FELIPE DE LEMOS

SPIDER MITE WEB PROTECTS PREY AND PREDATOR ALIKE

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

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Aos familiares, que sempre me apoiaram!

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BIOGRAFIA

Felipe de Lemos, filho de Fátima Maria de Lemos e Adilson Pereira Nunes nasceu em 4 de fevereiro de 1985 em Itaguaí, Rio de Janeiro.

Em março de 2003 iniciou o curso de graduação em Agronomia pela Universidade Federal de Viçosa. Durante a graduação foi bolsista de Iniciação Científica sob orientação do Prof. Angelo Pallini. Em janeiro de 2009, Felipe graduou-se como Engenheiro Agrônomo pela mesma instituição.

Em Março de 2009 ingressou no curso de mestrado em Entomologia pela Universidade Federal de Viçosa também sob a orientação do Prof. Angelo Pallini, submetendo-se à defesa da dissertação em fevereiro de 2011.

SUMÁRIO

RESUMO

LEMOS, Felipe de, M.Sc., Universidade Federal de Viçosa, fevereiro de 2011. **Teia de ácaros fitófagos protege tanto a presa quanto o predador**. Orientador: Angelo Pallini Filho. Co-Orientadores: Madelaine Venzon e Arnoldus Rudolf Maria Janssen.

Em todo o mundo lavouras de tomate sofrem o ataque de pragas. Uma das principais pragas do tomate é o ácaro vermelho *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae). Uma característica marcante de *T. evansi* é a sua alta produção de teia sobre suas plantas hospedeiras. Essa teia pode afetar diretamente os inimigos naturais, reduzindo a sua eficiência de predação ou indiretamente alterando seu comportamento de busca. No entanto, alguns ácaros predadores da família Phytoseiidae podem ser bem adaptados para lidar com a teia dos ácaros fitófagos. Para selecionar agentes de controle biológico eficientes é importante compreender as interações diretas e indiretas entre os organismos envolvidos no agroecossistema em questão. Nesta dissertação, foi investigado o papel da teia produzida por *T. evansi* como mediadora de interações diretas e indiretas com seus inimigos naturais. No Capítulo 1 foram investigados os efeitos da teia produzida por *T. evansi* em suas interações com o ácaro predador *Phytoseiulus longipes* Evans (Acari: Phytoseiidae). Foi avaliado se fêmeas de *T. evansi* reconhecem sinais químicos do predador e se estes sinais podem induzir os ácaros a produzirem mais teia. Os resultados mostram que a presa não produz mais teia em resposta a estímulos de risco de predação, no entanto, passa a depositar uma maior parcela de seus ovos suspensos na teia, longe da superfície da folha. Esses ovos suspensos sofreram uma menor predação por *P. longipes* do que os ovos que se encontravam na superfície da folha. Entretanto a taxa de predação de *P. longipes* sobre ovos de *T. evansi* em discos com e sem teia não diferiram, indicando que esse predador não é afetado negativamente pela teia de sua presa. No segundo capítulo foi estudado se a teia produzida por *T. evansi* pode afetar o comportamento de forragemamento de *P. longipes* modulando a sua preferência entre

ambientes e presas. Estudou-se também se ocorre predação intraguilda entre *P. longipes* e outro ácaro predador *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae), que podem co-ocorrerem sobre as mesmas plantas. Observou-se que *P. longipes* prefere as metades de discos de folhas que apresentavam uma estrutura mais complexa (mesmo quando eles não tinham alimento) em relação às metades dos discos com ovos e sem teia de *T. evansi*. Esta preferência por ambientes de estrutura complexa pode ser explicada pela menor taxa de predação intraguilda de *P. macropilis* observada em discos de tomate com teia de ácaros. A presença da teia reduziu a capacidade predatória de *P. macropilis*. A diferença entre ambos os predadores para lidar com a teia de ácaros fitófagos pode ser explicado em parte pelas diferenças no comprimento das setas dorsais j3, z2 e z4, que são mais longas em *P. longipes*. Com relação à preferência de *P. longipes* por espécies de presas, observou-se que os ácaros predadores escolheram *T. evansi* a *T. urticae*, tanto em presença quanto ausência de teia. Esses resultados indicam que a teia de *T. evansi* pode mediar o comportamento de forrageamento de ácaros da família Phytoseiidae de acordo com seus níveis de adaptações morfológicas e comportamentais. Em linhas gerais, pode-se concluir que os ácaros predadores *P. longipes* são bem adaptados para lidar com a grande quantidade de teia produzida por *T. evansi*. Além de não evitar plantas com elevada quantidade de teia, esse ácaro predador pode se beneficiar desse ambiente complexo, reduzindo a competição com outros predadores. No entanto, a presença do predador estimula *T. evansi* a mudar seu comportamento de oviposição, reduzindo a eficiência predatória de *P. longipes*. Assim, ao alterar o seu comportamento de oviposição em resposta aos sinais dos predadores, as fêmeas de *T. evansi* utilizam mais eficientemente sua teia para protegerem sua prole.

ABSTRACT

LEMOS, Felipe de, M.Sc., Universidade Federal de Viçosa, February of 2011. **Spider mite web protects prey and predator alike**. Adviser: Angelo Pallini Filho. Co-advisers: Madelaine Venzon and Arnoldus Rudolf Maria Janssen.

Worldwide, tomato suffers from herbivory. One of the most important tomato pests is the red spider mite *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae). A striking characteristic of *T. evansi* is its high production of web over its host plants. The web can directly affect natural enemies by reducing its predation efficiency or indirectly by changing its foraging behaviour. However some Phytoseiidae can be well adapted to cope with spider mite webbing. To select efficient biologicol control agents, it is important to understand the direct and indirect interactions between the organims involved in the agroecosystem. In this dissertation, I investigated how the web produced by *T. evansi* mediates direct and indirect interactions with its natural enemies. In Chapter 1 I investigate the effects of the web produced by *T. evansi* on its interactions with the predatory mite *Phytoseiulus longipes* Evans. We tested whether spider mite females recognize predator cues and whether these can induce the spider mites to produce denser web. We found that the prey did not produce denser web in response to such cues, but laid more eggs suspended in the web, away from the leaf surface. These suspended eggs suffered less predation by *P. longipes* than eggs that were laid on the leaf surface, under the web. However the predation rate of *P. longipes* on eggs of *T. evansi* on discs with and without web was not different, indicating that this predatory mite is not negatively affected by the spider mite web. In the second Chapter I studied whether the complex web produced by spider mites could affect the foraging behaviour of *P. longipes* modulating its preference between patches and prey. Was also studied if there is intraguild predation between the predatory mites *P. longipes* and *Phytoseiulus macropilis* (Banks)

