stands out among them because its web is extremely dense (Ferragut and Escudero 1999) and a featured showed to prevent other spider mites from profiting from the down-regulated defences (Sarmento et al. 2011b). The spider mite web has several functions, such as protection against adverse climatic conditions and natural enemies (Gerson 1985; Sabelis and Bakker 1992). However, other organisms can also use it on their own benefit; some thrips and predatory mites for example, can use spider mite web as protection against their natural enemies (Pallini et al. 1998; Venzon et al. 2000). Nevertheless, it is not known how the down-regulate defences of *T. evansi*, the spider mites and their web affect other herbivores pest of tomato crops, such as whiteflies and thrips, both main pest of tomato crops worldwide (Buntin et al. 1993; Reitz 2009; Yang et al. 2015; Mouden et al. 2017).

In order to understand these interactions, herbivore assays are often carried out to access the performance of herbivores feeding on plants with their defensive traits induced or suppressed. These assays can be performed on whole plants (Stout and Duffey 1996; Agrawal 2000) or detached plant parts, such as leaves, leaflets and leaf discs (Dussourd and Hoyle 2000; Sarmento et al. 2011; Oliveira et al. 2015). Regarding this assessment of herbivore performance, a simple issue that always arises is whether to carry out the experiment on whole plants or on detached plant parts. Both methods have pros and cons and the choice of which one to use needs to be made taking in to account some aspects, such as the organisms involved (plants and herbivores) and the availability of resources. For example, some evaluations require a microscope what it is difficult when dealing with a whole plant. Also, working with a whole plant usually results in large loss of replicate during the experiment (escape of herbivores, contaminations, etc.), which demand large number of plants and space. On one hand, working with detached plant parts is usually easier; but on the other hand, they are wounded during the detachment which can trigger the production of defensive traits in the plants (Graham et al. 1986; Ryan 1990; Karban and Baldwin 1997). These traits are often similar to those induced by herbivory, and can also negatively affect the performance of herbivores (Howe et al. 1996; Stratmann and Ryan 1997; Ryan 2000; Strassner et al. 2002; Glawe et al. 2003; Major and Constabel 2006) which can affect the results of the test. In addition, the systemic induction of defensive traits is limited on detached plant parts (Cipollini and Sipe 2001; Cipollini et al. 2004).

In this thesis, I studied the direct and indirect effects of plant defence changes due infestation with *T. urticae* and *T. evansi*, as well as the influence of their presence and their web, on other arthropods that simultaneously share the same tomato plants. In chapter one, I investigated whether herbivores assays carried out on intact plants differ from those carried out on detached plant parts. I therefore, investigated the effects of plants with defences induced or suppressed on the subsequent performance of an herbivore on intact plants and on leaf discs. In chapter two, I investigated the attraction of arthropods to plants with defences suppressed by *T. evansi* and induced by *T. urticae* in field, with infested plants covered or not by spider mite web. In the third chapter, I investigated the effect of indirect and direct tomato plants defences (induced or suppressed), and spider mite web on host plant choice and performance of the whitefly *Bemisia tabaci*. At last, in the fourth chapter, I investigated whether tomato plants infested by *T. urticae* or *T. evansi*, with or without web, affect the plant host preference and performance of *F. occidentalis*.

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CHAPTER 1

Consequences of using detached plant parts on the study of plant resistance against herbivores

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Abstract

Attacked plants often have increased levels of defensive traits. These defences can provide the plants a robust resistance against herbivores. Herbivore performance assay is often used to investigate plant resistance and it is normally carried out either on whole plants or detached plant parts, such as leaves, leaflets and leaf discs. However, when performed on detached plant parts, the traits of resistance might suffer variation due to the mechanical wounding inflicted. To the best of our knowledge, there is no systematic comparison of performance bioassays on intact plants and plant parts. We therefore investigated the effects of induced or suppressed plant defence on the subsequent performance of an herbivore on intact plants and on leaf discs. We first manipulated plant defences by infesting intact tomato plants either with the spider mite *Tetranychus urticae*, which induces plant defence in tomato plants, or *Tetranychus evansi*, which suppresses plant defence. Subsequently, circular arenas were prepared surrounded by non-drying glue (Bio-Controle®), either on intact plants or on detached leaf discs. Spider mites, eggs and web were removed from the arenas and one female of *T. evansi*, aged two days after reach adult stage, was released to evaluate oviposition. The type of arena and plant infestation affected the oviposition rate of *T. evansi*.

T. evansi laid fewer eggs on detached leaf discs than on whole plants. However, there was no significant interaction between previous plant treatment and the type of arena used: the oviposition rate of *T. evansi* was lower on plants infested with the inducer *T. urticae* than on plants infested with the suppressor *T. evansi* and uninfested plants, independently of whether plants have been detached or not. We conclude that both whole plants and leaf discs can be used to evaluate the effects of plant defences on the spider mites.

Keywords: induced plant defences, mechanical wounding, *T. evansi*, *T. urticae*, performance, leaf discs, whole plant.

Introduction

In response to herbivory, plants developed a broad range of defensive mechanisms to protect themselves against herbivores attack (Walling 2000; Kessler and Baldwin 2002; Glazebrook 2005; Howe and Jander 2008; Wu and Baldwin, 2010). These mechanisms are categorized as direct defence, when the defensive trait directly affects the herbivore (Karban and Baldwin 1997; Howe and Jander 2008, Alba et al. 2015); and indirect defence, when the defensive trait affects the herbivore indirectly by attracting their natural enemies to plants, which consequently reduces herbivory (Price et al. 1980; Drukker et al. 1995; Janssen 1999; Thaler 1999). The combination of the effects of direct and indirect plant defences can provide the plants with a robust resistance to arthropod herbivores (Gatehouse 2002; Kessler et al. 2004).

Traits of resistance can be produced continuously on plants at a basal level, characterizing the constitutive defences; or can be increased when the plant is under herbivore attack, so called induced defences (Karban and Baldwin 1997; Baldwin and Preston 1999; Sabelis et al. 2001, 2007). They can vary from morphological structures to the synthesis of secondary metabolites, which all negatively affect herbivores (Karban and Baldwin 1997; Walling 2000; Kessler and Baldwin 2002; Howe and Jander 2008; Alba et al. 2011, 2015). The expression of these traits can affect both attacker and later-arriving herbivores, for example, by disrupting their feeding, growth and reproduction (Green and Ryan 1972; Broadway and Duffey 1986; Howe et al. 1996; Karban and Baldwin 1997; Walling 2000; Lawrence and Koundal 2002; Pompermayer et al. 2003; Kant et al. 2004). Nevertheless, some herbivores developed sophisticated mechanisms against plant defences, such as resistance to defensive traits or the ability to suppress the induction of traits of resistance in plants (Musser et al. 2002; Lawrence et al. 2008; Kant et al. 2008; Sarmento et al. 2011a, Alba et al. 2015, Godinho et al. 2015).

To study the effect of induced or suppressed plant defence on herbivores, it is necessary to access the performance of herbivores feeding on plants with their defensive traits induced or suppressed. To this end, herbivore performance assays are performed either on whole plants (Stout and Duffey 1996; Agrawal 2000 a, b) or detached plant parts, such as leaves, leaflets and leaf discs (Dussourd and Hoyle 2000; Sarmento et al. 2011; Oliveira et al. 2015). Regarding this assessment of herbivore performance, a simple issue that often arises is whether to carry out the experiment using whole plants or on detached plant parts. In order to choose properly, some aspects such as the research question, the organisms involved and the availability of resource might be considered. For example, evaluations of development and oviposition of small herbivores can be a challenge when the assay is performed in a whole plant. These evaluations require the use a microscope what it is difficult when dealing with a whole plant. Likewise, assays performed on whole plants demand large number of plants in order to reduce variability which often is higher on these assays due the large loss of replicate during the experiment: escape of herbivores, contaminations, etc. Consequently, these assays will demand more work, time, space and facilities to locate and evaluate the plants. Herbivore assays performed on detached plant parts are generally easier to locate, manipulate, transport and evaluate.

However, when detached plant parts are used, the induced defences can change because of the mechanical wounding inflicted. Similar to herbivory, mechanical wounding can also induce trait of resistance in plants by for example increasing the expression of defensive genes and proteins (Graham et al. 1986; Ryan 1990; Karban and Baldwin 1997; reviewed in Reymond and Farmer 1998). These inductions of defensive traits may negatively affect the performance of herbivores that subsequently feed on these plants. Thus, the herbivore evaluated on herbivore assays performed on detached plant parts can be affected, especially because some of these defensive genes and proteins induced by mechanical wounding are the same as those induced by herbivory (Howe et al. 1996; Stratmann and Ryan 1997; Ryan 2000;

Strassner et al. 2002; Glawe et al. 2003, Major and Constabel 2006). However, there is a lack of information about the effect of mechanical wounding on plants with defences previously suppressed by an herbivore.

Mechanical wounding is also an integral part of other methods used on study of plant resistance, such as molecular and physiological assays. In this case, plant samples need to be detached from plants in order to measure genetic, molecular and physiological activity (e.g. Kessler and Baldwin 2002; Howe and Jander 2008; Zheng and Dicke 2008; Sarmento et al. 2011a; Alba et al. 2015; Oliveira et al. 2015; Godinho et al. 2015). Moreover, the plant parts collected are immediately frozen in liquid nitrogen (i.e. Alba et al. 2015; Ataide et al. 2016), this will stop or reduce the molecular activities, including those induced by mechanical wounding. However, in herbivore performance assays, the detached plant parts are used in experiments that may take considerably longer (i.e. Sarmento et al. 2011). This means that the herbivore evaluated in the assay will be exposed to the plant response to mechanical wounding for some period. Additionally, when performed on leaf discs, the possible effects of mechanical wounding might be even stronger because of the bigger wounded area compared to leaf or leaflet. The wounded area on leaf discs is equivalent to the leaf disc circumference that often varies from 2 to 5 cm^2, while on leaves and leaflets it is the equivalent of the stem cross-section of the leaf or leaflet, which can range from 0.1 to 0.5 cm^2.

Another important aspect is that the defensive traits induced by herbivores attack are produced not only locally, but also systemically throughout the plant (Karban and Myers 1989; Ryan 1990; Karban and Baldwin 1997; Bostock 2005). Herbivory can induce local defence responses on wounded regions, on undamaged regions in the attacked leaves and in the other intact leaves. On herbivore performance assay, herbivory can induce or suppress defensive traits systemically on whole plants, but when performed on detached plant parts the systemic induction of defensive traits is limited (Cipollini and Sipe 2001; Cipollini et al. 2004). Thus, unlike assays performed on whole plants, the systemic plant responses to the second herbivore attack are excluded when herbivore assays are performed on detached plant parts.

In order to choose the most suitable method for each situation, it is important to investigate whether the mechanical wounding promoted in plant tissue during detachment from the plants can interact with induction or suppression of traits of resistance in plants affecting the performance of the tested herbivore. Here, we investigated the consequence of detaching plant parts on the study of induction and suppression of plant defence in a well-known model system, consisted of tomato plants and two herbivore spider mites. The plants were previous infested either with the spider mite *Tetranychus urticae*, which induces traits of resistance in plants; and the spider mite *T. evansi*, which suppresses traits of resistance. We, therefore, investigated whether the effects of induced or suppressed defences of tomato plants on the performance of the herbivore *Tetranychus evansi* differ when evaluated on detached leaf discs and on whole plants.

Material and methods

Rearing methods

All the rearings and experiments performed here took place at the University of Amsterdam, Amsterdam, the Netherlands. *T*omato plants (*Solanum lycopersicum*, var. Santa Clara I-5300) were grown in plastic pots (Ø = 14 cm) using a commercial substrate. The plants were kept in a climate room under controlled conditions (25 ± 1 °C, 60-70 % RH, 16:8 h L: D), fertilized (EC fertilizer NPK (Ca, Mg), 15-5-15 (+7+3)) once a week, and watered by filling the trays under the pots with water three times per week. The spider mites *T. urticae* and *T. evansi*, both Viçosa strains as previsouly refered by Alba et al. 2015, were reared on detached tomato leaves. The rearing arenas consisted of big (40 x 25 cm) and small (18 x 12 cm) trays, with small trays placed upside down inside big trays. Big trays were filled with tap water, thus isolating small trays to avoid contamination. A tomato leaf containing spider mites was placed on the small tray with the petiole touching the water in the big tray to maintain leaf turgor. A fresh tomato leaf was added to the rearing three times per week. Once a month the arenas were cleaned, the water in the big trays was replaced, and old leaves removed. *T. evansi* and *T. urticae* rearings were kept in separate climate rooms to avoid cross contamination.

Performance of T. evansi

We evaluated the performance of *T. evansi* on whole plants and on leaf discs cut from plants previously infested by *T. urticae*, previously infested by *T. evansi* and noninfested plants. Tomato plants, 45 days old with four completely developed leaves were used in the experiment. To obtain different levels of plant resistance, we previously infested plants with the spider mites *T. urticae*, which induces traits of resistance in tomato plants (Ozawa et al. 2000; Li et al. 2002; Kant et al. 2004, Sarmento et al. 2011a, Alba at al. 2015), and *T. evansi*, which suppresses plant resistance (Sarmento et al. 2011a, Alba et al. 2015, Oliveira et al. 2015, Gondinho et al). Hence, we had six treatments from the combination of two types of arenas, and three levels of plant infestation. The arenas consisted on a circular area delimited on a whole plant leaf or on a leaf disc cut from a detached leaflet; and the treatments were uninfested plants (control), plant infested with *T. evansi* and plants infested with *T. urticae*.

Plants were infested by releasing 20 adult female spider mites on each leaflet (totalizing 400 spider mites per plant) for four days, which is sufficient time to affect plant defences (de Oliveira et al. 2015). On the fourth day, a circle (Ø = 2.4 cm) was drawn with a pen on abaxial surface of the leaflets one, three and five of the third and fourth leaves of the plants. Subsequently, the circles of infested plants were cleaned by carefully removing spider mites, their eggs and web using a soft brush. After that, a thin bordering line of non-drying glue (Bio-Controle®) was applied on the circumference of the drawn circle (on the top of the drawn line, Fig. 1). Subsequent, half of the plants were left intact while leaf discs were cut from the drawn circle from the other half by using a circular cutter (Ø = 3 cm), so we had arenas on whole plants and leaf discs (Fig. 1). The leaf discs were cut around the circle of non-drying glue (Bio-Controle®) from outside, making sure that they had the same size as the circles on intact plants (Fig. 1). From whole plant treatments, we also removed the spider mites from the outside of the arena to avoid further induction or suppression of defences. Subsequently, an adult female of *T. evansi* was released on each arena, whole plants and leaf discs, and its oviposition was evaluated after three days. These females were taken from a cohort of two days old. To prepare this cohort, we transferred around 30 adult males and 50 tello-chrysalis of *T. evansi*, last quiescent stage before adult, from the rearing to tomato leaflets arranged on wet cotton wool on Petri dishes.

The experiment was performed in six blocks through time, each composed of a group of six plants; three levels of plant treatment crossed with the two arena types. From each plant, there were six arenas that received one adult female of *T. evansi* each. We accessed the interaction between arena and treated plants to verify whether the type of arena affected the performance of *T. evansi* on treated plants. We averaged the *T. evansi* oviposition rate per plant, and analysed these averages using a generalized linear model (GLM) with a Gaussian error distribution, with plant infestation and arena as fixed factors. Non-significant interactions and factors were removed from the model until obtaining a minimal adequate model. Contrasts among treated plants and arenas were assessed using least-square means (function lsm of the lsmean package in R, Lenth 2016), using the general linear hypotheses function (glht of the multcomp package in R, Hothorn et al. 2008). All the statistical analyses were performed with R (R Development Core Team 2015).

Results

The oviposition rate of *T. evansi* was affected by the type of arena (Fig. 2, GLM: $F_{(1,32)} = 6.69$, $P = 0.014$) and plant infestation (Fig. 2, GLM: $F_{(2,32)} = 7.12$, $P = 0.0028$). *T. evansi* laid fewer eggs on detached leaf discs than on whole plants arenas (Fig. 2). However, there was no significant interaction between previous plant infestation and the type of arena used (GLM: Deviance = 7.29, *df* = 2,32, *P* = 0.68): the oviposition rate of *T. evansi* was lower on plants infested with the inducer *T. urticae* than on plants infested with the suppressor *T. evansi* and uninfested plants, independent of the type of arena (Fig. 2).

Discussion

Bioassays performed either on whole plants or detached plants parts are often used in studies of effect of plant resistance on herbivores (Jones and Firn 1979; Jones et al. 1981; Jones and Coleman 1988; Sarmento et al. 2011a, Oliveira et al. 2015). The decision of which to use is usually made based on the research question, the organisms involved (plants and herbivores) and the availability of resource. Here we showed that plant infestation and the type of arena, whole plants or leaf disc, affected the performance of *T. evansi*: the females laid fewer eggs on detached leaf discs than on whole plant arenas. However, there was non-effect of the type of arena on *T. evansi* response to plant resistance: the oviposition rate of *T. evansi* was lower on plants infested with the defence inducer *T. urticae* than on plants infested with the defence suppressor *T. evansi* and uninfested plants on both arenas.

Detached leaf discs are often used in herbivore assays aiming to evaluate herbivore preference, consumption and performance (Jones and Firn 1979; Lewis and Van Emden 1986; Sarmento et al. 2011a, Oliveira et al. 2015). Nevertheless, substantial changes regarding to plant resistance to herbivores occur in the plant tissue when plant parts are detached. Plants respond to mechanical wounding by, for example, increasing expression of defensives genes and proteins (Graham et al. 1986; Ryan 1990; Howe et al. 1996; reviewed in Reymond and Farmer 1998; Glawe et al. 2003). Some of these genes and proteins are the same as those induced by herbivory (Howe et al. 1996; Stratmann and Ryan 1997; Ryan 2000; Strassner et al. 2002; Glawe et al. 2003), thus, herbivore assays performed on detached plant parts can maybe ne affected by the changes in plant resistance induced after a mechanical. Consequently, in some cases herbivore assays performed on detached plants parts can show different results from those carried out on whole plants (e.g. Barnes 1963; Risch 1985).

Alternatively, it is well known that the traits of plant resistance against herbivores induced after herbivory are produced not only locally, but also systemically throughout the plant (Karban and Myers 1989; Ryan 1990; Heil and Bostock 2002; Heil and Silva Bueno 2007). Likewise, the spider mite *T. evansi* also suppresses traits of resistance systemically (Sarmento et al. 2011) within one day of infestation (Oliveira et al. 2015). In the herbivore assay performed here, we released one female of *T. evansi* on each arena to be evaluated. It is possible that this female suppressed the systemic defensive traits on whole plants, unlike on detached leaf discs where systemic defences are limited (Cipollini and Sipe 2001; Cipollini et al. 2004). Additionally, when detached from the plant, plant parts accelerate the process of senescence and do not long receive nutrients that are systemically translocated through the plant. Thus, detached plant parts will have a slightly lower quality to herbivores that increases with time, which may affect the spider mite tested. The combination of these systemic issues and the mechanical wounding inflicted on leaf discs might explain the lower oviposition of *T. evansi* observed on leaf discs compared to whole plants was. Thereby, regarding the systemic defences, it would be also interesting to test the spider mite *T. urticae* which induces traits of resistance in tomato plants.

However, even though the females of *T. evansi* laid more eggs on intact whole plants than on leaf discs, their response to herbivory-induced/suppressed defence was not affected by the type of arena. Plants infested with *T. urticae* were expected to have their defences induced, which reduce herbivores performance; while plants infested by *T. evansi* to have their defences suppressed, which increase herbivores performance (Sarmento et al. 2011a; Oliveira et al. 2015; Godinho et al. 2015; Alba et al. 2015). Here, we did find the expected results for induced resistance: oviposition rate of *T. evansi* was lower on plants infested with the inducer *T. urticae* than on plants infested with the suppressor *T. evansi* and then on uninfested plants. However, we did not find evidence for plant defence suppression by *T. evansi*, which occasionally occurs due to variability on heritable factors in the mites from primary infestation (Knegt et al. unpublished data). Genetic features and behaviour are common characteristics that often suffer variation on an herbivore population along the time, which is even more evident on herbivores with short life cycle like spider mites. Nevertheless, these results did not vary with the type of arena indicating that both intact whole plants and leaf discs are suitable to evaluate the effects of herbivore-induced defences on herbivores.

Herbivore assays carried out on detached plant parts can be very practical and convenient in cases where there are no restrictions regarding the research question, organisms involved and the availability of resource. Nevertheless, the consequences of detaching samples from plants must be considered. Here, we showed that the type of arena affected the herbivore oviposition. However, it did not affect the herbivore responses to induced traits of resistence. We thereby conclude that both whole plants and leaf discs can be used to evaluate the effects of tomato plant defences on the performance of these spider mites.

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Figure legends

Figure 1 (a) Detached leaf disc (Ø = 3 cm) showing the drawn circular arena bordering with a thin line of non-drying glue (Bio-Controle®) (Ø = 2.4 cm). (b) Leaflet from an intact whole plant showing the drawn circular arena bordering with a thin line of non-drying glue (Bio-Controle®) (Ø = 2.4 cm).

Figure 2 Mean oviposition rate (± SE) of *T. evansi* on two different arenas, whole plants (first bars) and leaf discs (second bars). Different bar colours represent the treatments: plants pre-infested with *T. evansi* (dark grey bars), uninfested plants (light grey bars) and pre-infested with *T. urticae* (white bars) (Contrast after a GLM, $P < 0.05$). Letters above bars indicate significant difference among treatments per arena. Asterisk above group of bars indicate significant differences among arenas (GLM, $P < 0.001$).

Figures

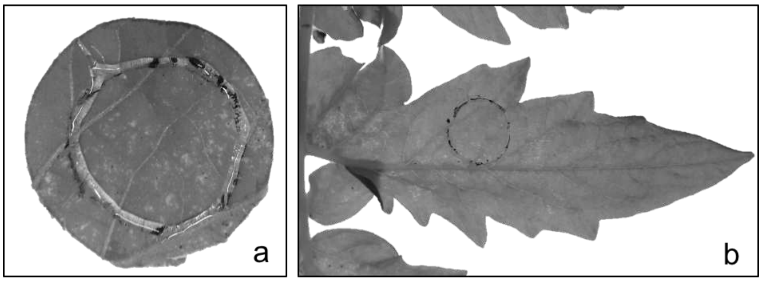

Figure 1

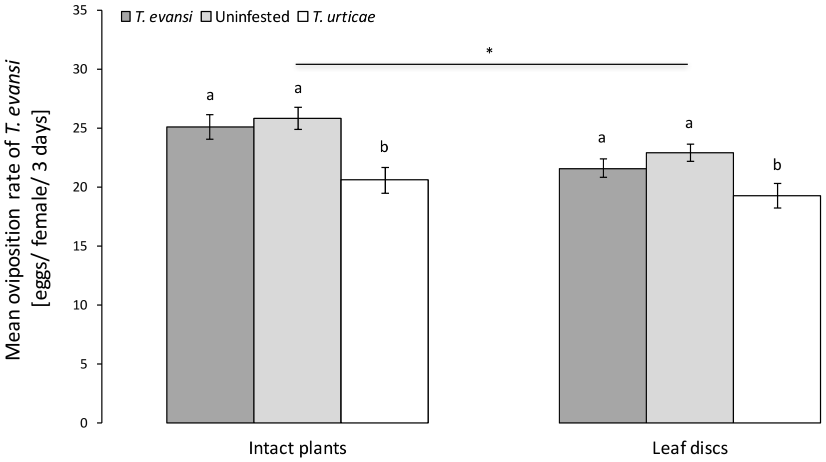
Mean oviposition rate of T. evansi [eggs/ female/ 3 days]
0
5
10
15
20
25
30
35
T. evansi
Uninfested
T. urticae
*
a
a
b
a
a
b
Intact plants
Leaf discs

Figure 2

CHAPTER 2

Effect of plant defences and spider mite web on the attraction of arthropods to plants

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Abstract

Plants attacked by herbivores often have their defences induced, which can decrease herbivore performance. An example is the spider mite *Tetranychus urticae* which induces defences in tomato plants. Herbivores thereby, can use cues from attacked plants to find suitable hosts, e.g. avoid plants with defences induced. Some herbivores can manipulate plant defences suppressing them, thereby increasing their performance, such as the spider mite *Tetranychus evansi*. It is known that tomato plants attacked by *T. evansi* are more attractive to conspecifics. Additionally, *T. evansi* also covers the plant with dense web for protection. Nonetheless, it is not well known whether other herbivores can also find these plants with defences suppressed and benefit from it. Hence, we investigated the attraction of arthropods to plants with defences suppressed by *T. evansi* and induced by *T. urticae* in field, with infested plants covered or not by spider mite web. Tomato plants were placed equidistant in a circle in an outdoor area and evaluated for three days. We evaluated the abundance of arthropods that arrived on plants, separating them in groups according to their general guild; herbivores and carnivores (natural enemies). Most of the herbivores captured on plants were whiteflies and leafhoppers, thus, we evaluated them separately from the other herbivores captured in considerable lower number. The natural enemies captured were generalist insects which usually do not feed on the spider mites we had on plants. Our results indicate that the infestation of tomato plants with the defence induced *T. urticae* and the suppressor *T. evansi* interfered on the attraction of leafhopper and whiteflies to plants, but not of natural enemies. The presence of web in combination with plant defence interfered on the attraction of whiteflies to plants: plants with defence suppressed were more attractive to whiteflies in absence of web, but not when plants were covered by spider mite web.

Keywords: Indirect defence, *Tetranychus evansi*, tomato, herbivores, natural enemies.

Introduction

Plants have several defence mechanisms to defend themselves against herbivory. When attacked by herbivores, their defences are induced (Karban and Baldwin 1997, Sabelis et al. 2001, 2007) which can affect herbivores directly or indirectly (Karban and Baldwin 1997; Agrawal 2000; Traw and Dawson 2002; Poelman et al. 2008). An example of direct defence is the production of antinutritive or toxic compounds by the attacked plant (Howe and Jander 2008). These induced defences can affect both, the attacker and later arriving herbivores (Karban and Baldwin 1997; Walling 2000; Howe and Jander 2008; Sarmento et al. 2011a; Alba et al. 2015; Alba et al. 2011, 2015), for example, by disrupting herbivore feeding, growth and reproduction (Green and Ryan 1972; Broadway and Duffey 1986; Karban and Baldwin 1997; Lawrence and Koundal 2002; Pompermayer et al. 2003; Kant et al. 2004). Indirect defence is the control of the attacker herbivore through the recruitment of natural enemies. Under herbivore attack, plants release a blend of volatile compounds that are attractive to the natural enemies of the herbivores, which indirectly affect the attacker herbivore (Price et al. 1980; Dicke and Sabelis 1988; Turlings et al. 1990; Heil 2008). These cues thereby, can also be used by other herbivores and natural enemies on their foraging in order to find suitable hosts/ prey (Dicke 1986; Dicke and Sabelis 1988; Dicke et al. 1993; Sabelis et al. 1998, 1999; Kessler and Baldwin 2001; Poelman et al. 2008).

The activation of induced defences changes the suitability of the plants to herbivores and consequently their attractiveness to them. Usually herbivores can perceive cues from attacked plants and associate them with host quality. Thus, they can avoid host plants where they would have reduced performance (Bleeker et al. 2009; Nombela et al. 2009; Saad et al. 2015), such as plants with defences already induced by previous herbivore infestation (Karban and Carey 1984; Karban and Baldwin 1997; Poelman et al. 2008; Nombela et al. 2009; Saad et al. 2015). However, the induced defences can eventually also make the plants more vulnerable to herbivores attack (Karban and Carey 1984; Karban and Baldwin 1997; Poelman et al. 2008; Bruessow et al. 2010). Likewise, natural enemies can also use cues from attacked plants to distinguish plants infested with different prey species and prefer plants infested with high-quality prey instead of an inferior or non-prey (Sabelis and van de Baan 1983; Dicke et al. 1988, 1990, Lesna and Sabelis 1999). Nevertheless, eventually they do not discriminate them (Turlings et al. 1993; Geervliet et al.1996; Shimoda and Dicke 1999) and are attracted to plants attacked by non-prey or non-host (Shimoda and Dicke 2000; van Poecke et al. 2003), which can negatively affect their development and reproduction (Sabelis and van de Baan 1983; Vet and Dicke 1992, Venzon et al. 2002).

The induction of plant defences after herbivory is a common plant response. The spider mite *Tetranychus urticae*, for example, induces defensive traits in tomato plants witch result in reduced performance of conspecific and other invasive spider mites (Sarmento et al. 2011, Godinho et al. 2015). However, in some specific cases attacked plants can also have their defences suppressed which benefit the herbivore (Musser et al. 2002; Lawrence et al. 2008; Kant et al. 2008; Sarmento et al. 2011a; Godinho et al. 2015). The tomato red spider mite *Tetranychus evansi* is an example of an herbivore able to suppress plant defences (Sarmento et al. 2011a; Alba et al. 2015; Oliveira et al. 2015). Unlike most herbivores, including the closely related spider mite *T. urticae*, *T. evansi* causes a reduction of defence compounds and defensive proteins in tomato plants to levels below of those found in undamaged plants, which result in increased performance of conspecifics (Sarmento et al. 2011a; Alba et al. 2015; Oliveira et al. 2015). Laboratory experiments showed that a few spider mites can also profit from this down-regulated defences and increase their performance on tomato plants that were previously damaged by *T. evansi* (Sarmento et al 2011a, b; Godinho et al. 2015). Hence, it is clear that the suppression of plant defences by *T. evansi* benefits *T. evansi* but also can benefit its competitors. *T. evansi*, however, might have strategies to prevent competitor spider mites to colonise the tomato plant and profit from its down-regulation of plant defences, such as, production of web (Sarmento et al. 2011b).

Tetranychidae are well known for covering their colonies with web (Saito 1983), which can have several functions such as protection against natural enemies and preventing competitors from colonising the plant (Gerson 1985; Sabelis and Bakker 1992; Morimoto et al. 2006). *T. evansi* stands out among the others Tetranychidae because of its high production of extremely dense web (Ferragut and Escudero 1999) that besides other functions also prevent competitor spider mites to colonise the tomato plant and profit from its down-regulation of plant defences (Sarmento et al. 2011b). Additionally, *T. evansi* induce the production of volatile compounds in tomato plants that are attractive to conspecifics compared to the volatiles produced by undamaged plants (Sarmento et al. 2011a; Dias et al. 2016). Nonetheless, it is not known whether other arthropods can also perceive and use these cues to locate plants with defence suppressed. We thereby investigated the attraction of arthropods to plants with defences suppressed by *T. evansi* and induced by *T. urticae* in field, with the infested plant covered or not by spider mite web.

Material and methods

Rearing methods

All the rearings and experiments performed here took place at the Federal University of Viçosa, Viçosa-MG, Brazil. Tomato plants (*Solanum lycopersicum*, variety Santa Clara I-5300) were grown in pots (2 L) using a commercial substrate. The plants were kept inside a greenhouse, fertilised with NPK (4-14-8) plus superphosphate and watered as needed. The spider mites *T. evansi* and *T. urticae* were reared on detached tomato leaves. The petiole of the tomato leaves was placed in a plastic tube filled with water to maintain leaf turgor. The tubes with the tomato leaves were kept in plastic trays filled with water to prevent mite escapes and invasion of other arthropods. Clean tomato leaves were added to these cultures as needed, old leaves were removed from the rearings, and the trays were cleaned once every two weeks. The cultures were kept in a climate-controlled room (25 ± 2 °C, 80 ± 10% relative humidity and 12 h light). The experiments were done in an experimental outdoor area located on the campus of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (20.7549° S, 42.8786° W). The area was delimited on one edge by cultivated plants (i.e. eucalyptus, orange and avocado trees) mixed with native vegetation and by an urban area on the other edge.

Attraction of arthropods

Tomato plants, 45 days old and with at least six completely developed leaves, were used in the experiment. Plants were infested by releasing 40 females of spider mites from the rearing on each leaflet, approximately 1220 mites per plant. To manipulate plant defences the plants were infested either with the spider mite *T. evansi*, which suppresses defences in tomato, or *T. urticae,* which induces defences. The experiment consisted in six plants and three treatments: (a) two uninfested plants; (b) two plants infested with *T. urticae*; and (c) two plants infested with *T. evansi*. The plants were kept in the greenhouse in separated cages covered with a fine mesh for four days, what is considered enough time for the spider mites infesting the plants and induce or suppress plant defences (Oliveira et al. 2015). Subsequently, they were transferred to the experimental area and placed equidistantly in a hexagon (Ø = 120 cm). The plants were alternated according to their treatment in a way that each plant had always a different sideway treatment. The plants occupied six positions of the wind rose: north, northeast, northwest, south, southeast and southwest.