(Acari: Phytoseiidae), which can co-occur on the same plants. We observed that *P. longipes* always preferred the leaf discs halves with more complex environmental structure (even when there was no food on it) to discs half with eggs and no web of *T. evansi*. The strong preference for patches with complex structure could be explained by the reduced rate of intraguild predation by *P. macropilis* observed on tomato discs with spider mite web. As observed, the presence of spider mite web reduced the predatory efficiency of *P. macropilis* in intraguild predation experiment. The difference between both predatory mites to cope with the web of spider mites could be explained in part by the differences in length of the setae j3, z2 and z4 that are longer in *P. longipesi*, but is needed manipulation of these traits. With respect to the preference of *P. longipes* by prey species, we observed that the predator chose *T. evansi* to *T. urticae,* both in the presence and absence of web. These results indicate that spider mite web can mediate the foraging behaviour of phytoseiid mites according to their level of morphological and behavioral adaptation to webbing. In general, we conclude that the predatory mite *P. longipes* is well adapted to cope with the higher web densities produced by *T. evansi*. Besides not avoid thus plants with high amounts of web, *P. longipes* can benefit from this complex environment by reducing competition with others predators. However the presence of the predatory mite stimulates *T. evansi* to change its oviposition behaviour, reducing the predation efficiency of *P. longipes*. Thus, by altering their oviposition behaviour in response to predator cues, females of *T. evansi* make better use of their web to protect their offspring.

GENERAL INTRODUCTION

Worldwide, crops suffer from herbivory. With tomato it is not different. The second most important vegetable crop in the world (FAOSTAT 2011), it is highly attacked by numerous arthropod pests (Bolland et al. 1998; Gallo et al. 2002). Among these are the phytophagous mites, especially the two-spotted spider mite *Tetranychus urticae* Koch and the red spider mite *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) (Moraes and Flechtmann 2008). These two spider mite species are distributed over large agricultural areas where they may inhabit the same crops or even the same plants (Escudero and Ferragut 2005).

Mites that feed on plants are referred as phytophagous, rather than herbivorous (Krantz and Lindquist 1979). On feeding, the stylet of *Tetranychus* mites penetrates the abaxial epidermis, reaching the palisade layer of plant tissue (Moraes and Flechtmann 2008). As a result of feeding on the cell contents, spider mites destroy the chloroplasts in the area restricted to where feeding took place (Moraes and Flechtmann 2008). The destruction of plant chloroplasts promotes a reduction of plant photosynthesis activity and consequently a crop productivity reduction.

The red spider mite *T. evansi* is a highly destructive pest with high rates of population increase over a wide temperature range (Bonato 1999; Gotoh et al. 2010). It is one of the major pests of solanaceous plants, especially tomato (Ferragut and Escudero 1999) and it has been recorded in South and North America, Africa, Europe and Asia (Ramalho and Flechtmann 1979; Blair 1989; Ferragut and Escudero 1999; Bolland and Vala 2000; Ho et al. 2004; Tsagkarakou et al. 2007; Gotoh et al. 2010). The red spider mite-infested leaves become bronze-coloured, with intensity proportional to the degree of internal damage.

Compared to other *Tetranychus* species, a striking characteristic of *T. evansi* is its high production of web over its host plants (Ferragut and Escudero 1999). The web built by spider mites over their host plants have many functions such as attachment to the leaf, dispersion between plants by ballooning, protection against adverse climatic conditions, like rain and wind, provision of a microclimate environment and nest sanitation (Gerson 1985; Bell et al. 2005; Kanazawa et al. 2010). Spider mite web produced over colonies can also protect against their natural enemies (Helle and Sabelis 1985; Saito 1985; Venzon et al. 2009). The web can directly affect natural enemies by reducing their predation efficiency (Venzon et al. 2009) or indirectly changing their behaviour (Furuichi et al. 2005). Due to the silk of the web consisting of protein (Hazan et al. 1975), the production must be costly, and it can therefore be expected that mites will fine-tune web construction to variation in the environment. Despite the lack of evidence (but see Li and Lee 2004 for an elegant example with spiders), it is also conceivable that web production shows phenotypic plasticity, for example, when spider mites perceive the presence of predators.

Structures present on the plants are well known for positively affect the natural enemies abundance by protecting them from predation and parasitism (Agrawal et al. 2000, Matos et al. 2006). When weave web over its hosts, spider mites alters the normal architecture of tomato plants. By modifying their microhabitat, spider mites act as "ecosystem engineers" (Jones et al. 1997). Ecosystem engineers are organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials (Jones et al. 1994). This alteration in the environment can negatively affect natural enemies of spider mites. However, some predatory mites like species of the genus *Phytoseiulus* are known to be well adapted to cope with the silk web spun by the phytophagous mites (Sabelis and Bakker 1992). Some predators can use silken mat over the plant trichomes to improve their dispersal between spider mite-infested leaves (van Haren et al. 1987). Other organisms can also profit from the spider mite web. For instance, thrips and predatory mites can use it as protection against their predators (Pallini et al. 1998; Roda et al. 2000; Venzon et al. 2000).

Usually, the red spider mites are controlled with acaricides, which results in problems of pest resistance and residue on the harvested products (Escudero and Ferragut 2005). Unlike traditional pest control with pesticides, biological control emerges as an important and sustainable strategy (Hajek 2004). The predators are so far the most studied organisms due their important effect on the dynamics of prey through removal of individuals (Hajek 2004). Predators can also have non-lethal effects on prey through the induction of changes in prey behaviour (Sih 1980; Sih 1982; Kats and Dill 1998; Lima 1998; Lemos et al. 2010). For example, prey may reduce time and energy spent on mating and foraging in order to hide or escape when they perceive predators (Lima 1998).

For successful biological control program, it is important to understand the interactions between predator and prey within the overall food web context in agricultural crops (Janssen et al. 1998). With the increased use of biological control agents, artificial food webs are created in agricultural crops and the interactions between plants, herbivores and natural enemies change from simple tritrophic interactions to more complex food web interactions. Therefore, herbivore densities will not only be determined by direct predator–prey interactions and direct and indirect defence of plants against herbivores, but also by other direct and indirect interactions (Janssen et al. 1998). Ecological processes in a food web can be mediated by interactions between the organisms involved. These interactions, such as intraguild predation, predator avoidance and antipredator behaviour, prey preference and foraging, and apparent competition are important in structuring arthropods communities (van

Veen et al. 2006). Most of the time, it is hard to separate the individual effects of these interactions in a simple food chain, even more in a complex food web.