Arthropods that naturally invaded the plants were collected every day at 8 a.m. and 6 p.m. for three consecutive days, using a pooter (Figure 1). A new pot was connected to the pooter on each plant evaluation to store the captured arthropods separated per plant.

Subsequent, the captured arthropods were transferred from the pooter to an Eppendorf filled with alcohol 70% and stored for later identification. In laboratory, we quantified the abundance of arthropods collected and classified them according with their order by using a microscope and identification keys. From it, we separated them in groups according with their guild: herbivores and carnivores (natural enemies). Those who did not fit in these groups, such as detritivores, were called as "other arthropods".

We had two separated experiments with and without spider mite web covering the infested plants. First of all, the spider mite web was kept on the infested plants and the experiment was done between January and May of 2014 with temperature varying from 29.9 °C to 16.9 °C and 74.2% of relative humidity. Secondly, the spider mite web was removed from the infested plants at the moment they were placed to the hexagon and the experiment was done between February and June of 2014 with temperatures ranging from 27.9°C to 17.3 °C and 81.8% of relative humidity. In the experiment without web, a soft brush was used to carefully remove the web from the plants; and eventually a few mites were also removed by accident in the process. The evaluations were done during three days, thus the new web produced by the spider mites that remained in the plants was also removed daily after the later evaluation at 6 p.m. Six replicates were done for each experiment, with and without web. Care was taken that each treatment had occupied each position twice on each replicate (Janssen 1999). The number of arthropods of each group captured on plants was analysed using a generalized linear mixed model (function glmer of the lme4 package, Bates et al. 2015) with replicate and position as random factor. Contrasts among treatments were assessed using least-squares means (function lsm of the lsmean package in R, Lenth 2016), and the general linear hypotheses (function glht of the multcomp package in R, Hothorn et al. 2008). All the statistical analyses were performed with R (R Development Core Team 2015).

Control of plant treatments

As mentioned before, the plants pre-infested with *T. urticae* were expected to be induced and those pre-infested with *T. evansi* were expected to be suppressed. Therefore, we checked plant defences through *T. evansi* performance (Sarmento et al. 2011a) on extra plants that received the same treatment as the plants used in the "Attraction of arthropods" experiment. Thus, we cut leaf discs (Ø = 2.4 cm) from the third and fourth leaves of the extra plants and used them to evaluated oviposition rate of *T. evansi*. The leaf discs were cleaned by removing all the spider mites, eggs and web. As next step, a female of *T. evansi* was released on each leaf disc. These females were taken from a cohort of two days old. To prepare this cohort, we transferred around 30 adult males and 50 tello-chrysalis of *T. evansi*, last quiescent stage before adult, from the rearing to tomato leaflets arranged on wet cotton on Petri dishes. Oviposition rate of *T. evansi* was evaluated daily for three days and analysed using a linear mixed effects model (function lme of the nlme package, Pinheiro et al. 2014), with replicate as a random factor and experiment as a factor. Contrasts among treatments were assessed using least-squares means on the general linear hypotheses.

Results

Control of plant treatments

In the control experiment, the oviposition rate of *T. evansi* differed significantly among treatments (Fig. 2, LME: $\chi^2 = 68.44$, d.f. = 7, $P < 0.0001$). Oviposition was higher on leaf discs previously attacked by *T. evansi* than on discs from clean plants, showing that the previous attack by *T. evansi* had indeed resulted in the suppression of plant defences (Fig. 2). Oviposition rate was lower on leaf discs from plants previous attacked by *T. urticae* than on discs from clean plants and from plants previous attacked by *T. evansi*, showing the induction of plant defence by *T. urticae* (Fig. 2). Together, this shows that our manipulation of plant defences through the previous infestation of the plants by the defence-inducing strain of *T. urticae* and by the defence-suppressing *T. evansi* was successful.

Attraction of arthropods

In both experiments, with and without spider mite web covering infested plants, a large number of arthropods from a few orders were collected on plants (Table 1, 2). Among the herbivores, whiteflies and leafhoppers were found in higher number compared with the others. Because of that, they were analysed separated from the other herbivores, so we had three groups of herbivores: whiteflies, leafhoppers and other herbivores. Just a few generalist natural enemies were found, such as, spiders, ants, wasps, bugs and beetles (Table 1, 2). A few arthropods captured did not fit in the group of herbivores or carnivores, we thereby classified them as guild not defined and called “other arthropods”.

In the experiment where infested plants had no web, there was a significant effect on total number of arthropod captured on plants (Fig. 3, GLMER: $\chi^2 = 69.48$, d.f. = 5, $P < 0.001$). Arthropods were more often on uninfested plants that on infested plants, and more often on plants infested with *T. evansi* than *T. urticae*. Among the herbivores, there was also a significant effect on the number of whiteflies (Fig. 3, GLMER: $\chi^2 = 63.73$, d.f. = 5, $P < 0.001$), leafhoppers (Fig. 3, GLMER: $\chi^2 = 10.64$, d.f. = 5, $P = 0.0049$) and other herbivores (Fig. 3, GLMER: $\chi^2 = 11.96$, d.f. = 5, $P = 0.0025$) captured on plants. The number of whiteflies was higher on uninfested plants than on the infested plants and higher on plants infested with *T. evansi* than on plants infested with *T. urticae*. Leafhoppers were more often found on uninfested plants than on plants infested with *T. urticae*. On plants infested with *T. evansi*, the number of leafhoppers captured was not significantly different from the other plants. The number of other herbivores captured was higher on uninfested plants than on infested plants. There was no significant difference on number of natural enemies (Fig. 3, GLMER: $\chi^2 = 0.46$, d.f. = 5, $P = 0.79$) and other arthropods captured on plants (GLMER: $\chi^2 = 4.20$, d.f. = 5, $P = 0.12$).

When infested plants were covered by web, the total number of arthropods captured on plants was significant different among treatments (Fig. 4, GLMER: $\chi^2 = 22.19$, d.f. = 5, $P < 0.001$). They were more often on uninfested than on infested plants. Among herbivores, the number of leafhoppers and whiteflies captured on plants were also significant different among treatments (Fig. 4, GLMER: $\chi^2 = 14.75$, d.f. = 5, $P < 0.001$; GLMER: $\chi^2 = 45.62$, d.f. = 5, $P < 0.001$). Leafhoppers were more often captured on uninfested plants than on plants infested with *T. urticae*, and non-significant different from the other on plants infested with *T. evansi*. Whiteflies were more often captured on uninfested than on infested plants. Number of natural enemies, other herbivores and other arthropods was not significant different among treatments, (Fig. 4, GLMER: $\chi^2 = 0.47$, d.f. = 5, $P = 0.79$; GLMER: $\chi^2 = 4.74$, d.f. = 5, $P = 0.093$; GLMER: $\chi^2 = 4.17$, d.f. = 5, $P = 0.12$).

Discussion

We showed here that cues from plants with defences induced by *T. urticae* or suppressed by *T. evansi* did not interfered on the attraction of natural enemies to plants, but it did herbivores such as leafhopper and whiteflies. A few groups of herbivores were captured of which whiteflies and leafhoppers were in higher number compared to the others, i.e. bugs. The presence of web in combination with plant defences also interfered on the attraction of whiteflies to plants: plants with defence suppressed were more attractive to whiteflies in the absence of web, but not when spider mite web covered the plants.

On both experiments, with or without spider mite web covering infested plants, we captured a few groups natural enemies on plants and they did not show preference for the plants (Fig 3, 4). Natural enemies usually use cues from attacked plants to locate prey (Elzen et al. 1983; Dicke and Sabelis 1988; Turlings et al. 1990). They can detect suitable prey choosing high-quality prey instead inferior or non-prey (Sabelis and van de Baan 1983; Dicke et al. 1988, 1990; Lesna and Sabelis 1999; Dias et al. 2016). However, often they also do not discriminate them (Turlings et al. 1993; Geervliet et al. 1996; Shimoda and Dicke 1999) and are attracted to plants attacked by non-prey or non-host (Shimoda and Dicke 2000; van Poecke et al. 2003) what might explain our findings. We did find natural enemies on plants even though they were not a specialist of the herbivores infesting the plants. They were basically a few individuals of generalist predators such as ants and spiders (Table 1, 2) and probably did not show preference for the plants because spider mites are not an important prey for them.

Herbivores also use cues from plants on their foraging, including those from attacked plants (Visser 1986; Dicke 1986; Pallini et al. 1997). These cues can help them to find suitable food (Mound 1962; Blackmer and Byrne 1993; Dias et al. 2016) and also to identify and avoid low quality plants such as plants with induced defences (Bernays and Chapman 1994; Sarmento et al. 2011a), which often negatively affect herbivores (Karban and Baldwin 1997; Sarmento et al. 2011a). We thereby expected herbivores to avoid plants attacked by *T. urticae*, which have defences induced and reduce the performance of conspecific and later-arriving herbivores (Fig. 2, Sarmento et al. 2011a). Furthermore, we expected herbivores to prefer plants attacked by *T. evansi*, which have defence suppressed and can increase the performance of conspecific and other invasive herbivores (Fig. 2, Sarmento et al 2011a; Godinho et al 2015) and then uninfested plants. Nevertheless, our results agreed only partially with these expectations. Whiteflies, the only herbivore described as pest of tomato plants that we found, were more often on uninfested than infested plants and more often on plants with defences suppressed by *T. evansi* than on plants with defences induced by *T. urticae* (Fig. 3). Probably, most of the whiteflies preferred uninfested plants instead of the plants already attacked in order to avoid competition. Often herbivores associate cues from attacked plants with the presence of competitors and avoid these plants in order to avoid competition (Pallini et al. 1997). Nonetheless, the preference of whiteflies for plants with defences suppressed over induced suggests that they may benefit from the down-regulation of tomato defences by *T. evansi*; and be negatively affected by defences induced by *T. urticae*.

Leafhoppers were also found in high number on plants; even though they are not a pest of tomato plants (Fig. 3). The area used in the assay had the ground covered by grass and several weed species, which are common hosts of leafhoppers (Morris 1974; Hawkins et al. 1979; Waloff 1980) and may have attracted them to the experimental area. The fact that they were also found in high number on the tomato plants, which are not a host for them, indicates that these herbivores probably had difficulties in properly selecting the host plant while foraging (Dempster 1969; O'Donnell and Coaker, 1975; Garcia and Altieri 1992; Theunissen et al. 1995. The presence of many species and weed around the experimental area could, for example, camouflage the host plant (Smith 1969; 1976) or masking the host plant odours (Tahvanainen and Root 1972). Additionally, the leafhoppers were more often found on uninfested plants than plants attacked by *T. urticae* (Fig. 3), what might be explained by the feed behaviour of these insects. Piercing-sucking insects like leafhoppers usually test several plants while foraging. Thus, although leafhoppers do not feed on tomato plants, it is possible that they have sensed the plants being affected by the defence compounds induced by *T. urticae*, what could have repelled them.

The spider mite web also affected the attractiveness of plants to whiteflies. When web covered infested plants, whiteflies were more often on uninfested plants than on infested plants (Fig. 4). The web produced by spider mites is supposed to protect them against abiotic (Hazan et al. 1975) and biotic agents (Hoy and Smilanick 1981), including preventing competitors from colonising the plant (Gerson 1985; Sabelis and Bakker 1992; Sarmento et al 2011b). Besides, the web produced by *T. evansi* also prevents competitors to profit from its down-regulation plant defence (Sarmento et al. 2011b). In the experiment where we removed the web from the infested plants, plants with defences suppressed by *T. evansi* were preferred over plants with defences induced by *T. urticae*. But when covered by the web, plants with suppressed defences were avoided just like plants with induced defences, suggesting that the web produced by both spider mites, *T. evansi* and *T. urticae*, might have a negative effect on whiteflies. Some arthropods have special body structures that allow them to break, enter and move around in the spider mite web (Gutierrez and Helle 1985; Sabelis and Bakker 1992; Roda et al. 2000; Montserrat et al. 2008; Shimoda et al. 2009; Lemos et al. 2015), but several others have not and are disturbed by the web (Gerson 1985; Sabelis and Bakker 1992). Invasive herbivores that are not adapted to inhabit plants with the spider mite web can be severely affected, for example, by being trapped, having reduced population growth and even die (Foott 1962, 1963; Slone and Croft 2001; Morimoto et al. 2006). Further investigation would be necessary to better understand the interaction of spider mite web with whiteflies. Additionally, natural enemies, leafhoppers, and other herbivores did not change their preferences when there was web covering the plants. Probably, the spider mite web does not affect them.

The ability of plants to lower the efficiency of herbivores foraging and recruiting natural enemies through their morphological and chemical characteristics can be very efficient in protect them against herbivory (Dicke 1986; Dicke and Sabelis 1988; Drukker et al. 1995; Pallini et al. 1997; Janssen 1999). However, this strategy can eventually have a reverse effect and make plants more vulnerable to herbivores attack (Karban and Carey 1984; Karban and Baldwin 1997; Poelman et al. 2008; Bruessow et al. 2010). In response to plant evolution, herbivores have also evolved and can maximize their fitness by using cues from plants to locate suitable plants and avoid unsuitable hosts and predation (Karban and Carey 1984; Karban and Baldwin 1997; Bleeker et al. 2009; Nombela et al. 2009; Bruce and Pickett 2011; Saad et al. 2015). These could bring important implications to plant fitness, which can be increased due the repellence of herbivores or attraction of natural enemies to plants

(Schuman et al. 2012); or decreased due the attraction of herbivores to plants. In conclusion, our results showed that the changes in plant defence due the infestation of plants with spider mites affected the attraction of leafhoppers and whiteflies to plants, but not to natural enemies. Likewise, the spider mite web in combination with plant defences affected the attraction of whiteflies to plants.

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Tables

Table 1 Classification and abundance of arthropods captured on plants from the experiments without web on infested plants. The arthropods captured were separated according to their order and guild, herbivores and carnivores (natural enemies). Those who did not fit on these two guilds were called non-defined and called "other arthropods" in the graphic.

Arthropod	Order	Guild	Abundance (#)	Web on plants
Whiteflies	Hemiptera	h	371	no
Leafhoppers	Hemiptera	h	272	no
Bugs	Hemiptera	h	39	no
Thrips	Thysanoptera	h	37	no
Aphids	Hemiptera	h	4	no
Moths	Lepdoptera	h	2	no
Spiders	Arachnida	c	61	no
Ants	Hymenoptera	c	15	no
Bugs	Hemiptera	c	3	no
Wasps	Hymenoptera	c	2	no
Beetles	Coleoptera	c	2	no
Flies	Diptera	nd	57	no
Beetles	Coleoptera	nd	25	no
Total			890	

Guild: h= herbivores, c= carnivore, nd = non-defined

Table 2 Classification and abundance of arthropods captured on plants, from the experiment with web on infested plants. The arthropods captured were separated according to their order and guild, herbivores and carnivores (natural enemies). Those who did not fit on these two guilds were called non-defined and called "other arthropods" in the graphic.

Arthropod	**Order**	**Guild**	**Abundance (#)**	**Web on plants**
Leafhoppers	Hemiptera	h	197	yes
Whiteflies	Hemiptera	h	110	yes
Bugs	Hemiptera	h	40	yes
Thrips	Thysanoptera	h	13	yes
Moths	Lepdoptera	h	3	yes
Aphids	Hemiptera	h	3	yes
Spiders	Arachnida	c	66	yes
Ants	Hymenoptera	c	9	yes
Wasps	Hymenoptera	c	7	yes
Bugs	Hemiptera	c	3	yes
Beetles	Coleoptera	c	2	yes
Flies	Diptera	nd	50	yes
Beetles	Coleoptera	nd	21	yes
Total			524	

Guild: h= herbivores, c= carnivore, nd = non-defined

Figure legends

Figure 1 Pooter, device used to collect arthropods in field, composed by a pot with a lid (Ø = 3.5 cm, 80 cm L), 2 tubes (20 cm and 40 cm) and a piece of nylon net (3 cm^2). The end of the shorter tube was positioned into the mouth of the collector. The end of the other tube was positioned a few centimetres of the target arthropod. Next, the air inside the pot was gently sucked (with the mouth) capturing the arthropod into the pot and up against the nylon net.

Figure 2 Mean (+- SE) oviposition rate of *T. evansi* on leaf discs from plants previously infested by *T. evansi*, uninfested and previously infested by *T. urticae*. Different letters above bars indicate significant differences in the oviposition of *T. evansi* among the treatments (contrasts after a lme, $P < 0.05$).