Phytophagous mites are attacked and fed upon by a diverse array of natural enemies including many relatively unspecialized groups such as insectivorous birds and mammals and web-building spiders, but also by guilds of more specialized natural enemies with narrower feeding preferences (van Veen et al. 2006). Many predatory mites were tested as biological control agents of *T. evansi*, but without great success (Escudero and Ferragut 2005). The inability of phytoseiids to develop adequately when feeding on *T. evansi* has already been demonstrated for different predatory mites (Moraes and Lima 1983; de Moraes and McMurtry 1985; Sarr et al. 2002; Oliveira et al. 2005, Vasconcelos et al. 2007). Other studies indicate that some Phytoseiidae mites are able to feed and complete their development on *T. evansi* but with a poor performance, suggesting inability to suppress *T. evansi* populations on commercial crops (Escudero and Ferragut 2005). Recently, the predatory mite *Phytoseiulus longipes* Evans (Acari: Phytoseiidae) was found associated with *T. evansi* in South Brazil (Furtado et al. 2006). Since then promising results have been observed with this species as a biological control agent of *T. evansi* (Badii et al. 1999; Furtado et al. 2007; Silva et al. 2010).

Species from the same trophic level may interact through competition for food, but in some cases also through intraguild predation (Polis et al. 1989). Intraguild predation can be defined as the killing and eating of species that otherwise use similar resource and are thus potential competitors (Polis et al. 1989; Janssen et al. 1998). However, as predation is an important process in the evolution of defense in prey organisms (Schmidt 1990), antipredator behaviour is expected to evolve. The high diversity and spread of defense mechanism among all the species is the results of the enormous selection pressure imposed on prey by their predators (Horita et al. 2003). To avoid predation, prey employ a large range of strategies, such as behavioural, morphological and life history changes (Havel 1987; Kats and Dill 1998; Tollrian and Harvell 1999; Montserrat et al. 2007; Chivers et al. 2008) that mediates both intraguild and *strictu sensu* predation. The first step in predator-induced changes in prey is the recognition of cues associated with predation risk. The ability of prey to distinguish high-risk predators from low-risk or harmless predators has important implications for the prey's reproductive success. Failing to recognize a predator is likely to increase the probability of capture during an encounter, and defensive responses to non-predators are likely to result in lost foraging and mating opportunities (Hirsch and Bolles 1980; Lima and Dill 1990; Chivers et al. 1996). Hence, it is important that prey accurately identify predator-related cues in order to assess the level of predation risk (Chivers et al. 1996).

In spider mites, the described anti-predator responses concern mainly the avoidance of patches with high predation risk (Grostal and Dicke 1999; Pallini et al. 1999; Magalhães et al. 2002; Choh and Takabayashi 2007), but recently attention has also been given to predator-induced diapause (Kroon et al. 2008) and reduction of oviposition (Oku et al. 2004; Skaloudova et al. 2007; Choh et al. 2010). Spider mites are also hypothesized to reduce predation risk through the production of a dense web (Sabelis and Bakker 1992; Horita et al. 2003; Oku et al. 2003; Shimoda et al. 2009).

Another important issue in food web studies is understanding the foraging and preference behaviour of natural enemies when faced with more than one prey specie on the same patch. Rarely a crop will be attacked by only one pest species. Usually, plants suffer of multiple infestations. Although the predatory mites of the genus *Phytoseiulus* are specialized in feeding on *Tetranychus* mites, it is expected that they do not prey indiscriminately but prefer some prey species over others (Dicke et al. 1988). When foraging on a leaf inhabited by a mixture of prey types, predatory mites have to decide which prey to accept and which to reject. The prey preference of predatory mites can be understood in terms of reproductive performance but also may have others causes (Dicke et al. 1988). Because food and habitat are closely linked for small herbivores that live on plants, food choice in the field may be constrained by the need to choose plants that provide less dangerous living sites (Duffy and Hay 1991). The ability to discriminate between prey is often achieved through learning; predators tend to favor profitable prey and to avoid harmful prey as a result of previous experiences with different prey types (Hansen et al. 2010). In predatory mites (due to blindness) the main prey feature used to learn about its quality as food are chemical cues. But they may also have innate prey preferences (Hansen et al. 2010).

In this dissertation, I investigated the role of web produced by *T. evansi* mediating direct and indirect interactions with its natural enemies. In chapter 1, it was studied how *T. evansi* can use its web against the predatory mite *P. longipes*. The importance of web as mechanism mediating antipredator behaviour was accessed. In the chapter 2, we focused on the foraging behaviour of *P. longipes* and the importance of spider mite web on its patches preference. A study of morphological characteristic based on chaetotaxy was also done to better discuss the specials skills of *P. longipes* in dealing with spider mite web.

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CHAPTER 1

Spider mite web mediates anti-predator behaviour †

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Abstract

Herbivores suffer significant mortality from predation and are therefore subject to natural selection on traits promoting predator avoidance and resistance. They can employ an array of strategies to reduce predation, ranging from changes in behaviour, morphology and life history. So far, the anti-predator response studied most intensively in spider mites has been the avoidance of patches with high predation risk. Less attention has been given to the dense web produced by spider mites, which is a complex structure of silken threads that is thought to hinder predators. Here, we investigate the effects of the web produced by the red spider mite *Tetranychus evansi* Baker & Pritchard on its interactions with the predatory mite *Phytoseiulus longipes* Evans. We tested whether female spider mites recognize predator cues and whether these can induce the spider mites to produce denser web. We found that the prey did not produce denser web in response to such cues, but laid more eggs suspended in the web, away from the leaf surface. These suspended eggs suffered less from predation by *P. longipes* than eggs that were laid on the leaf surface under the web. Thus, by altering their oviposition behaviour in response to predator cues, females of *T. evansi* protect their offspring.

Keywords: predation risk, oviposition behaviour, *Tetranychus evansi*, *Phytoseiulus longipes*, tomato, ecosystem engineers

Introduction

Predation is one of the main factors determining the distribution and dynamics of prey populations. It has an effect on the dynamics of prey through removal of individuals. Predators can also have non-lethal effects on prey through the induction of changes in the prey (Sih 1980, 1982; Kats and Dill 1998; Lima 1998). To avoid predation, prey employ a large range of strategies, such as behavioural, morphological and life history changes (Havel 1987; Kats and Dill 1998; Tollrian and Harvell 1999; Montserrat et al. 2007; Chivers et al. 2008). However, these changes come with a cost. For example, prey may reduce time and energy spent on mating and foraging in order to hide or escape when they perceive predators (Lima 1998). Since these responses will reduce prey reproduction in one way or another, predators also exert non-lethal effects on prey, thus also affecting their distribution and dynamics.

The first step in predator-induced changes in prey is the recognition of cues associated with predation risk. Prey are known to use auditory, visual and chemical senses to perceive cues from predators or predator products (Janssen et al. 1998; Pallini et al. 1998; Grostal and Dicke 1999; Venzon et al. 2000; Stumpner and von Helversen 2001; Agarwala et al. 2003; Nomikou et al. 2003; de Bruijn et al. 2006; Lohrey et al. 2009). The ability of prey to distinguish high-risk predators from low-risk or harmless predators has important implications for the prey’s reproductive success. Failing to recognize a predator is likely to increase the probability of capture during an encounter, and defensive responses to non-predators are likely to result in lost foraging and mating opportunities (Hirsch and Bolles 1980; Lima and Dill 1990; Chivers et al. 1996). Hence, it is important that prey accurately identify predator-related cues in order to assess the level of predation risk (Chivers et al. 1996).

In spider mites, the anti-predator responses described thus far mainly concern the avoidance of patches with high predation risk (Grostal and Dicke 1999; Pallini et al. 1999; Magalhães et al. 2002; Choh and Takabayashi 2007), but recently attention has also been given to predator-induced diapause (Kroon et al. 2008) and reduction of oviposition (Oku et al. 2004; Skaloudova et al. 2007; Choh et al. 2010). Spider mites are also hypothesized to reduce predation risk through the production of a dense web (Sabelis and Bakker 1992; Oku et al. 2003; Horita et al. 2004; Shimoda et al. 2009). Moreover, the web has been hypothesized to serve various other purposes, such as attachment to the leaf and protection against adverse climatic conditions (Gerson 1985). Indeed, by modifying their microhabitat, spider mites act as "ecosystem engineers" (Jones et al. 1997). Other organisms can also profit from the spider mite’s web; thrips and predatory mites can also use it as protection against their predators (Pallini et al. 1998; Roda et al. 2000; Venzon et al. 2000).

Little is known of the within-species variation in the production of web. Because the silk of the web consists of protein (Hazan et al. 1975), the production must be costly, and it can therefore be expected that mites will fine-tune web construction to variation in the environment. Indeed, natural populations of *Tetranychus urticae* harbour genetic variation in web production (Tien et al. 2009). It is also conceivable that web production shows phenotypic variation, for example, spider mites may start producing denser web when perceiving the presence of predators. In this article, we investigate this, using the red spider mite *Tetranychus evansi* Baker & Pritchard and the predatory mite *Phytoseiulus longipes* Evans.

Compared to other *Tetranychus* species, the red spider mite is a highly destructive crop pest, with high rates of population increase over a wide temperature range (Bonato 1999). It is one of the major pests of solanaceous plants, especially tomato (Ferragut and Escudero 1999) and has been recorded in South and North America, Africa, Spain, Portugal, Greece and Taiwan (Ramalho and Flechtmann 1979; Blair 1989; Ferragut and Escudero 1999; Bolland and Vala 2000; Ho et al. 2004; Tsagkarakou et al. 2007). A striking characteristic of *T. evansi* is its high production of web compared with other *Tetranychus* species (Ferragut and Escudero 1999).

Recently, the predatory mite *Phytoseiulus longipes* was found associated with *T. evansi* in South Brazil (Furtado et al. 2006). It shows potential as a biological control agent (Furtado et al. 2007). However, for a successful biological control program, it is important to understand the interactions between predator and prey within the overall food web context in agricultural crops (Janssen et al. 1998). The non-lethal effects of predators on prey might be an important component of predator–prey interactions in food webs because they can be as large as lethal effects (Schmitz 1998).

Here, the objective is to understand how the web of *T. evansi* mediates predator-prey interactions. To this end, we tested whether the spider mites recognize cues of predation and whether these cues induce them to produce more web or to change other aspects of their behaviour. Increased web production might impede effective control of the spider mites by predators. We subsequently studied the effect of these changes in behaviour of the red spider mite on predation of eggs by the predatory mite.

Material and Methods

Tomato plants (*Solanum lycopersicum* var. Santa Clara I-5300) were weekly sown in a commercial plant substrate (Bioplant®, Bioplant Misturadora Agrícola LTDA, Nova Ponte, MG) in a polystyrene tray (8 x 16 cells) in a greenhouse. Trays with seedlings were kept inside a cage with a fine mesh to avoid contamination with herbivores. Upon reaching twenty days of age, we transplanted the seedlings to pots (5L) with a mixture of soil, bovine manure and mineral fertilizer (NPK 04-14-08) in larger mesh-covered cages in a greenhouse.

A population of *T. evansi* was obtained in 2002 from a natural infestation of tomato plants in a greenhouse in Viçosa, Minas Gerais, Brazil. The spider mites were reared on tomato leaves with their petiole inserted in a PVC tube filled with water to prevent desiccation of the leaves. Tubes with infested leaves were kept in PVC trays filled with detergent and water (1:25, v/v), which served to prevent mite escapes and invasion of mites and other non-flying arthropods. The mass culture was maintained in a room (25 ± 3 ºC, 70 – 90 % relative humidity) with controlled photoperiod (12:12 L:D).

The laboratory culture of the predatory mite *P. longipes* was started with individuals that were kindly sent by Dr Gilberto de Moraes from the University of São Paulo, Brazil. Colonies were maintained in a climate cabinet (28 ± 2 ºC, 80 ± 10 % relative humidity), fed daily with *T. evansi* on tomato leaves from the spider mite culture. All experiments were carried out in a climate cabinet (25 ± 1 ºC, 80 ± 10 % relative humidity).

Effect of predator cues on oviposition and web production

Small discs (Ø = 1.2 cm) were cut from tomato leaves and were exposed to predators by releasing 10 individuals of *P. longipes* per disc. Control leaf discs were cut at the same time from the same plant to exclude effects of plant quality and did not receive predators. Ten leaf discs were arranged in a regular pattern on wet cotton wool in a Petri dish. After 24 h, we gently removed all predatory mites and their eggs from the discs, leaving only other cues from the predators. A fine brush was used for manipulations of the mites, which was cleaned in water after removing the predators. Using a thin brush, one female of a cohort of *T. evansi*, aged 3 – 5 days since turning adult, was placed on each disc. After 48 hours we measured oviposition and web production. We counted the number of prey eggs both suspended in the web and on the leaf surface under the web.

Subsequently, web density was quantified with a technique adapted from Sabelis (1981). We collected a small sample of red clay soil (oxisol) that was dried in an incubator. The dried soil was subsequently run through a series of sieves and the fraction of particles with a diameter of 0.177 mm was stored for further use. To quantify the web density, we sprinkled soil particles over a leaf disc with web, using a fine brush. The red colour of the soil particles contrasts well with the green leaf tissue. The particles were light enough to remain attached to the sticky spider mite silk. Some particles would adhere to the silk, whereas others would drop through the web of silken strands and land on the surface of the leaf disc. With increasing web density, more particles remain suspended in the web (R. Sarmento, A. Janssen, pers. obs). We therefore defined web density as the percentage of particles that was found suspended in the web. Particles were counted and their position (in the web or on the leaf) scored using a stereo microscope.