Figure 3 Total numbers of different groups of arthropods captured on plants during the 3 days of evaluations. Bars indicate the total number of arthropods captured on clean plants (first bar), plants infested by *T. evansi* without its web (second bar) and plants infested by *T. urticae* without its web (third bar). Different letters above bars indicate significant differences among plants with different treatments (contrasts after GLMER). Within each bar, groups are presented in different colours; dark grey bars show numbers of leafhopper, grey bars show the number of whiteflies, light grey bars show number of natural enemies, striped bars show herbivores excluding whiteflies and leafhoppers, and white bars show others. Within bar fragments of the same colour, different letters indicate significant differences among the three plant treatments (contrasts after GLMER).

Figure 4 Total numbers of different groups of arthropods captured on plants during the 3 days of evaluations. Bars indicate the total numbers of arthropods captured on clean plants (first bar), plants infested by *T. evansi* with its web (second bar) and plants infested by *T. urticae* with its web (third bar). Different letter above bars indicate significant differences in total numbers of arthropods captured among treatments (contrasts after GLMER). Within each bar, groups are presented in different colours; dark grey bars show numbers of leafhopper, grey bars show number of whiteflies, light grey bars show number of natural enemies, striped bars show herbivores excluding whiteflies and leafhoppers, and white bars show others. Within bar fragments of the same colour, different letters indicate significant differences among the three treatments (contrasts after GLMER).

Figures

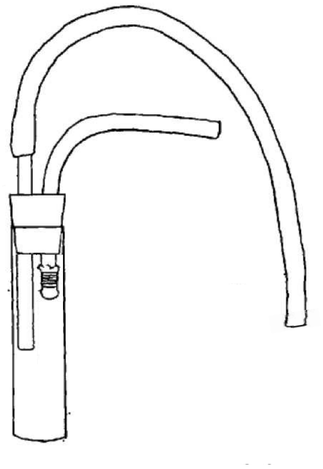

Figure 1

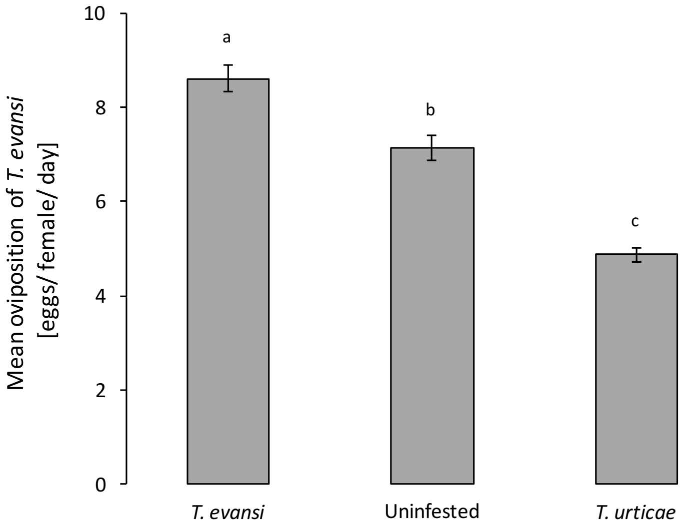
10
8
6
4
2
0
Mean oviposition of *T. evansi*
[eggs/ female/ day]
a
b
c
T. evansi
Uninfested
T. urticae

Figure 2

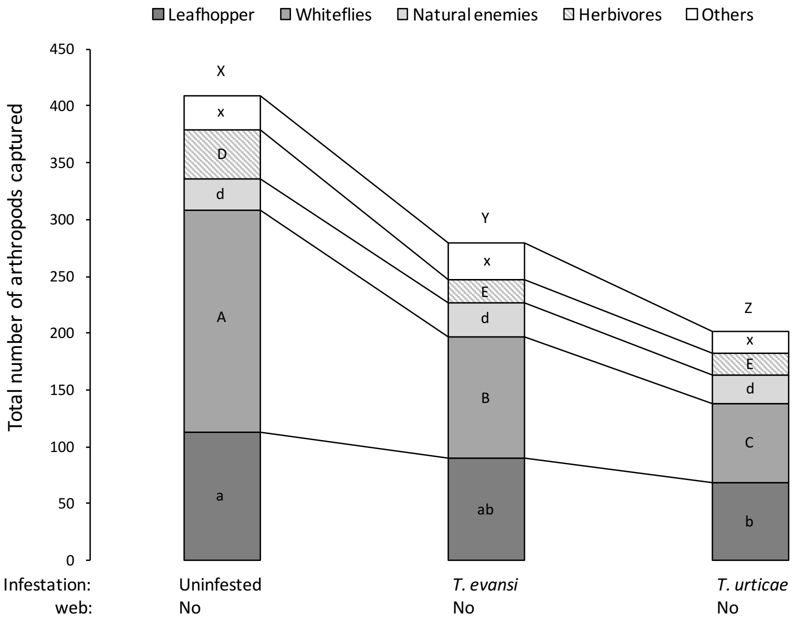
Leafhopper
Whiteflies
Natural enemies
Herbivores
Others
Total number of arthropods captured
0
50
100
150
200
250
300
350
400
450
X
x
D
d
A
a
Y
x
E
d
B
ab
Z
x
E
d
C
b
Infestation:
web:
Uninfested
No
T. evansi
No
T. urticae
No

Figure 3

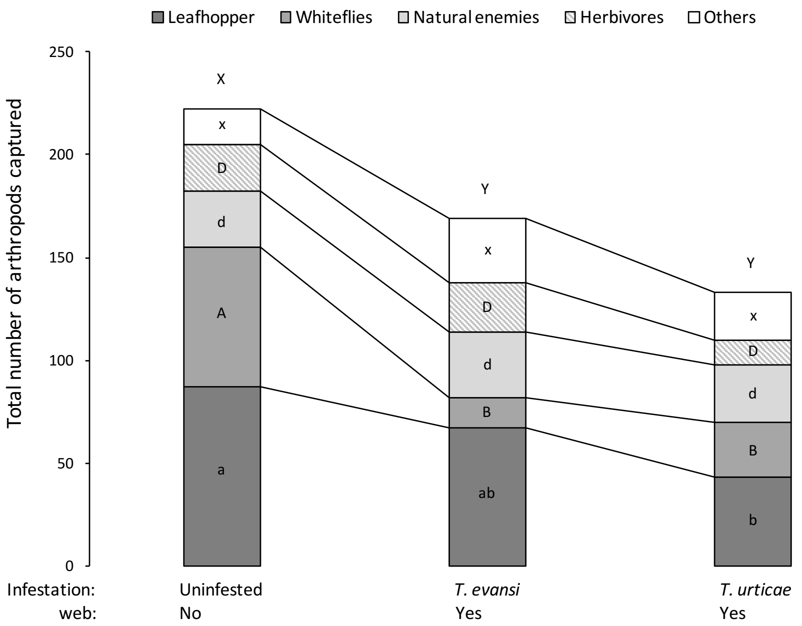
Leafhopper
Whiteflies
Natural enemies
Herbivores
Others
Total number of arthropods captured
250
200
150
100
50
0
X
x
D
d
A
a
Y
x
D
d
B
ab
Y
x
D
d
B
b
Infestation:
web:
Uninfested
No
T. evansi
Yes
T. urticae
Yes

Figure 4

CHAPTER 3

Plant defences and spider mite web affect host plant choice and performance of *Bemisia tabaci*

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Abstract

Plants may increase their defences in response to herbivory, which often negatively affect herbivore performance. Unlike most herbivores, *Tetranychus evansi* does not induce plant defences but suppresses them, causing decreases of defence compounds instead. *T. evansi* also covers its feeding sites on plants with a high-density of web, which, among other functions, is hypothesised to prevent other herbivores to benefit from the suppression of plant defences. Here, we investigated the effect of indirect and direct tomato plants defences, reduced defences, and spider mite web on host plant choice and performance of the whitefly *Bemisia tabaci.* Whiteflies were more often recaptured on uninfested plants and plants suppressed by *T. evansi* without web than on induced plants and plants with web. Also, they preferred plants infested with *T. urticae* with web than plants infested with *T. evansi* with web. Mortality of juvenile stages of *B. tabaci* was higher on infested plants with web than on uninfested plants. Moreover, most of the whiteflies that became an adult on plants with web died stuck on it, especially on *T. evansi* web. In the absence of web, the survival of juveniles was high on uninfested and plants with defence suppressed than on with defences induced. We conclude that whiteflies preferred plants where they had higher survival of juvenile and recently emerged adults, which was uninfested and plants with defence suppressed without web.

Keywords: whiteflies, *T. evansi*, suppressed defences, spider mite web.

Introduction

Plants often suffer from herbivory. So, they have evolved several mechanisms to defend themselves against herbivore attack. They possess the so-called constitutive defences, present continuously, but they will also mount defences after being attacked by herbivores; the so-called induced defences (Karban and Baldwin 1997; Sabelis et al. 2001, 2007). Besides being constitutive or induced, plant defences can affect herbivores in two different ways, direct or indirect. Direct defences are, for example, production of antinutritive or toxic compounds (Duffey and Stout 1996; Howe and Jander 2008) that may affect herbivores performance (Karban and Baldwin 1997). When induced by herbivores, these defences can negatively affect the attacker herbivore and also later arriving herbivores (i.e. reducing their development) (Ohgushi 2005). However, it can eventually also turn the plants more vulnerable to attacks by other herbivores (Karban and Carey 1984; Karban and Baldwin 1997; Poelman et al. 2008; Bruessow et al. 2010).

Indirect defences involve the natural enemies of the herbivores (Price et al. 1980; Dicke and Sabelis 1988; Turlings et al. 1990; Heil 2008) and thus affect herbivores indirectly. An example of indirect defences is the production of volatiles cues by attacked plants (Dicke and Sabelis 1988; Turlings et al. 1990; Sabelis et al. 1998; Kessler and Baldwin 2001). Among many functions (Baldwin 2010), these volatiles can also be attractive to natural enemies (Dicke and Sabelis 1988) resulting in increased attack of herbivores and lower plant damage (Drukker et al. 1995; Janssen 1999; Thaler 1999). However, not only natural enemies rely on plant volatiles to find a suitable patch, herbivores can also perceive and use them on their foraging (Dicke 1986; Sabelis et al. 1999). Once herbivores perceive volatiles cues, they can respond or not to them, being attracted or repelled (Dicke 1986; Dicke et al. 1993; Pallini et al. 1997; Poelman et al. 2008). Very often, volatiles cues from attacked plants also serves as indicative of the presence of competitors (Pallini et al. 1997) or indicator of host plant quality (Bernays and

Chapman 1994) to herbivores (both co and heterospecific) during their foraging (reviewed by Dicke and Vet, 1999).

Nevertheless, some herbivores do not induce plant defences but suppress them to levels even lower that uninfested plants (Musser et al. 2002; Lawrence et al. 2008; Sarmento et al. 2011a, Godinho et al. 2015). The tomato red spider mite *Tetranychus evansi* is an example of such herbivore able to suppress plant defences (Sarmento et al. 2011a; Alba et al. 2015; Oliveira et al. 2015). Unlike most herbivores, including the closely related spider mite *T. urticae*, *T. evansi* causes a reduction of defence compounds to levels below those found in undamaged tomato plants (Sarmento et al. 2011a; Alba et al. 2015; Oliveira et al. 2015). The induced defence responses in tomato plants are regulated by phytohormones such as salicylic acid (SA) and jasmonic acid (JA). The SA is responsible for the regulation of pathways involved in plant defence mostly against biotrophic pathogens (Vlot et al. 2009), and JA against necrotrophic pathogens (Glazebrook, 2005) and herbivores (Walling 2000; Howe and Jander 2008). *Tetranychus urticae* induces defences regulated by both pathways, SA and JA (Ozawa et al. 2000; Kant et al. 2004; Sarmento et al. 2011a; Alba at al. 2015), while *T. evansi* suppresses both (Sarmento et al. 2011a, Alba at al. 2015). Hence, conspecifics and a few other herbivores as the spider mites *T. urticae* (Sarmento et al. 2011a, b; Oliveira et al. 2015) and *T. ludeni (*Godinho et al. 2015) have increased performance on plants with defences suppressed by *T. evansi*.

Additionally, plants attacked by *T. evansi* produce volatile compounds that are attractive to conspecifics compared to the volatiles produced by undamaged plants (Sarmento et al. 2011a; Dias et al. 2016). Thus, other herbivores could also use this volatiles to locate plants with suppressed defences. Therefore, it is possible that other herbivores locate plants attacked by *T. evansi* and benefit from the suppressed plant defences. In return, *T. evansi* might have strategies to prevent competitors from taking advantage of its plant defence suppression, such as, for example, production of web (Sarmento et al. 2011b). Spider mites from the family Tetranychidae are well known for covering their colonies with the web (Saito 1983) that can have several functions such as protection against natural enemies and preventing competitors from colonising the plant (Gerson 1985; Sabelis and Bakker 1992). *T. evansi* stands out among the others Tetranychidae because its production of extremely dense web (Ferragut and Escudero 1999), that also prevents competing spider mites from attacking the plant and profiting from its downregulations of plant defences (Sarmento et al. 2011b).

Except for the spider mites *T. urticae* and *T. ludeni*, it is not known how the suppression of plant defences by *T. evansi* affects other herbivores that also feed on tomato plants. Among the herbivores that can co-occur with *T. evansi* on tomato plants, the whitefly *Bemisia tabaci* Gennadius 1889 biotype B (Hemiptera: Aleyrodidae) stands out as being the main pest of tomato plants worldwide (Buntin et al. 1993). Interactions among whiteflies and other herbivores on a shared host plants have been widely studied (Hoddle et al. 1998; Inbar and Gerling 2008; Baldin et al. 2013), but it is not known how sharing the host plant with a plant defence suppressor spider mites would affect the whitefly *B. tabaci*. Here, we investigated whether the indirect and direct tomato plant defences induced by *T. urticae* or suppressed by *T. evansi* affect the *B. tabaci* host plant choice and performance. We also assessed the effect of spider mite web on preference and performance of the whitefly.

Material and methods

Rearing methods

All the rearings and experiments performed here took place at the Federal University of Viçosa, Viçosa-MG, Brazil. Tomato plants (*Solanum lycopersicum*, variety Santa Clara I-5300) were grown in pots (2 L) using a commercial substrate. The plants were kept inside a greenhouse, fertilised with NPK (4-14-8) plus superphosphate, and watered as needed. All the experiments were performed with tomato plants when they reached the phenological grow stage of six leaves completely developed (expanded). We worked with two species of spider mites, the plant defence inducer *T. urticae* and the plant defence suppressor *T. evansi*, both Viçosa strains as previsouly refered by Alba et al. (2015). They were reared on detached tomato leaves. The petiole of the tomato leaves was placed in a plastic tube filled with water to maintain leaf turgor. The tubes with the tomato leaves were kept in plastic trays filled with water to prevent mite escapes and invasion of other arthropods. Clean tomato leaves were added to these cultures as needed, old leaves were removed from the rearings and the trays were cleaned once every two weeks. The cultures were kept in a room at 25 ± 2 °C, 80 ± 10% relative humidity and 12 hours of light. The whitefly *Bemisia tabaci* Gennadius 1889 biotype B (Hemiptera: Aleyrodidae) was reared on whole tomato plants of the same variety as above. These plants were maintained inside a cage (60 x 60 x 120 cm) covered with a thin screen to avoid escapes of whiteflies and invasion by other arthropods. An uninfested tomato plant with at least four completely developed leaves was added to the rearing once every two weeks. The oldest plants were removed from the cage once a month. The rearing was kept in a shelter under natural climate conditions for the region of Viçosa, Minas Gerais, Brazil (20.7549° S, 42.8786° W).

Host plant choice

Tomato plants grown in pots (2 L) with six completely developed leaves were used for the experiments. We had here three basic plant treatment: uninfested plant, plants infested with *T. evansi* and infested with *T. urticae.* To infest the plants, we released 40 adults of spider mites collected from the rearing, on each leaflet of each leaf of the plants. Three days after infestation, the spider mite web on infested plants was either left intact or was carefully removed with a soft brush, which also resulted in the removal of a few spider mites. We choose this period of infestation (three days) because it gives sufficient time for the spider mites inducing or suppressing plant defences (Oliveira et al. 2015). The plants were placed in a cage consisting of a frame (160 x 160 x 120 cm) covered with a fine mesh. The cage could be opened on all sides, which facilitated access to the plants and minimised the disturbance as a result of sampling. The cage was placed in an outdoor experimental area located on the campus of the Federal University of Viçosa under natural conditions of light, humidity and temperature.