The experiment was done in two blocks, one with 30 and one with 20 females of control and treatment each. Data from spider mites that had died during the experiment were discarded (26 % of all mites). This resulted in 22 and 17 replicates for clean leaf discs and 23 and 12 replicates for leaf discs with predator cues for the two blocks respectively. There was no difference in this mortality due to treatment (Generalized Linear Model with binomial error distribution: deviance = 0.834, d.f. = 1, P = 0.361).

Effect of web and position of prey eggs on their survival

As will be shown in the Results section, spider mites laid more eggs suspended in the web when exposed to predator cues than in the absence of such cues. This experiment served to study predation of eggs being positioned in the web or on the leaf surface. We treated leaf discs with predators prior to allowing spider mites to produce web and oviposit to obtain leaf discs with spider mite eggs suspended in the web. In an attempt to increase the proportion of eggs suspended in the web, we used larger leaf discs (Ø = 1.7 cm), more predators and a longer exposure of discs to predators (48 h) than in the previous experiment. Thirty leaf discs were first exposed to 15 adult female predators. Because the discs did not contain food for the predators, many of them escaped from the disc. We therefore added 15 more individuals on the second day. This was to ensure that leaf discs would contain ample amounts of cues of predatory mites. As a control, discs without predator cues were used (44 replicates). After 2 days, the predators were removed as above, and the discs without (control) or with predator cues were infested with 15 females of *T. evansi* for 3 days. As in the previous experiment, the effect of predator cues on oviposition behaviour of the spider mite was assessed by counting the number of prey eggs on the leaf surface and suspended in the web. Web density was not quantified because of its possible interference with the behaviour of the predators that were subsequently released on the leaf discs.

On all leaf discs, the adult spider mites were killed with a fine insect needle, but the eggs and web were left intact. The web was subsequently removed from 20 of the 44 control discs without predator cues. The removal of the web will have resulted in the removal of a few eggs that were laid suspended in the web, but in the absence of predator cues, the large majority of eggs was positioned on the leaf surface (see Results section). The web on the discs with predator cues was left intact. This resulted in three treatments: (1) leaf discs with prey eggs but without web and without predator cues (controls without web, N = 20), (2) discs with prey eggs, predominantly on the leaf surface, and with web but without predator cues (controls with web, N = 24), and (3) discs with predator cues and with prey eggs both on the leaf surface and suspended in the web (predator treatment, N = 30). A young adult, mated female predatory mite was released on each disc and the remaining prey eggs and the eggs laid by the predator on both leaf surface and suspended in the web were counted after 24 hours. The experiment was done in 3 blocks through time: once with 12 replicates of each treatment and control; once with 6 replicates of the predator treatment and the control with web and 5 of the control without web; and a third time with 12 replicates of the predator treatment, 6 of the control with web ad 3 of the control without web.

Statistical analysis

We observed that web density and oviposition varied considerably through time (F. Lemos and R.A. Sarmento, pers. obs.). Because experiments were done in blocks through time, with each treatment in each block, we used linear mixed effects models (LMER, library lme4, R-Development-Core-Team 2006), with block as random variable. For quantification of web production, we used the numbers of soil particles on the disc surface and in the web as dependent variable and a quasibinomial error distribution to correct for overdispersion (Crawley 2007). Total numbers of spider mite eggs on discs with cues of predators and clean discs were compared with a LMER with a quasi-Poisson error distribution to correct for overdispersion. The distribution of data on eggs of *T. evansi* on the leaf surface or suspended in the web was strongly non-normal due to zero inflation, even after transformations; we therefore used the more conservative nonparametric Wilcoxon rank sum test to compare the fraction of eggs suspended in the web. Predation rates and oviposition rates of *P. longipes* were also compared with LMER with quasi-binomial and quasi-Poisson errors respectively. Models were simplified by removing non-significant interaction terms and factors, until a minimal adequate model was obtained (Crawley 2007). All the analyses were done using the statistical software R 2.6.2 (R Development Core Team 2006) and the models were checked for heteroscedasticity and normality of errors according to Crawley (2007).

Results

Effect of predator cues on oviposition and web production

Predator cues did not significantly affect web production by *T. evansi* (LMER: $Chi^2 = 1.82$, d.f. = 1, P = 0.18). Overall, 24.2 ± 1.34 % of the particles was found suspended in the web on leaf discs without predator cues and 26.1 ± 1.50 % was in the web in the presence of predator cues.

The oviposition of red spider mite females was not significantly affected by the presence of predator cues (Figure 1, LMER: $Chi^2 = 2.66$, d.f. = 1, P = 0.10). However, the spider mites did lay more eggs suspended in the web on discs with predator cues (Figure 1, Wilcoxon rank-sum test: W = 214.5, $P < 0.0001$). In the absence of predator cues, all eggs were laid on the leaf disc surface, whereas 29.2 ± 5.50 % of the eggs were found suspended in the web in the presence of predator cues. Because there were no predators but only predator cues present when the spider mites were allowed to oviposit and construct web, we conclude that predator cues changed the oviposition behaviour of *T. evansi*.

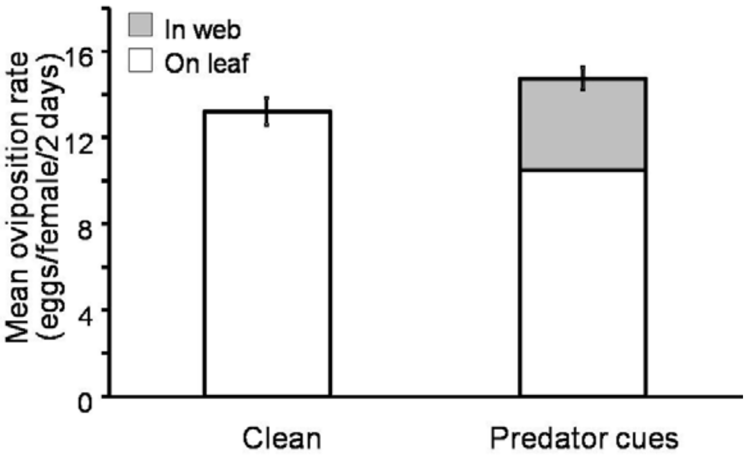

Figure 1. Mean oviposition rates of single *T. evansi* females during two days on leaf discs that were either clean or contained cues of the predatory mite *P. longipes*. White parts of the bars show the number of eggs on the leaf surface, grey parts show the eggs that were suspended in the web. Error bars show the S.E.M. of the mean total number of eggs (on the leaf surface plus in the web).

Effect of web and position of prey eggs on predation

To assess the effects of the changed oviposition behaviour of *T. evansi* as described above, we compared egg predation on discs with all eggs on the disc surface with that on discs with a proportion of the eggs suspended in the web. In order to obtain leaf discs with suspended eggs, we repeated the above experiment with slightly larger leaf discs and more predator cues. Again, we found more eggs suspended in the web when predator cues were present than on clean leaf discs (Figure 2, Wilcoxon rank-sum test: W = 19, $P < 0.0001$). On average, 36.1 ± 4.86 % of the eggs were laid in the web on discs with predator cues while 0.71 ± 0.27 % was found in the web on clean discs. We now found significantly lower oviposition rates in the presence of predator cues (Figure 2, LMER: $Chi^2 = 45.5$, d.f. = 1, $P < 0.0001$).