Our most important question concerns the effect of activation or suppression of plant defences by *T. evansi* and *T. urticae* respectively, over a third herbivore species. To ensure that our treatments indeed manipulated plant defences, we measured the performance of *T. evansi* on spared plants treated as mentioned above; uninfested, infested with *T. evansi* and infested with *T. urticae*. Four days after plant infestation, i.e. at the time that the experimental plants were used for host plant choice, leaf discs (Ø = 1.5 cm) were cut from the fifth and sixth leaves of these spared plants and used to evaluate the oviposition rate of *T. evansi* (Sarmento et al. 2011a). Subsequently, the leaf discs were cleaned by removing the adult spider mites, nymphs, eggs and their web. A female of *T. evansi*, aged 12 days old, was released on each leaf disc and its oviposition evaluated daily for three days. Likewise, this same trial was also done in the following experiment "Whitefly development". Mean oviposition rate of *T. evansi* was analysed using a linear mixed-effects models (function lme of the nlme package, Pinheiro et al. 2014), with treatment (plants infested with *T. urticae*, *T. evansi* and uninfested plants) and experiment (host plant choice and whitefly development) as fixed factors, and replicates as a random factor. Contrasts among treatments were assessed using least-square means using the general linear hypotheses test. All the statistical analyses were performed with R (R Development Core Team 2015).

Subsequently, we tested whitefly host plant choice in two experiments. First, we gave the whiteflies a choice among plants with five different treatments: (1) plants infested with *T. evansi* with web; (2) plants infested with *T. evansi* without web; (3) plants infested with *T. urticae* with web; (4) plants infested by *T. urticae* without web; and (5) uninfested plants (Control). After spider mite web was removed from plants of the respective treatments (2 and 4, above), all plants were placed equidistantly in a pentagon (Ø = 80 cm) inside the cage. Second, we gave whiteflies a choice between four plants with two treatments; two plants infested with *T. evansi,* and two plants infested with *T. urticae,* in both treatments the web was kept. These plants were placed equidistantly in a square (L = 80 cm) inside the cage. In both experiments, care was taken that plants of each treatment occupied a different position in each replicate to control any unforeseen directionality in the searching behaviour of the whiteflies (Janssen 1999).

One day after placing the plants in the cage (four days after plant infestation), the preference of whiteflies was accessed. To this end, 100 females of whiteflies were collected individually in a pipette tip around 18:00 hours in the afternoon of the previous day. Each tip was closed with metal thumbtack (Nomikou et al. 2003) and stored in a pipette tip box. Fifteen hours later, the pipette tip box was placed in the centre of the arena. A magnetic strip was used to simultaneously open all pipette tips to release the whiteflies (Meng et al. 2006). Previous tests had shown that almost all the whiteflies left the tips to find a host plant after four hours (Dias CR, personal observation). We, therefore, counted the whiteflies that arrived on plants after four and 24 hours. The whiteflies observed on plants were not recaptured in order to also observe whether they change their choice after the first contact with the plants. Thus, the second observation included individuals that: remained on plants from the previous evaluation; arrived later on plants; and have exchanged plants since the last assessment. The first experiment was replicated five times and the second experiment four times. The number of whiteflies observed on plants after four and 24 hours was analysed using a generalised linear mixed model (function glmer of the lme4 package, Bates et al. 2015) with treatment and time of evaluation (four and 24h after whiteflies release) as fixed factors, and replicate and position of the plants as a non-nested random factor. Contrasts among treatments were assessed using least-squares means (function lsm of the lsmean package, Lenth 2016), using the general linear hypotheses test (function glht of the multcomp package, Hothorn et al. 2008).

Whitefly development

The plants used in the experiments were treated similar to the described for "Host plant choice". We performed three experiments, each with three treatments: (1) plants infested with *T. evansi*; (2) with *T. urticae*; and (3) uninfested plants. In the first experiment, we had plants infested with 40 spider mites per leaflet and covered by the web, spider mites and eggs. However, to complete their immature development at 25 ºC (the condition of our experiments), the whitefly *B. tabaci* takes around 14 ± 20 days (Fancelli and Vendramim 2002). This is enough time to the population of spider mite increase to the point of almost overexploits the tomato plants infested with 40 spider mites per leaflet, high infested plants. Because of that, we decided to use lower infestation on the subsequent experiments, 10 spider mites per leaflet. Hence, in the second experiment, plants were infested with 10 spider mites per leaflet and covered by web, spider mites and eggs. In the third experiment, plants were infested with ten spider mites per leaflet, but a leaf area on which whitefly performance was assessed was clean, thus contained no web or spider mites. This was done in the following manner: a circle (Ø = 1.5 cm) consisting of a thin barrier of non-drying glue (Bio-Controle®) was drawn on the abaxial surface of each leaflet of the fifth and sixth leaves of each plant four days after plant infestation. In the treatment without web, the non-drying glue was applied before plant infestation. Thus, the spider mites released on plants could walk, lay eggs and produce web on the whole plant except inside the evaluation arena. On the fourth day after plant infestation, one whitefly crawler (i.e. mobile stage aged one day) was released in the centre of each arena. The arenas were checked 24 hours later to verify that crawlers were still there to eliminate losses of whiteflies due to manipulation. Only a few crawlers were missing, and there were no differences in the numbers of crawlers missing among treatments. The juveniles were observed daily until all of them reached adulthood or died. On treatments with web, we observed that newly emerged adults were often getting stuck and dying in the spider mite web. Thus, we also evaluated the number of adults dead trapped in the web. Each experiment was replicated four times. Each plant had ten arenas, so we evaluated 40 individuals per treatment. The mean developmental time of whitefly juveniles, the proportion of emerged adults and proportion of adults stuck in spider mite web were tested using a linear mixed effect model (function lme of the nlme package in R, Pinheiro 2015) with treatment as fixed factor and replicate as a random factor. Contrasts among treatments were assessed using least-square means (function lsm of the lsmean package in R, Lenth 2016), using the general linear hypotheses (function glht of the multcomp package in R, Hothorn et al. 2008). All the statistical analyses were performed with R (R Development Core Team 2015).

Results

Host plant choice

We found in our experiments the expected induction and suppression of defences of the tomato plants caused by the spider mites, according to results observed by Sarmento et al. (2011a). The oviposition rate of *T. evansi*, evaluated on spared plants from the experiments *Host plant choice* and *Whitefly development*, was significantly different among treatments (Fig. 1, LME: $\chi^2 = 68.44$, d.f. = 7, $P < 0.0001$). Oviposition was higher on leaf discs of plants previously attacked by *T. evansi* than on discs from clean plants, showing that the previous attack by *T. evansi* had indeed resulted in the suppression of plant defences (Fig. 1). The oviposition rate was lower on leaf discs from plants previously attacked by *T. urticae* than on discs from clean plants or from plants previously attacked by *T. evansi*, showing the induction of plant defence by *T. urticae* (Fig. 1). Together, this shows that our manipulation of plant defences through the previous infestation of the plants by the defence-inducing strain of *T. urticae* and by the defence-suppressing *T. evansi* was successful.

In the first host plant choice experiment, the number of whiteflies observed on plants differed significantly among plants that received different treatments (Fig. 2, GLMER: χ^2 = 62.17, d.f. = 4, $P < 0.001$). The number of whiteflies observed on plants after 4 or 24h did not differ significantly (GLMER: χ^2 = 0.49, d.f. = 1, $P = 0.48$), meaning that most of the whiteflies were probably already on plants after four hours of their release. The highest numbers of whiteflies were observed on clean plants and plants infested with *T. evansi* without the web, and lower numbers were observed on plants infested with *T. urticae* with or without web and *T. evansi* with the web. In the second host plant choice experiment, when the whiteflies had a choice between plants with *T. urticae* and their web and plants with *T. evansi* and their web, they were more often observed on plants infested with *T. urticae* (Fig. 3, GLMER: χ^2 = 16.56, d.f. = 1, $P < 0.001$). The number of whiteflies observed on plants after 4 or 24h did not differ significantly (GLMER: χ^2 = 0.38, d.f. = 1, $P = 0.53$).

Whitefly development

In the first experiment where plants were highly infested by spider mites (40 spider mites per leaflet) and covered by web, the mean developmental time of juvenile stage of *B. tabaci* was significantly affected by the treatments (Fig. 4, LME: χ^2 = 11.85, d.f. = 3, $P = 0.0027$). Whitefly juveniles developed faster on uninfested plants than on plants infested with *T. evansi*, and it was no significantly different from that on plants infested with *T. urticae* (Fig. 4 first bars). The proportion of emerging adults was also significantly different among the plant's treatments

(Fig. 5 first bars, LME: χ^2 = 15.75, d.f. = 3, *P* < 0.001). More juveniles of whitefly became adults on uninfested plants than on plants infested either with *T. urticae* or *T. evansi.* Additionally, most of the adults that emerged from infested plants were found dead, stuck on the spider mite web (Fig. 6), being more often in *T. evansi* than in *T. urticae* web (Fig. 6 first bars, LME: χ^2 = 4.22, d.f. = 3, *P* = 0.04).

In the second experiment where plants were low infested by spider mites (10 spider mites per leaflet) and covered with web, the mean developmental time of immature stages of *B. tabaci* was affected by the treatments (Fig. 4, LME: χ^2 = 9.15, d.f. = 3, *P* = 0.010). Development of juveniles was faster on plants infested with *T. urticae* than on plants infested with *T. evansi*, and non-significantly different from that on uninfested plants (Fig. 4 second bars). Proportion of juveniles that reached adulthood was significantly different among treatments (Fig. 5, LME: χ^2 = 7.34, d.f. = 3, *P* = 0.025). More juveniles became adults on uninfested plants than on plants infested with *T. urticae*, and non-significantly different from that on plants infested with *T. evansi* (Fig. 5 second bars). Also, a high number of newly emerged adults were found dead stuck in the spider mite web. There was no significant difference between the proportion of adults died stuck in the web of *T. urticae* and *T. evansi* (Fig. 6, LME: χ^2 = 0.96, d.f. = 3, *P* = 0.33).

In the third experiment where plants were low infested (10 spider mites per leaflet) without web, mean developmental time of *B. tabaci* juveniles did not differ significantly among treatments (Fig. 4, LME: χ^2 = 1.50, d.f. = 3, *P* = 0.47). However, the proportion of adults emerging was significantly affected by the treatments (Fig. 5, LME: χ^2 = 12.57, d.f. = 3, *P* = 0.0019). The proportion of adults was higher on uninfested plants and plants infested with *T. evansi* than on plants infested with *T. urticae* (Fig. 5).

Discussion

We show here that induction and suppression of plant defences, as well as the presence of the spider mite web, affected the host choice and performance of the whitefly *B. tabaci*. These results suggest that the changes in plant defence due spider-mite plant infestation and web might affect whiteflies, which was indeed what we found. Herbivores often avoid plants they could have reduced performance, such as herbivore-induced plants (Karban and Carey 1984; Karban and Baldwin 1997; Poelman et al. 2008; Nombela et al. 2009; Tan and Liu 2014; Saad et al. 2015). Tomato plants attacked by *T. urticae* have their defences induced (Li et al. 2002; Ament et al. 2004; Sarmento et al. 2011a) though activation of both defensive pathways, jasmonic acid (JA) and salicylic acid (SA) (Ozawa et al. 2000; Kant et al. 2004, Sarmento et al. 2011a, Alba at al. 2015). These signalling pathways may cross-talk, interfering with each other, either positively or negatively (Beckers and Spoel 2006), thus, they can have a positive, negative or non-effects on later-arriving herbivores (Karban and Baldwin 1997; Poelman et al. 2008; Bruessow et al. 2010). It has been shown that defences induced by *T. urticae* in tomato plants negatively affect conspecifics and few other spider mites (Sarmento et al. 2011a, Oliveira et al. 2005, Godinho et al. 2015). We herewith showed that they also affected the whitefly *B. tabaci* host choice and performance. Our results indicated that whiteflies preferred uninfested plants than plants infested by *T. urticae* (Fig. 2), where their juvenile survival was substantially reduced (number of adults in Fig. 5 third bars), probably because of the defences induced by *T. urticae*.

In contrast, tomato plants attacked by *T. evansi* have their defences suppressed (Sarmento et al. 2011a, Alba at al. 2015), which occur independently of the JA-SA antagonism with reduction of both phytohormones accumulation (Alba et al. 2015). Thereby, herbivores perform better and are more attracted to plants infested by *T. evansi* than plants infested by *T. urticae* and uninfested plants (Fig. 1, Sarmento et al. 2011a; Oliveira et al. 2015; Godinho et al. 2015; Dias et al. 2016). We showed here that *B. tabaci* preferred plants with defences suppressed by *T. evansi* (without web) than plants with defences induced by *T. urticae* (Fig. 2). In agreement with whitefly choice, the survival of juveniles *B. tabaci* was significantly higher on plants with defence suppressed than on plants with defence induced (Fig. 5, Proportion of adults, third bars).

Nevertheless, *B. tabaci* did not show significant preference between plants with defences suppressed by *T. evansi* and uninfested plants (Fig. 2). We demonstrated in the *T. evansi* performance experiment that our plants infested with *T. evansi* were suppressed and resulted in higher performance of herbivores compared to uninfested plants and plants infested by *T. urticae* (Fig. 1), confirming previous findings (Sarmento et al. 2011a; Oliveira et al. 2015). Although it has been shown that other herbivores can also benefit from *T. evansi* suppression increasing their performance compared to uninfested plants (Sarmento et al. 2011a; Oliveira et al. 2015; Godinho et al. 2015), we did not find significant differences either in development time (Fig. 4 third bars) or survival (Fig. 5, third bars) of juveniles *B. tabaci* between uninfested and plants with defence suppressed (infested with *T. evansi* without web). These results suggest that uninfested plants and plants infested with *T. evansi* without the web are both equally suitable for *B. tabaci*.

However, when there was spider mite web on infested plants, uninfested plants were preferred over plants infested with *T. evansi* (Fig. 2). Overall, *B. tabaci* was more often found on plants without the web than on plants with the web, except for plants with defence induced where they were less often on both, with and without the web (Fig. 2). These results indicate that the spider mite web also might have an effect on whiteflies, which was further confirmed by the development experiments. The production of web is a common characteristic of spider mites from the family Tetranychidae (Saito 1983) and can have several functions including preventing competitors from colonising the plant (Gerson 1985; Sabelis and Bakker 1992; Morimoto et al. 2006). A few natural enemies and herbivores can enter the spider mite web and use it to self-protection (Pallini et al. 1998; Roda et al. 2000; Venzon et al. 2000; Lemos et al. 2010, 2015), but often they are harmed. We showed that the spider mite web has a strong adverse effect on whiteflies, both juveniles and adults. The survival of whiteflies juveniles was significantly reduced on infested plants with the web than on uninfested plants (Fig.5, first bars). Additionally, only 26% of the crawlers became adult (Fig. 5 first bars) on high-infested plants (40 spider mites per leaflet), and most of them died stuck in the spider mites web (Fig. 6).

Furthermore, the presence of web seems to turn plants with defence suppressed less attractive to herbivores, and also less suitable for their establishment on plants. Whiteflies did not show preference for uninfested or plants with defence suppressed when there was no web on infested plants, but they preferred uninfested plants when plants with defence suppressed were covered by web (Fig. 2). In the absence of web, the survival of *B. tabaci* juveniles was reduced only on induced plants (Fig. 5 third bars). In contrast, in the presence of web, the survival of *B. tabaci* juveniles was reduced on both induced and plants with defence suppressed (Fig. 5, first bars). We also showed that plants with defence suppressed only had a positive effect on whiteflies when there was no spider mite web on infested plants, in the presence of web whiteflies had reduced performance. These results corroborate the idea that the web of *T. evansi* has a protective function against invasions by other herbivores, thus preventing those heterospecific from profiting from the down-regulated defences (Sarmento et al. 2011b).

Additionally, *Bemisia tabaci* was less often found on plants infested with *T. evansi* with web than on plants infested with *T. urticae* with web (Fig. 3), even though plants infested with *T. urticae* are induced and plants infested with *T. evansi* are suppressed (Fig. 1, Sarmento et al. 2011; Oliveira et al. 2015). These results suggest that the web produced by *T. evansi* probably has a stronger effect on whiteflies then *T. urticae* web, which was corroborated by the development experiments. Most of the adults of *B. tabaci* died stuck in the *T. evansi* web comparing to *T. urticae* web, on plants highly infested (Fig. 6). Also, on low infested plants with web, the development of *B. tabaci* juveniles was slower on plants infested with *T. evansi* than on plants infested with *T. urticae* (Fig. 4 second bars). Although the production of web is a common characteristic of Tetranychidae (Saito 1983), *T. evansi* stands out by its extreme production of web (Baker and Pritchard 1960; Ferragut and Escudero 1999) that can be denser than *T. urticae* web (Sarmento et al. 2011b), which may explain its stronger effect on whiteflies.