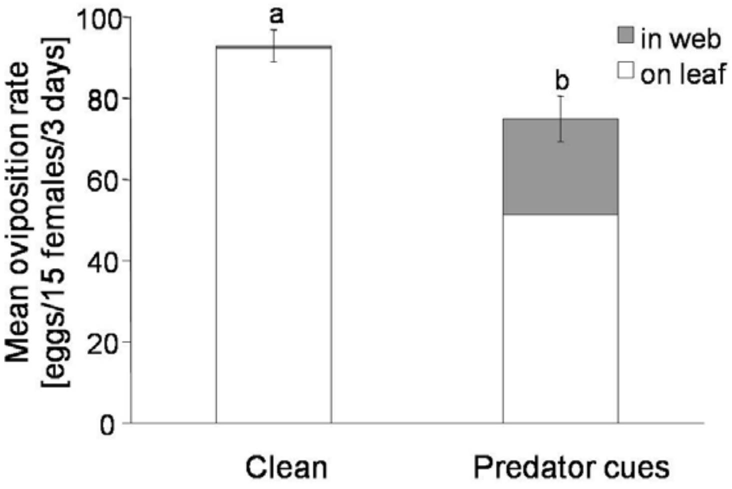

Figure 2. Mean oviposition rates of groups of 15 adult females of *T. evansi* either on clean tomato leaf discs or on discs with cues of the predatory mite *P. longipes*. White parts of the bars show the number of eggs on the leaf surface, grey parts show the eggs that were suspended in the web. Error bars show the S.E.M. of the mean total number of eggs (on the leaf surface plus in the web). Different letters above the vertical bars denote a significant difference in total numbers of eggs between the treatments ($P < 0.05$).

Predation by *P. longipes* was not affected by the presence of web or cues of conspecifics (Figure 3, LMER: $\text{Chi}^2 = 2.06$, d.f. = 2, $P = 0.36$), showing that the lower density of prey eggs in the treatment with predator cues (Figure 2) did not affect predation. We subsequently analyzed the predation rate of eggs of *T. evansi* on the leaf surface and in the web in the treatment with predator cues only (the other two treatments had too few eggs in the web). The overall percentage of suspended eggs that was killed was 32.8 ± 4.3 %, whereas 50.2 ± 3.4 % of all eggs on the leaf surface were consumed, and this difference was highly significant (LMER: $\text{Chi}^2 = 35.3$, d.f. = 1, $P < 0.0001$). Hence, depositing eggs suspended in the web, away from the leaf surface, reduces the risk of predation.

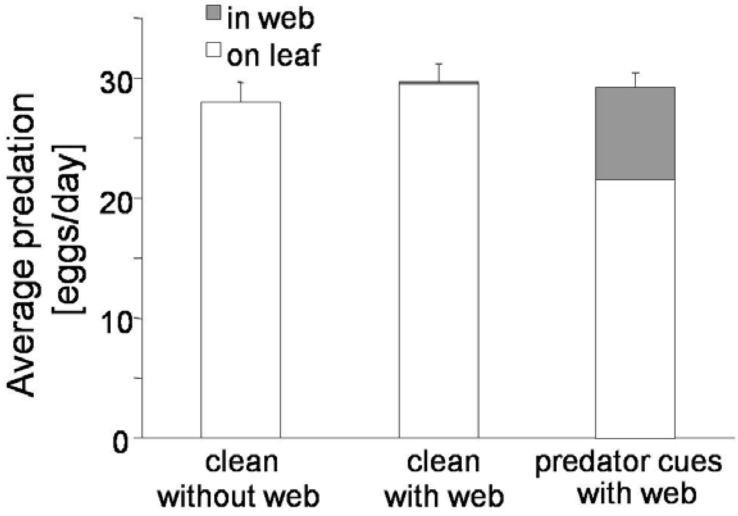

Figure 3. Mean predation rate by *P. longipes* on eggs of the spider mite *T. evansi* in the presence of predator cues and web produced by the spider mite or in the absence of predator cues (clean) with or without web. White bars show predation of eggs from the leaf surface, grey bars predation of eggs that were suspended in the web. Error bars indicate standard errors of the mean total predation (in web plus on leaf).

Discussion

We found no effect of the presence of predator cues on the density of web produced by females of *T. evansi*. Using the same method of estimating web density, preliminary experiments showed that the production of web by this spider mite is flexible, depending on the ecological setting (R.A. Sarmento, pers. obs.). However, spider mite web in general does not protect against all species of predator. For example, the predatory mite *Phytoseiulus persimilis* can cope with the silken web produced by its prey, the two-spotted spider mite *T. urticae*. Adaptations of predatory mites to web of their prey are hypothesized to be linked to the morphology and the pattern of the mid-dorsal and lateral setae of the predators (Sabelis and Bakker 1992), and to their ability to cut silken strands of the web (Shimoda et al. 2009). Because both predation rates and oviposition rates of *P. longipes* were not reduced by the web of *T. evansi*, we suggest that this predator has evolved to counter this defence of its prey, and is a potential candidate for biological control of this upcoming pest. This is in agreement with observations by Furtado et al. (2006), who state that the movement of *P. longipes* was not impeded on tomato leaves covered with the web of *T. evansi*. An increase in web production would perhaps not result in decreased predation by *P. longipes*. It is still possible, therefore, that cues of predator species that are hindered by the web would induce an increase in web production. This clearly requires further research.

Predator cues did elicit another behavioural response in the prey, i.e. the spider mites oviposited more frequently on the silken strands of the web, away from the leaf surface. This indicates that they perceived the predator cues. The apparent change in oviposition behaviour resulted in a lower risk of predation on these eggs. Hence, *T. evansi* preferentially oviposits on the leaf surface, and alters this behaviour only when perceiving predator cues. This can be understood given the life style of spider mites: they feed on the leaf parenchyma (Helle and Sabelis 1985), and usually oviposit on the leaf surface. Ovipositing away from their food source would probably involve costs in terms of time and energy spent by the females in moving from the leaf surface into the web. Moreover, larvae that emerge suspended in the web need to find the leaf surface. All these costs may go at the expense of fitness-related components such as egg production, development and survival. Oku et al. (2003) found a similar use of web by spider mites; quiescent stages of the spider mite *T. kanzawai* had a reduced predation risk on top of their web than under it, but had a lower probability of being guarded by males, and therefore possibly a lower probability of mating.

We found lower oviposition of the spider mites on leaf discs with predator cues in the experiment on the effect of web and position of prey eggs on predation (Figure 2), but not in the experiment on the effect of predator cues (Figure 1). This may be due to the higher concentration of predator cues in the second experiment and the longer period over which oviposition was measured (3 days vs. 2 days in the first experiment). The reduced oviposition is another indirect effect of predators on the prey (Oku et al. 2004), and may result in lower population growth rates, thus further contributing to the suppression of populations of this pest.

It is unclear why the spider mites laid only around 30% of their eggs in the web and not all of them. Perhaps the concentration of predator cues or the physical presence of a predator determines the extent of the behavioural change. Alternatively, the effect of suspending eggs in the web may be frequency dependent; possibly, the predators would concentrate their searching in the web if all the eggs were suspended. The eggs that were laid on the leaf surface may act to distract the attention of the predator, thereby rendering the suspended eggs relatively safe. If so, there should be an optimum oviposition strategy where spider mites balance predation risk of eggs on and away from the leaf surface against the costs of laying eggs suspended in the web.

The effects of predator presence or predator cues on behaviour of prey are well documented for a diverse array of vertebrate and invertebrate prey (Lima and Dill 1990; Lima and Bednekoff 1999). Such adaptive responses include morphological changes, life history shifts and behavioural responses (Havel 1987; Lima and Dill 1990; Kats and Dill 1998; Tollrian and Harvell 1999); these last two being reported more frequently. It is known that spider mites can use chemical cues to recognize and avoid patches with predation risk (Grostal and Dicke 1999; Pallini et al. 1999). Here we show that such cues can also induce another type of antipredator behaviour, *i.e.* a change in the site of oviposition within patches.

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CHAPTER 2

Spider mite web mediates preference behaviour of the predatory mite *Phytoseiulus longipes* Evans

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Abstract

Structures present on plants are well known to positively affect natural enemy abundance through protection from predation and parasitism. On the other hand, some structures as spider mite web are expected to be a mechanism of prey defence against predators. The red spider mite *Tetranychus evansi* Baker & Pritchard produces a high amount of web which seems to protect it against predators. Nevertheless, some phytoseiid predators can be well adapted to cope with spider mite web. The objective of this work was to understand whether the complex web produced by spider mites could affect the foraging behaviour of the predatory mite *Phytoseiulus longipes* Evans modulating its preference between patches and prey. In a first experiment series, tomato leaf discs were divided by the central midrib in two treatments. It was offered to adult females of *P. longipes* the choice between (i) eggs of *T. evansi* without web *versus* eggs and web of *T. evansi*; (ii) eggs of *T.*

evansi without web *versus* web of *T. evansi* without eggs; and (iii) eggs of *T. evansi versus* eggs of *T. evansi* and cotton fibers. After that we evaluated the intraguild predation of *Phytoseiulus macropilis* (Banks) (on leaf discs with and without web of either of *T. evansi* or *T. urticae*) on eggs and juveniles of *P. longipes*. Subsequently, we measured the length of dorsal shield setae of both predatory mites aiming to correlate their ability to cope with spider mite web with the chaetotaxy. In the final experiment, we observed the preference of adult females of *P. longipes* for *T. evansi* or *T. urticae* in the presence and absence of its web. We observed that *P. longipes* always preferred leaf discs halves with more complex structure even when they did not consume any food. This strong preference for patches with complex structure could be explained by the reduced rate of intraguild predation by *P. macropilis* observed on tomato discs with web of both *T. evansi* and *T. urticae*. The difference between both predatory mites to cope with the web of spider mites could be explained in part by the differences in length of the setae j3, z2 and z4 that are longer in *P. longipes*. With respect to preference of *P. longipes* for prey species, we observed that the predatory mites chose *T. evansi* to *T. urticae,* both in presence and absence of web. The results indicate that spider mite web can mediate the foraging behaviour of phytoseiid mites according to their level of morphological and behavioral adaptation to webbing.

Introduction

Structures present on the plants are well known to positively affect natural enemy abundance, protecting them from predation and parasitism (Agrawal et al. 2000, Matos et al. 2006). On the other hand, some structures as web produced by spider mites over its host plants are expected to be a defence mechanism against predation (Helle and Sabelis 1985; Saito 1985). This defence can be functional, affecting the predator’s performance (Venzon et al. 2009) or behavioral, affecting the preference of the predatory mites (Furuichi et al. 2005). However, predators of the genus *Phytoseiulus* can be well adapted to cope with spider mite web being less or not hampered (Sabelis and Bakker 1992). It has been hypothesized that the ability of predatory mites to cope with the web produced by spider mites is correlated with their chaetotaxy (Sabelis and Bakker 1992). The long dorsal setae can act as a pantograph, allowing the predator to walk through the sticky silken threads (Shimoda et al. 2009). Besides acting as antipredation mechanism, the web serves to various other purposes such as attaching to the leaf, protecting against adverse climatic conditions, as cue to males find females to mate, dispersion between plants by ballooning and nest sanitation (Bell et al. 2005; Gerson 1985; Kanazawa et al. 2010). Others organisms can also profit from environmental changes promoted by the spider mite web. For instance, thrips and predatory mites can use it as protection against their predators (Pallini et al. 1998; Roda et al. 2000; Venzon et al. 2000).

Little is known of the within-species variation in the production of web. Because the silk of the web consists of protein (Hazan et al. 1975), the production must be costly and it can therefore be expected that mites will fine-tune web construction to variation in the environment. Indeed, natural populations of *Tetranychus urticae* Koch (Acari: Tetranychidae) harbor genetic variation in web production (Tien et al. 2009). It is also conceivable that web production shows phenotypic variation, for example, spider mites may start producing denser web when perceiving the presence of predators. With *Schizotetranychus recki* Ehara inhabiting the hairy leaves of a dwarf bamboo, *Sasa senanensis*, all quiescent stages, including eggs, appear within web boxes in the leaf hairs of their host plant. This habit is thought to be a trait involved with predator avoidance considering that five predatory mites species are unable to penetrate the web box of *S. recki* (Horita et al. 2003).

Compared to other *Tetranychus* species, the red spider mite *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) is a highly destructive crop pest with high rates of population increase over a wide temperature range (Bonato 1999; Gotoh et al. 2010). It is one of the major pests of solanaceous plants, especially tomato (Ferragut and Escudero 1999) and it has been recorded in South and North America, Africa, Spain, Portugal, Greece, Taiwan and Japan (Blair 1989; Bolland and Vala 2000; Ferragut and Escudero 1999; Gotoh et al. 2010; Ho et al. 2004; Ramalho and Flechtmann 1979; Tsagkarakou et al. 2007). The red spider mite is distributed over large areas where they may share the same crops or even the same plants with other spider mites such as *T. urticae* (Escudero and Ferragut 2005). Another striking characteristic of *T. evansi* is its high production of web compared with other *Tetranychus* species (Ferragut and Escudero 1999).