Host preference and herbivore performance are essential components in host-range ecology and evolution and are expected to have a positive correlation (Fry 1989). Thus, herbivores usually choose their host plants based on their suitability (Bernays and Chapman 1994), preferring plants where they will have better performance (Karban and Carey 1984; Poelman et al. 2008; Tan and Liu 2014; Saad et al. 2015). A plant already attacked may have its defences manipulated which can interfere on the population growth of later invasive herbivore (Karban and Carey 1984; Agrawal et al. 2000; Saad et al. 2015). As result, the population dynamic of arthropods on plants can also be affected. A previous colonization of tomato plants by spider mites would probably affect the establishment of whiteflies populations. Here we show that plant defences and the web produced by spider mites affected the herbivore host plant choice and performance. Whiteflies preferred plants where they had higher survival of juvenile and recently emerged adults, which was on uninfested and plants with defence suppressed without web.

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Figures legends

Figure 1 Mean (± SE) oviposition rate of *T. evansi* on leaf discs from plants previously infested by *T. evansi*, previously infested by *T. urticae*, and uninfested plants. Bars with different letters differ significantly (contrast after a lme, $P < 0.05$).

Figure 2 Average number (± SE) of *B. tabaci* observed on uninfested plants, plants infested with *T. evansi* without its web (*T. evansi* - web), plants infested with *T. urticae* without its web (*T. urticae* - web), plants infested with *T. evansi* with its web (*T. evansi* + web) and plants infested with *T. urticae* with its web (*T. urticae* + web), after four (grey bars) and 24 hours (white bars). Different letters above bars indicate significant differences in the average of whiteflies observed on each plant (contrast after a GLMER, $P < 0.05$). Uppercase letters indicate differences among the number of whiteflies observed on plants after four hours, and lowercase after 24 hours.

Figure 3 Average number (± SE) of *B. tabaci* observed on plants infested with *T. evansi* with its web (light grey bars) and plants infested with *T. urticae* with its web (dark grey bars), 24 hours after their release. Different letters above bars indicate significant differences in the proportion of whiteflies observed on plants (contrast after a GLMER, $P < 0.05$).

Figure 4 Mean development time (± SE) of *B. tabaci*, from first stage of nymph until adult, on uninfested plants (white bars), plants infested with *T. urticae* (dark grey bars) and plants infested with *T. evansi* (light grey bars). Each group of bars shows the levels of plants infestation, number (#) of spider mites per leaflet, and whether there were spider mites and their web on evaluation arenas. Different letters above bars indicate significant differences in the developmental time of juveniles of whiteflies within each group of bars (contrast after LME, $P < 0.05$).

Figure 5 Proportion (± SE) of *B. tabaci* juveniles that reached adulthood on uninfested plants (white bars), plants infested with *T. evansi* (light grey bars) and plants infested with *T. urticae* (dark grey bars). Each group of bars shows the levels of plants infestation, number (#) of spider mites per leaflet, and whether there were spider mites and their web on the evaluation arena. Different letters above bars indicate significant differences in the proportion of juveniles of whitefly that developed into adult within each group of bars (contrast after LME, $P < 0.05$).

Figure 6 Proportion (± SE) of adults of *B. tabaci* that were found dead in the spider mite web. Both groups of plants were covered with spider mite web. Different letters above bars indicate significant differences in the mortality of adults of whiteflies in the web within each group of bars (contrast after LME, $P < 0.05$).

Figures

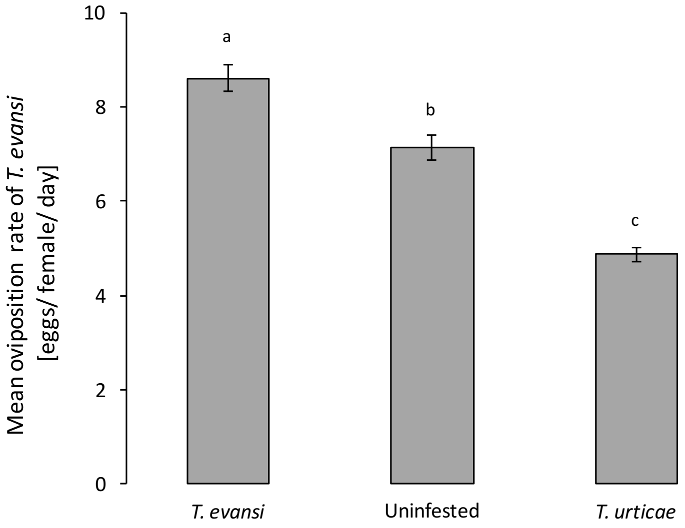

Figure 1

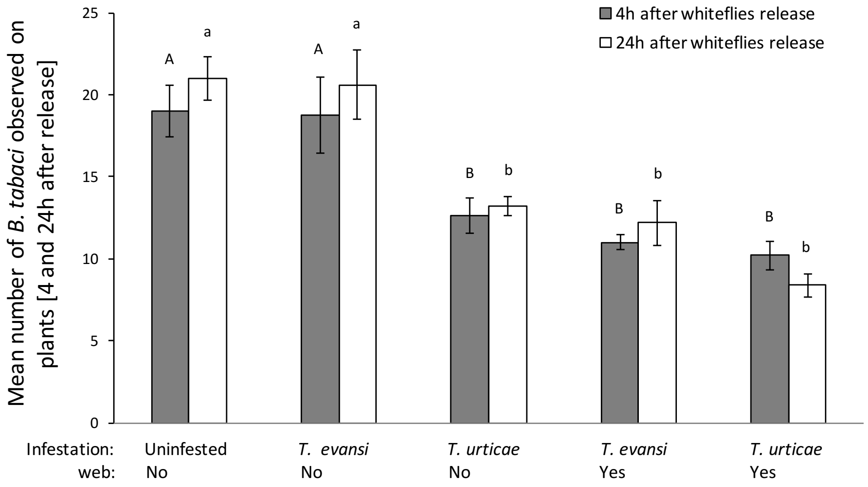
Mean number of B. tabaci observed on plants [4 and 24h after release]
0
5
10
15
20
25
4h after whiteflies release
24h after whiteflies release
A
a
A
a
B
b
B
b
B
b
Infestation:
web:
Uninfested
No
T. evansi
No
T. urticae
No
T. evansi
Yes
T. urticae
Yes

Figure 2

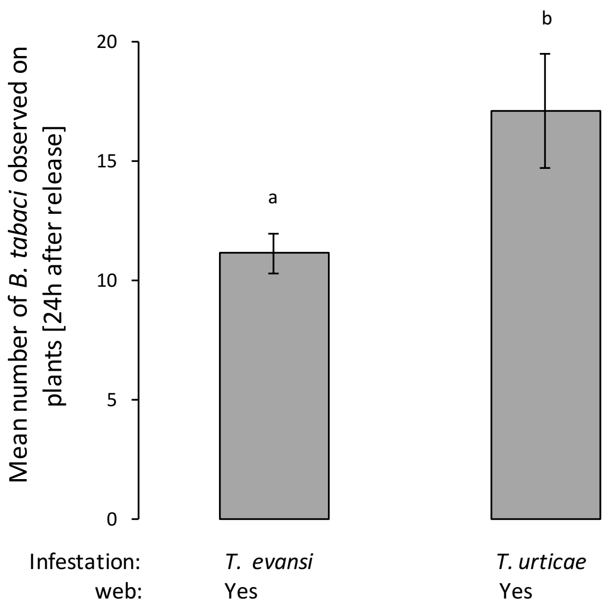
Mean number of *B. tabaci* observed on plants [24h after release]
0
5
10
15
20
a
b
Infestation:
web:
T. evansi
Yes
T. urticae
Yes

Figure 3

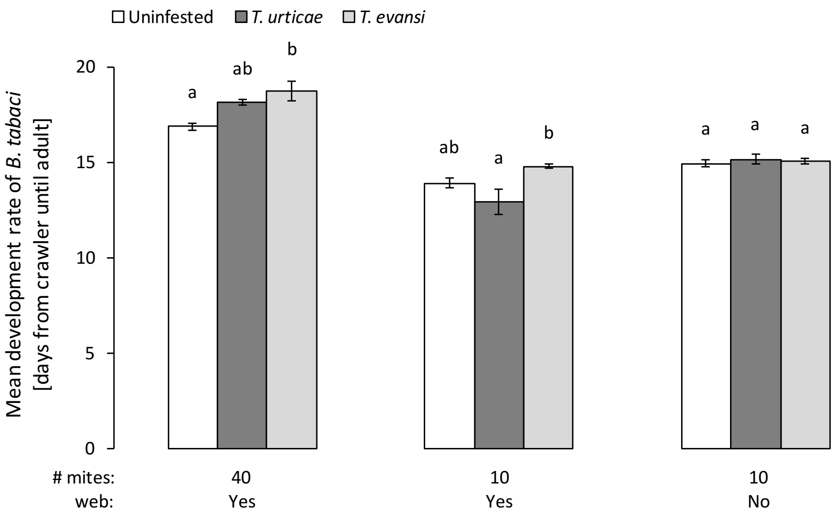
☐ Uninfested
▨ *T. urticae*
☐ *T. evansi*
Mean development rate of *B. tabaci* [days from crawler until adult]
20
15
10
5
0
a
ab
b
ab
a
b
a
a
a
mites:
web:
40
Yes
10
Yes
10
No

Figure 4

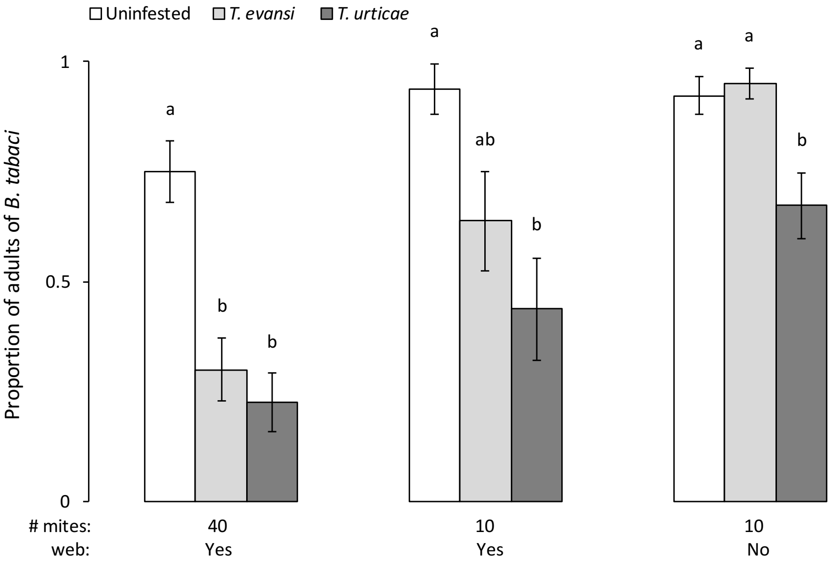
☐ Uninfested
☐ *T. evansi*
■ *T. urticae*
Proportion of adults of *B. tabaci*
1
0.5
0
a
b
b
a
ab
b
a
a
b
mites:
web:
40
Yes
10
Yes
10
No

Figure 5

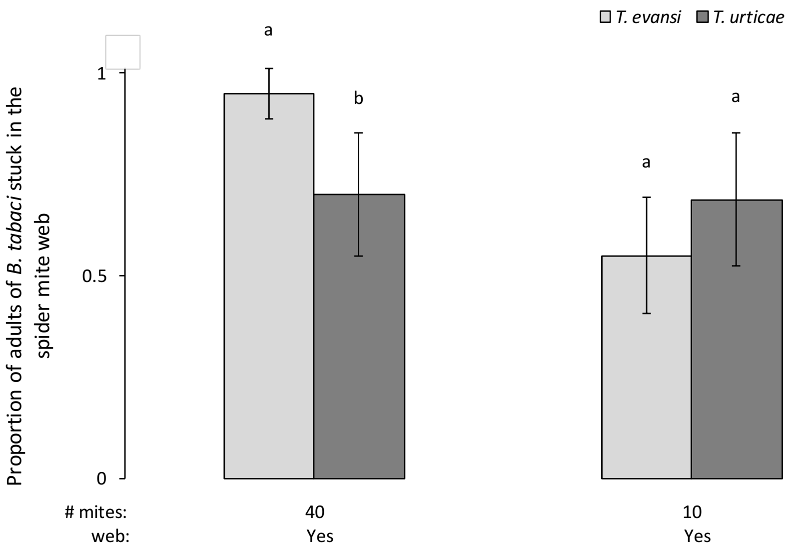
T. evansi
T. urticae
Proportion of adults of B. tabaci stuck in the spider mite web
1
0.5
0
a
b
a
a
mites:
web:
40
Yes
10
Yes

Figure 6

CHAPTER 4

Preference and performance of *Frankliniella occidentalis* on plants infested with defence-inducer or suppressor spider mites

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Abstract

Plants have several defence mechanisms to defend themselves against herbivores attack, but some herbivores have strategies to overcome these plant defences. For example, the spider mite *Tetranychus urticae* induces defence traits in tomato plants, while *T. evansi* suppress them. These spider mites also cover their colony with web, which can also affect invasive herbivores. Another important worldwide pest of tomato plants is the thrips *Frankliniella occidentalis*, which besides feeding on plants also feeds on pollen and mite eggs. Pests interactions in a shared host can be very important on the matters of population ecology and practical applications as biological control. We thereby, investigated whether the changes in plant defence due infestation of plants with *T. urticae* and *T. evansi*, as well as the presence of the spider mites and their web, affects the plant host preference and performance of *F. occidentalis*. The thrips were more often found on plants infested with *T. evansi* with web than on plants infested with *T. evansi* without web. The performance of *F. occidentalis* was not affected by the plant treatments. We thereby conclude that *F. occidentalis* was not affected by the presence of the spider mites on plants and by their interference on plant defences.

Nevertheless, the presence of spider mite web on plants infested with *T. evansi* made the plant more attractive to *F. occidentalis*, but did not affect the performance and survival of juveniles.

Keywords: Induced defence, suppressed defence, *Tetranychus evansi*, spider mite web.

Introduction

As self-defence against herbivory, plants have several defence mechanisms that are continuously produced (constitutive defences) or produced only after the encounter with herbivores (induced defences) (Karban and Baldwin 1997; Sabelis *et al*. 2001, 2007). Attacked plants accumulate phytohormones that are involved in regulation of plant defences against herbivores such as the Jasmonic acid (JA) (Walling 2000; Howe and Jander 2008; Thaler et al. 2012). The accumulation of JA can result in the production of defensive proteins such as protease inhibitor and polyphenol oxidases in plants (Ryan 1990; Howe et al. 1996; Glawe et al. 2003). Such chemical defences disrupt herbivore feeding, growth and reproduction (Green and Ryan 1972; Broadway and Duffey 1986; Howe et al. 1996; Lawrence and Koundal 2002; Pompermayer et al. 2003; Kant et al. 2004). However, there are also some herbivores that do not induce plant defences but suppress it instead (Musser et al. 2002; Lawrence et al. 2008; Kant et al. 2008; Sarmento et al. 2011a; Godinho et al. 2015). In such cases, the levels of defensive compounds are lower in attacked plants than in uninfested plants resulting in increased performance of herbivores on already infested plants (Sarmento et al. 2011a; Alba at al. 2015; Godinho et al. 2015; Oliveira et al. 2015).

The manipulation of plant defences by herbivores can interfere with the dynamics of arthropods on plants. For instance, the induction of defences by a previous herbivore can impact the population of later-arriving herbivores (Ohgushi 2005). The induced accumulation of defensive compounds and/or defensive proteins can reduce other herbivore performance, which may decrease their preference and abundance on the plant, i.e. the thrips *Frankliniella occidentalis* (Thaler et al. 1996; Blauth et al. 1998; Abe et al. 2008, 2009). Herbivores can perceive and use cues from attacked plants on their foraging (Visser 1986; Dicke 1986; Sabelis et al. 1999) and associate them with host quality (Bernays and Chapman 1994; Dicke and Vet 1999). They can for example, use these cues as indicative of induced defences and presence of competitors (Bernays and Chapman 1994; Pallini et al. 1997), being then either attracted or repelled by them (Dicke 1986; Dicke et al. 1993; Pallini et al. 1997; Poelman et al. 2008).

In tomato plants, several pests co-occur (Kennedy 2003; Lange and Bronson 1981) affecting each other in many ways. The two spotted spider mite *Tetranychus urticae*, for example, induces Salicylic acid (SA) and JA defences pathways in tomato (Ozawa et al. 2000; Li et al. 2002; Kant et al. 2004; Sarmento et al. 2011a; Alba at al. 2015). However, its co-related tomato red spider mite *Tetranychus evansi* suppresses both defensive mechanisms in tomato (Sarmento et al. 2011a; Alba et al. 2015; Oliveira et al. 2015). Consequently, *T. evansi* makes their attacked tomato plants more suitable and attractive to conspecifics compared to plants previous attacked by *T. urticae* and non-attacked plants (Sarmento et al. 2011a; Alba at al. 2015; Godinho et al. 2015; Oliveira et al. 2015; Dias et al. 2016). Likewise, other herbivores also showed increased performance on plants previous attacked by *T. evansi*, including the spider mites *T. urticae* and *T. ludeni* (Sarmento et al. 2011b; Godinho et al. 2015) indicating that the suppression of plant defences benefits not only *T. evansi* but also its competitors. In return, *T. evansi* has strategies to prevent competitors from taking advantage of plants with defence suppressed, which is, covering its colonies with web (Sarmento et al. 2011b).

Tetranychidae mites are well known for covering their colonies with a silken web (Saito 1983), which has numerous functions such as protection against adverse climatic conditions, natural enemies and competitors (Gerson 1985; Sabelis and Bakker 1992; Sarmento et al. 2011b). The complex structure of this web can affect the foraging efficiency of small arthropods (Sabelis 1985; Oku et al. 2003, 2004), inhibit their movement on plants (Putman 1962; McMurtry et al. 1970; Trichilo and Leigh 1986) and entrap them which may result in their death (McMurtry and Scriven 1964; Trichilo and Leigh, 1986; Osakabe 1988). However, some arthropods can also benefit from this web. Some species of thrips and predatory mites, for example, can use it as protection against their natural enemies (Pallini et al. 1998; Venzon et al. 2000; Lemos et al. 2015). In the case of *T. evansi,* it stands out among the other Tetranychidae for covering its host plant with an extremely dense silken web (Ferragut and Escudero 1999), which has also the function of preventing other mites to colonise the plants and profit from its down-regulated defences (Sarmento et al. 2011b). It is unknown how the manipulation of plant defences by spider mites and its combination with spider mites web can affect other important insect pests of tomato plants, such as for example the thrips *F. occidentalis*.

The western flower thrips *F. occidentalis* is the main pest of several crops, being one of the most destructive sucking pests of vegetables, fruits and ornamental crops worldwide (Parrella 1995; Kirk and Terry 2003; Reitz 2009; Reitz et al. 2011; Yang et al. 2015; Mouden et al. 2016). They are omnivorous and can feed on plant tissue, pollen and eggs of other arthropods (Pimm and Lawton 1978; Hunter and Ullman 1989; Trichilo and Leigh 1986). Thrips can cause direct damage to plants by feeding on leaves, flowers and fruits (Tommasini and Maini 1995; Shipp et al. 1998; Medhurst and Steiner 2001; Coll et al. 2006; Zalom et al. 2008) and indirectly by transmitting viruses, of which the "tomato spotted wilt virus" is the most important (De Jager et al. 1995; Maris et al. 2003; Whitfield et al. 2005; Webster et al. 2011; Zhao et al. 2014).

Western flower thrips are often found co-occurring with spider mites having an important role as predator of spider mite eggs (Lincoln et al. 1953; Gonzalez and Wilson 1982; Agrawal and Karban 1997). The consumption of the spider mite eggs can benefit the thrips because it can decrease their larval development time, increase larval survival and oviposition rate (Milne and Walter 1997). The thrips *F. occidentalis* mainly feeds on leaf parenchyma and plant pollen, but also on eggs of *T. urticae* (Trichilo and Leigh 1986; Pallini et al. 1998; Janssen et al. 1998; Agrawal and Klein 2000). This omnivory interaction is called intraguild predation, when a species (*F. occidentalis*) eats another species (*T. urticae*) that share the same resource (tomato plant) and are thus potential food competitor (Polis et al. 1989; Janssen et al. 2007; Lucas et al. 2009).

The diet selection of omnivores can be complex and evolve several foraging strategies, nutritional requirements, and availability of non-plant food (Schoener 1971; Westoby 1978). The proportion of spider mite eggs in the thrips diet often depends on the quality of the host plant; thrips predation is usually higher on less suitable plants (Trichilo and Leigh 1988; Wilson et al. 1996; Agrawal et al. 1999; Janssen et al. 2003; Magalhaes et al. 2005) such as plants with defences already induced by a previous herbivore (Agrawal et al. 1999; Omer et al. 2001). Plants infested with the spider mite *T. urticae* have the JA-regulated plant defences induced, which can restrict thrips performance and host plant selection (Delphia et al. 2007; Abe et al. 2009). Nonetheless, it is unknown how the downregulation of tomato plant defences by *T. evansi* in combination with the presence of the spider mites and their web can affect the thrips. We thereby investigated the effects of the induction or suppression of tomato plant defences, by *T. urticae* and *T. evansi* respectively, as well as the presence of the spider mites and their web on the performance and host plant selection of western flower thrips *F. occidentalis.*

Material and methods

Rearing Methods

All the rearings and experiments performed here took place at the University of Amsterdam, Amsterdam, the Netherlands. Tomato plants (*Solanum lycopersicum*) of the variety Santa Clara I-5300 were grown in pots (Ø = 14 cm) using a commercial substrate. The plants were kept in a climate room under controlled conditions (25 ± 1 °C, 60-70 % RH, 16:8 h L: D), fertilized once a week (EC fertilizer NPK (Ca, Mg), 15-5-15 (+7+3)) and watered by filling the trays under the pots with water three times per week. These plants were used for mite cultures and experiments.

Tetranychus evansi and *T. urticae* (Acari: Tetranychidae), both populations originally from Viçosa-MG Brazil, were reared on detached tomato leaves. The rearing arenas consisted of big (40 x 25 cm) and small (18 x 12 cm) trays with small trays placed upside down inside big trays. Big trays were filled with tap water, thus isolating small trays to avoid contamination. A tomato leaf with spider mites was placed on the small tray with the petiole touching the water inside the big tray to maintain leaf turgor. A clean tomato leaf was added to the rearing three times per week. Once a month, the arenas were cleaned, the water in the big trays was replaced and old leaves that had no spider mites were removed. The cultures of *T. evansi* and *T. urticae* were kept in separate climate rooms (25 ± 1 °C, 60-70 % RH, 16:8 h L: D) to avoid cross-contamination.

Frankliniella occidentalis was reared on entire tomato plants and placed in a cage covered by a fine mesh to avoid thrips escapes and contamination with other arthropods. A small amount of pollen of *Ricinus communis* was added on the tomato leaves twice per week. Hence, *F. occidentalis* could feed on plants and pollen. Plants were watered twice per week and fertilized once per week. An undamaged plant was added to the rearing once per month. Old plants were kept in the cage until they were completely filled, upon which the oldest plant was removed. This procedure allowed nymphs and adults of *F. occidentalis* to move from the old to the new plants, and provided sufficient time for the pupae, which are predominantly in the soil, to develop. The rearing was kept in a climate room with controlled conditions (25 ± 1 °C, 60-70 % RH, 16:8 h L: D).

Host plant choice

We first tested the *F. occidentalis* host choice by giving it five options, in sequence, according with the results obtained we decided whether would be other combinations of host plants to be tested. Thus, we tested the host choice of *F. occidentalis* in four different combinations of tomato plants, placed equidistantly on a circle (Ø = 76 cm) or side by side (distance = 76 cm) when there were two options. At the first experiment, *F. occidentalis* had five options of plants: (i) uninfested plants (control); (ii) plants infested with *T. evansi* with its web; (iii) plants infested with *T. evansi* without web; (iv) plants infested with *T. urticae* with its web and (v) plants infested with *T. urticae* without web. In the second experiment, *F. occidentalis* had three options of plants: (i) uninfested (control); (ii) infested with *T. evansi* with its web; and (iii) infested with *T. urticae* with its web. Five replicates were done for the first and second experiments. In the third and fourth experiments, plants were located side by side (distance = 76 cm) so *F. occidentalis* had 2 options of plants: (i) infested with *T. evansi* with its web *vs* (ii) infested with *T. evansi* without web; and (i) infested with *T. urticae* with its web *vs* (ii) infested with *T. urticae* without web. Four replicates were done for each combination of the third and fourth experiment.

Tomato plants grown in pots (2 L) with at least four completely developed leaves were used in the experiments. The plants were infested by releasing 20 females of one of the spider mite species on each leaflet. The treated plants were kept in a greenhouse in separate cages to prevent contamination. The plants were placed equidistantly on a circle (Ø = 76 cm) inside a cage (200 x 83 x 100 cm) in a greenhouse on the third day after plant infestation at 6 p.m. The next step of the experiment was done 15 hours later, on the fourth day after plant infestation, to avoid that the stress caused by the plants manipulation and transport would interfere on the results. The cage was used in order to avoid any contamination with other arthropods from others compartment of the greenhouse, as well to avoid escape of *F. occidentalis*. On the fourth day after plant infestation, the web of the infested plants from the treatments without web was carefully removed with a soft brush, which could also accidentally remove few spider mites. Posteriorly, one hundred females of *F. occidentalis* were collected from the rearing unit using four pipette tips (25 females in each) and released at the centre of the circle of plants.

Four days of infestation is considered enough time for the spider mites to suppress or induce plant defence (Oliveira et al. 2015). *F. occidentalis* that arrived on plants were recaptured during the first six hours with intervals of one hour between samplings. To control any unforeseen directionality in the searching behaviour of the thrips, care was taken that each treatment occupied a different position in each replicate (Janssen 1999). The total number of *F. occidentalis* recaptured on plants in each experiment was analysed using a generalized linear mixed model (function glmer of the lme4 package in R, R Development Core Team 2013, Bates et al. 2013) with position as a non-nested random factor. Contrasts among treatments were assessed using least-square mean (function lsm of the lsmean package, Lenth 2016), using the general linear hypothesis test (function glht of the multcomp package, Hothorn et al. 2008).

Performance of F. occidentalis

Tomato plants with similar development stage and treatment as described above were used to evaluate the juvenile development of *F. occidentalis*: plants infested by *T. evansi* with web; infested by *T. evansi* without web; infested by *T. urticae* with web; infested by *T. urticae* without web; and uninfested plants. Four days after plant infestation, a leaf disc (Ø = 2 cm) was cut from each leaflet of the third and fourth leaves of the plants. The leaf discs were placed on wet cotton wool in a small Petri dish (Ø = 2 cm, L = 2.5 cm). Spider mites, their eggs and web were kept on the discs for the two treatments with web, and were removed for the treatments without web using a soft brush. A first-instar larva of *F. occidentalis* was released on each leaf disc and the container was closed with a lid that allowed air exchange through a thin mesh covering an opening in its center (Ø = 1 cm). Every three days, a new group of plants with the respective treatments was used to prepare new leaf discs to replace the old ones. The stage of *F. occidentalis* was observed daily until reaching adulthood. Four replicates were done where each replicate was composed by a group of five plants (treatments), and 10 individuals of *F. occidentalis* were evaluated per plant. We averaged the developmental time and the proportion of juveniles that became adult per plant and analysed using a generalized linear model (GLM) with a Gaussian error distribution (R Development Core Team 2015).

Control of treatments

Extra plants were infested to verify whether plant defences had changed as a result of the treatments through measuring the oviposition rate of *T. evansi* as above. Plants infested with *T. urticae* are expected to have their defences induced and plants infested with *T. evansi* suppressed (Sarmento et al. 2011; Oliveira et al. 2015). Therefore, as a control of the effect of the treatments, plant defences were verified through evaluation of the performance of *T. evansi* (Sarmento et al. 2011) on extra plants that received the same treatments as in the experiments described above, “host plant choice” and “performance of *F. occidentalis*”. Hence, an herbivore assay was done on extra plants at the same time as the main experiments. Four days after plant infestation, leaf discs (Ø = 2.4 cm) were cut from the third and fourth leaves of each extra plant. The leaf discs were cleaned (removing mites, eggs and web) and placed in a Petri dish filled with cotton wool soaked in water. Subsequently, an adult female of *T. evansi* was released on each leaf disc. These females were taken from a cohort of two days old. To prepare this cohort, we transferred around 30 adult males and 50 tello-chrysalis of *T. evansi*, last quiescent stage before adult, from the rearing to tomato leaflets arranged on wet cotton on Petri dishes. The oviposition of *T. evansi* was evaluated daily for three days. We averaged the *T. evansi* oviposition rate per plant, and analysed these averages using a linear mixed effects model (function lme of the nlme package, Pinheiro et al. 2014), with replicate as a random factor. Contrasts were assessed as described above. All statistical analyses were performed with R (R Development Core Team 2013).

Results

Control of plant treatments

The plant treatments significantly affected oviposition of *T. evansi* (Fig. 1, LME: $\chi^2 = 11.33$, d.f. = 2, $P = 0.0035$). Oviposition was higher on uninfested plants and plants infested with *T. evansi* than on plants infested with *T. urticae*. However, there were no significant differences in the oviposition of *T. evansi* on uninfested plants and plants infested with *T. evansi*.

Host plant choice

When *F. occidentalis* had five options of host plant, they distributed themselves significantly different from random (Fig. 2, GLMER: $\chi^2 = 9.78$, d.f. = 4, $P = 0.044$). *F. occidentalis* was more often found on plants infested with *T. evansi* covered by web than without web. For all the other combination of plants, three options and two options of host plant, we did not observe significant differences on host plant preferences of *F. occidentalis*: plants infested with *T. evansi* with web, *T. urticae* with web and uninfested plants (Fig. 3, GLMER: $\chi^2 = 0.26$, d.f. = 2, $P = 0.88$); plants infested with *T. evansi* with or without web (Fig. 4, GLMER: $\chi^2 = 1.38$, d.f. = 1, $P = 0.24$); and plants infested with *T. urticae* with or without web (Fig. 4, GLMER: $\chi^2 = 46.47$, d.f. = 1, $P = 0.94$).

Performance of F. occidentalis

The juvenile developmental period and number of juveniles which active adulthood of thrips did not differ significantly on plants that had previously received different treatments with spider mites (Fig. 5, GLM: Deviance = 14.17, d.f. = 4, $P = 0.65$; Fig. 6, GLM: Dev = 0.40, d.f. = 4, $P = 0.94$).

Discussion

The plants we infested with *T. urticae* had their defences induced as we expected, but the plants infested by *T. evansi* did not showed signs of suppression: performance of *T. evansi* on these plants were not significant different from uninfested plants (Fig. 1). Although it is well known that *T. evansi* suppresses defences on tomato plants (Sarmento et al. 2011a; Oliveira et al 2015; Godinho et al 2015), in some cases, this suppression is not observed probably due to variability on heritable factors in the mites used to infest the plants (Knegt et al. unpublished data). Thus, we had here plants with defences induced by *T. urticae* and non-induced plants: uninfested plants and infested with *T. evansi*.

Our results showed that *F. occidentalis* was not affected by the presence of spider mites or induction of plant defences by *T. urticae*, but it was more attracted to plants with the web of *T. evansi*. The spider mite *T. urticae* induces Salicylic acid (SA) and JA defences pathways on tomato plants (Ozawa et al. 2000; Li et al. 2002; Kant et al. 2004; Sarmento et al. 2011a; Alba at al. 2015), which can also negatively affect other arthropods that feed on tomato including the thrips *F. occidentalis* (Thaler and Duffey 1996; Blauth et al. 1998; Abe et al. 2008; Abe et al. 2009). The spider mite *T. evansi* thereby, either suppress or do not induce these pathway (Sarmento et al. 2011a; Alba et al. 2015; Oliveira et al. 2015; Knegt et al. unpublished data). We then expected that *F. occidentalis* would be negatively affected by defences induced by *T. urticae* compared with non-induced plants. However, there was no significant difference on the performance and survival of juveniles of *F. occidentalis* between plants with defences induced by *T. urticae* and non-induced (Fig. 5, 6), which might also explain why they did not show preference between these plants (Fig. 2). This result suggests that the changes in plant defences due the infestation of plants with the spider mites did not affect thrips. It has been showed for other crops, like pepper and cotton, that when feeding on leaves from plants with defences induced by previous spider mite, thrips has reduced larvae development (Maharijaya et al. 2012) and reduced its natural abundance on plants compared with non-induced plants (Agrawal et al. 2000). However, most of the studies, in agreement with our findings, showed that induced plant defences did not affect the developmental time of thrips stages and particularly its population size (Trichillo and Leigh 1988; Soria and Mollema 1995; Agrawal and Klein 2000; Alabi et al. 2004; Maris et al. 2004).

Another important factor to be considered is that *F. occidentalis* does not only feed plant tissue but also feed on spider mites eggs. In some crops, thrips are considered important pest causing several damages (Parrella 1995; Kirk and Terry 2003; Reitz 2009; Reitz et al. 2011; Yang et al. 2015; Mouden et al 2016), but in others it is considered an important predator of spider mites eggs (Lincoln et al. 1953; Wilson et al. 1991). According with the foraging theory, omnivores are expected to balance their diet in order to fit nutritional needs (Coll and Ridgway 1995; Agrawal and Klein 2000; Eubanks and Denno 2000). Thrips have the strategy of feeding less on plant and more on non-plant food when plants are of bad quality (Trichilo and Leigh 1988; Wilson et al. 1996; Agrawal et al. 1999; Janssen et al. 2003; Magalhaes et al. 2005), such as plants with defences already induced by a previous herbivory (Agrawal et al. 1999; Omer et al. 2001).

The thrips *F. occidentalis* is negatively affected by the defences induced by herbivory (Thaler and Duffey 1996; Blauth et al. 1998; Abe et al. 2008; Abe et al. 2009). However, when it feeds on spider mites eggs thrips shows decreases in larval developmental time, and increases in larval survivorship and oviposition (Milne and Walter 1997). We however, did not find significant differences in the development time and survival of *F. occidentalis* among plants infested with spider mites and their web and uninfested plants. Likewise, *F. occidentalis* did not avoid plants with defence induced with or without spider mites and web. Although we did not measure the consumption of plant and spider mite eggs by thrips it is possible that *F. occidentalis* feed more on spider mite eggs than on plant tissue (Trichilo and Leigh 1988;

Wilson et al. 1996; Agrawal et al. 1999; Janssen et al. 2003; Magalhaes et al. 2005), since plants were induced and could reduce herbivores/omnivores performance (Thaler and Duffey 1996; Blauth et al. 1998; Abe et al. 2008; Abe et al. 2009).

We showed here that the spider mite web from *T. evansi* had an effect on thrips. The presence of web on plants previous infested with *T. evansi* affected the attractiveness, but not performance, of *F. occidentalis*. Thrips were more often found on plants infested with *T. evansi* with web than without web (Fig. 2), but their performance and juvenile survival did not differ significantly among these treatments (Fig. 5, 6). Tetranychidae are well known for covering their colonies with the web (Saito 1983). This web may (Hazan et al.1975; Linke 1953; Davis 1952; Yano 2008) serve as protection against natural enemies and prevent competitors from colonising the plant (Helle and Sabelis 1985; Gerson 1985; Sabelis and Bakker 1992). *T. evansi* stands out among the others Tetranychidae because its production of extremely dense web (Ferragut and Escudero 1999) that also prevents competing spider mites from attacking the plant and profiting from its downregulations of plant defences (Sarmento et al. 2011b). However, some arthropods can break, enter and move in the spider mites web using it in their favour as their own protection against predation (Gutierrez and Helle 1985; Sabelis and Bakker 1992; Roda et al. 2000; Montserrat et al. 2008; Shimoda et al. 2009; Lemos et al. 2015). This thrips species was reported to be able to enter and move into the spider mite web, which is used by their larvae stage as refuge to escape from their predators (Pallini, Janssen and Sabelis 1998; Venzon et al. 2000). Likewise, the thrips is also predator of mite eggs. So, being able to enter into the web it also gives them free access to this non-plant food. Probably, *F. occidentalis* can use also the web produced by *T. evansi* as protection, but further studies are necessary to investigate deeper how is the interactions between *F. occidentalis* and the web of *T. evansi.*

Omnivores are present in most of the natural communities; it is thereby essential to understand the complexities of their diet selection to comprehend their dynamic in the field (Polis at al. 1989; Polis 1991; Polis and Strong 1996; Polis and Winemiller 1996). In agroecosystems, they can be either beneficial, through their consumption of herbivores, or detrimental, through their consumption of plants (Rosenheim et al. 1995; Agrawal and Karban 1997; Coll and Ruberson 1998). Usually, the host plant quality is what determines the trophic role taken by omnivore insects like thrips (Agrawal et al. 1999; Agrawal and Klein 2000). From the plant perspective, the omnivore trade-off between feeding on the plant or on herbivore eggs has important fitness consequences. As strategy, some plants induced defensive responses which reduce their quality to omnivores forcing them to feed on herbivores (Agrawal et al. 1999). We, however, showed here that the omnivore was not affected by the presence of the spider mites on plants and by their interference on plant defences.

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Figure Legends

Figure 1 Mean (± SE) oviposition rate of *T. evansi* on uninfested plants and plants pre-infested with *T. evansi* or *T. urticae*. Different letters above bars indicate significant differences among treatments (Contrast after a LME, $P < 0.05$).

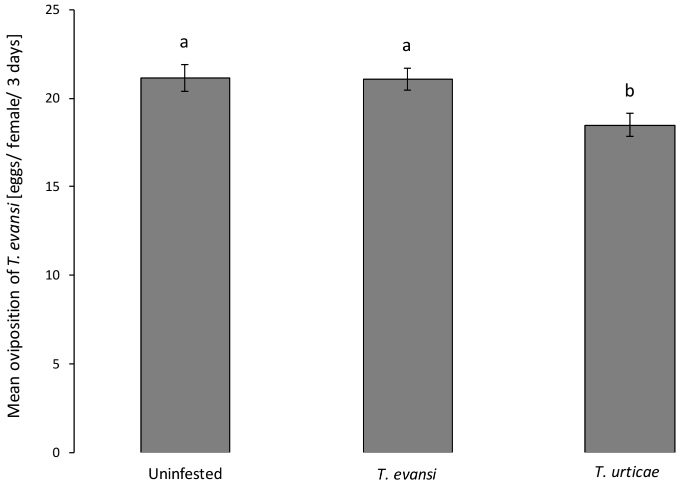
Figure 2 Proportion (± SE) of *F. occidentalis* recaptured on plants: infested with *T. evansi* with web, infested with *T. urticae* without web, uninfested, infested with *T. urticae* with web and infested with *T. evansi* without web. Letters above bars indicate significant differences among treatments (Contrast after a GLMER, $P > 0.05$).

Figure 3 Proportion (± SE) of *F. occidentalis* recaptured on plants: infested with *T. evansi* with web, uninfested, infested with *T. urticae* with web. Letters above bars indicate no significant differences among treatments (GLMER, $P > 0.05$).

Figure 4 Proportion (± SE) of *F. occidentalis* recaptured on plants. First bars show the *F. occidentalis* choices between a plant infested with *T. evansi* with web (grey bar) and a plant infested with *T. evansi* without web (white bar). Second bars show the choices of *F. occidentalis* between a plant infested with *T. urticae* with web (grey bar) and a plant infested with *T. urticae* without web (white bar). Letters above bars indicate no significant differences among treatments (GLMER, $P > 0.05$).

Figure 5 Mean (± SE) developmental time of *F. occidentalis* on plants: uninfested, infested with *T. evansi* with web, infested with *T. urticae* with web, infested with *T. evansi* without web and infested with *T. urticae* without web. Letters above bars indicate no significant differences among treatments (GLM, $P > 0.05$).

Figure 6 Proportion (± SE) of adults *F. occidentalis* on plants: uninfested, infested with *T. evansi* with web, infested with *T. urticae* with web, infested with *T. evansi* without web and infested with *T. urticae* without web. Letters above bars indicate no significant differences among treatments (GLM, $P > 0.05$).



Figures

Figure 1

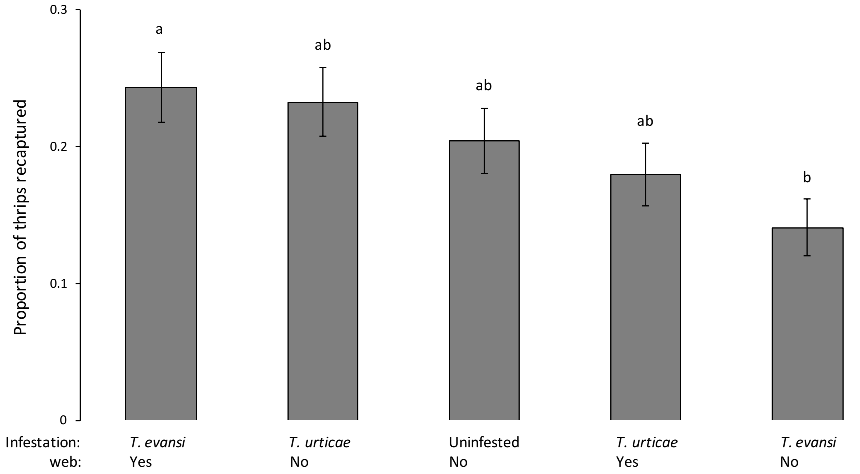

0.3
0.2
0.1
0
Proportion of thrips recaptured
a
ab
ab
ab
b
Infestation:
web:
T. evansi
Yes
T. urticae
No
Uninfested
No
T. urticae
Yes
T. evansi
No

Figure 2

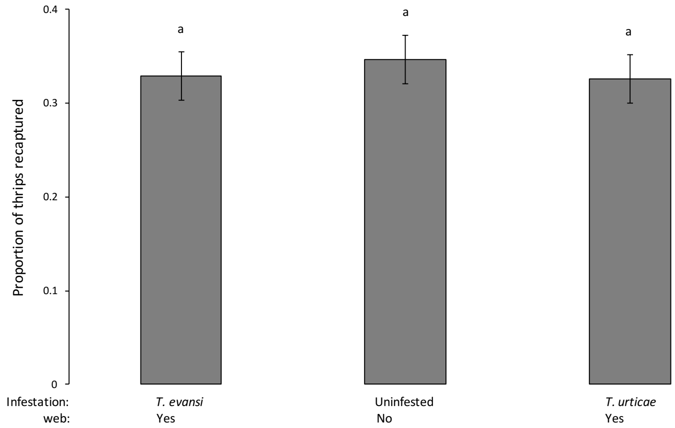

a
a
a
0.4
0.3
0.2
0.1
0
Proportion of thrips recaptured
Infestation:
web:
T. evansi
Yes
Uninfested
No
T. urticae
Yes

Figure 3

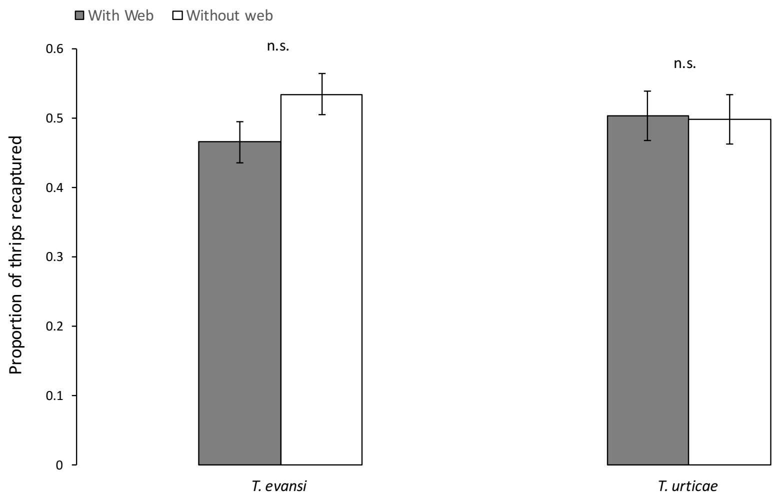

With Web
Without web
Proportion of thrips recaptured
0.6
0.5
0.4
0.3
0.2
0.1
0
n.s.
n.s.
T. evansi
T. urticae

Figure 4

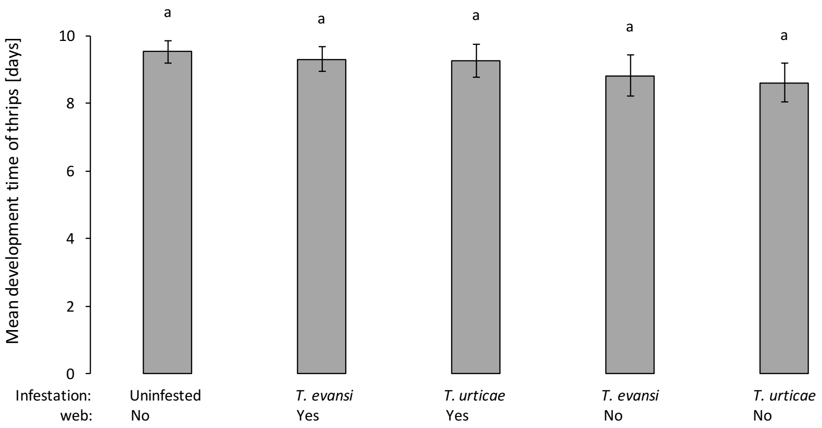

a
a
a
a
a
Mean development time of thrips [days]
0
2
4
6
8
10
Infestation:
web:
Uninfested
No
T. evansi
Yes
T. urticae
Yes
T. evansi
No
T. urticae
No

Figure 5

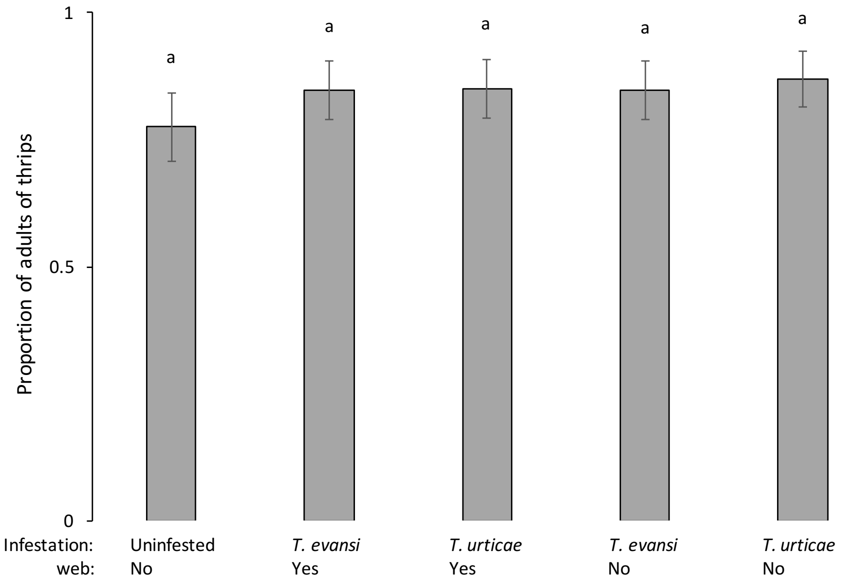

1
0.5
0
Proportion of adults of thrips
a
a
a
a
a
Infestation:
web:
Uninfested
No
T. evansi
Yes
T. urticae
Yes
T. evansi
No
T. urticae
No

Figure 6

GENERAL CONCLUSION

The type of arena and plant infestation affected the herbivore oviposition. *T. evansi* females evaluated on whole plants showed higher oviposition than those evaluated on detached leaf discs. However, the type of arena did not affect the herbivore responses to induced defences. On both arenas, *T. evansi* showed lower oviposition on plants infested with the defence-inducer *T. urticae* than on plants infested with the defence suppressor *T. evansi* and uninfested plants. These findings indicate that both whole plants and leaf discs can be used to evaluate the effects of tomato plant defences on the spider mite performance.

In field conditions, induced plant defences interfered on the host choice of herbivores but not of natural enemies. Herbivores responded to cues from their host plants and avoided potential unsuitable plants: infested plants covered with web and plants with defences induced. The presence of web in combination with plant defences also interfered on the herbivore host choice: plants with defence suppressed were more attractive to whiteflies in absence of web, but not when plants were covered by spider mite web.

Plant defences and spider mite web affected whiteflies host plant choice and performance. Whiteflies preferred plants where they had higher survival of juvenile and recently emerged adults, which was uninfested and plants with defence suppressed without web. *F. occidentalis* was not affected by the presence of the spider mites and by their changes in plant defences. Nevertheless, the presence of spider mite web on plants infested with *T. evansi* made these plants more attractive to *F. occidentalis*, but did not affect the performance and survive of its juveniles.