Traditionally, red spider mites have been controlled with acaricides, causing pest resistance and leaving residues in the environment and on agricultural products (Escudero and Ferragut 2005). Many predatory mites were tested as biological control agents of *T. evansi*, but without great success. Recent attempts to control the pest through mass releases of predatory mites, mainly *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), on tomatoes in some locations of the Mediterranean coast and the Canary Islands, have been unsuccessful (Escudero and Ferragut 2005). Studies indicate that predatory mites as *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) and *P. persimilis* are able to feed and complete their development on *T. evansi* but with a low performance compared with others spider mites (Escudero and Ferragut 2005). This result indicates a poor ability of the two tested predators (*N. californicus* and *P. persimilis*) to suppress *T. evansi* populations on commercial crops (Escudero and Ferragut 2005). The inability of phytoseiids to develop adequately when feeding on *T. evansi* has already been demonstrated for *Euseius concordis*

(Chant) (Acari: Phytoseiidae) by Moraes and Lima (1983) and by eight predatory mite species, including *P. persimilis, N. californicus* and *Phytoseiulus longipes* Evans (Acari: Phytoseiidae), by de Moraes and McMurtry (1985). The low capacity to feed and develop on *T. evansi* was also observed by *Phytoseiulus fragariae* Denmark & Schicha (Acari: Phytoseiidae) (Vasconcelos et al. 2008). These studies revealed that none of the tested phytoseiid species were effective predators of the tomato red spider mite. In all cases the oviposition and survival rates of predatory mites were low on this prey.

A strain of the predatory mite *P. longipes* was found associated with *T. evansi* in South Brazil (Furtado et al. 2006). It has shown potential as a biological control agent of *T. evansi* (Badii et al. 1999; Furtado et al. 2007; Silva et al. 2010). Moreover *P. longipes* can walk very well in web of *T. evansi* (Furtado et al. 2007) and its predation rate is not negatively affected by webbing of this spider mites (Lemos et al. 2010). However, for a successful biological control program, it is important to understand the interactions between predators and prey within the overall food web context in agricultural crops (Janssen et al. 1998). Interactions such as intraguild predation can reduce the efficiency of the biological control program. Moreover, it is important to access the foraging behaviour and prey preference of natural enemies when confronted with multiple pest infestation.

During foraging, the pedipalps of *Phytoseiulus* mites are in frequent contact with the substrate. In this way, the predator may perceive prey cues deposited on the substrate, such as feaces, silk, exuviae and other residues. Spider mite silk is a very important cue for *P. persimilis* to find its prey, *T. urticae* (Schmidt 1976). The mechanism that explains this perception is still unclear. In *P. persimilis,* the peripheral olfactory system consists of olfactory sensilla on the tarsus of the first pair of legs (Akkerhuis et al. 1985; van Wijk et al. 2006). The pedipalps of *P. persimilis* also harbour sensille but these are more likely involved in gustation (Akkerhuis et al. 1985; van Wijk et al. 2006). Probably the receptors on these sensilla are responsable for recognizing prey cues in *Phytoseiulus* mites.

Predatory mites of the genus *Phytoseiulus* are specialized in feeding on spider mites of the genus *Tetranychus*. However it is expected that these predatory mites discriminates between prey species and exhibits a preference between them (Dicke et al. 1988). The prey preference of predatory mites can be understood in terms of reproductive performance but also by other reasons (Dicke et al. 1988). When foraging on a leaf inhabited by a mixture of prey types, predatory mites have to decide which prey to accept and which to reject.

The objective of this work was to understand whether the complex web produced by *T. evansi* mediates direct and indirect interactions with its natural enemies. To this, first we studied how the presence of complex structures on patches affects the preference behaviour of *P. longipes*. We also explored indirect interactions mediated by *T. evansi* web via intraguild predation between its natural enemies. In a third part we studied the morphological differences between predatory mites as adaptations to cope with prey webbing..

Material and Methods

Plants and mite rearing

Tomato plants (*Solanum lycopersicum* var. Santa Clara I-5300) were weekly sown in a commercial plant substrate (Bioplant®, Bioplant Misturadora Agrícola LTDA, Nova Ponte, MG) in a polystyrene tray (8 x 16 cells) in a greenhouse. Trays with seedlings were kept inside a cage covered with fine mesh to avoid contamination with other herbivores. After twenty days, seedlings were transplanted to pots (5L) containing a commercial plant substrate (Bioplant®, Bioplant Misturadora Agrícola LTDA, Nova Ponte, MG). The plants were fertiirrigated weekly with a mixture of 50g of N-P-K (20-05-20) and 100g of simple superphosphate dissolved on 20 L of water. Pots were kept inside larger mesh-covered cages in a greenhouse.

A population of *T. evansi* was obtained in 2002 from a natural infestation on tomato plants in a greenhouse in Viçosa, Minas Gerais, Brazil. *Tetranychus urticae* population was started with individuals collected on bean plants under natural infestation (*Phaseolus vulgaris* L.) in a greenhouse in Viçosa. Both spider mites populations were reared on tomato leaves that had their petioles inserted in a PVC tube filled with water to prevent desiccation of the leaves. Tubes with infested leaves were kept in PVC trays filled with detergent and water (1:25, v/v), which served to prevent mite escape and invasion of mites and other non-flying arthropods. The rearing of each spider mite species were maintained in separated room (25 ± 3 ºC, 70 – 90 % relative humidity) with controlled photoperiod (12:12 L:D).

The rearing of the predatory mite *P. longipes* was started with individuals that were sent by Dr Gilberto de Moraes from the University of São Paulo, Brazil, in 2007. The population of *P. macropilis* was obtained in 2008 from field population observed on common bean plants attacked by two-spotted spider mites. The predatory mites were reared on tomato leaves infested with spider mites. Both colonies species were maintained in separate rearing rooms (25 ± 3 ºC, 70 – 90 % relative humidity). *P. longipes* were fed daily with *T. evansi* on tomato leaves taken from the spider mite culture and *P. macropilis* were fed in the same way but with *T. urticae* infested leaves. All experiments were carried out in a rearing room (25 ± 3 ºC, 70 - 90 % relative humidity) with controlled photoperiod (12:12 L:D).

Effect of environmental structure on patch preference of P. longipes

Leaf discs (2.4 cm of Ø) were cut from the basal part of tomato leaflets and regularly arranged on wet filter paper, positioned on wet foam inside a tray (12.5 x 7.5 x 2.5 cm). Leaf discs were cut so that the midrib divided the leaf disc into two halves. Then a thin yarn of wet cotton wool was placed along de midrib with the ends touching the wet filter paper. This connection with the water kept the cotton yarn wet and impeded the spider mites from crossing between the two halves. Subsequently, each leaf disc half was infested with 60 *T. evansi* females and they were left for 24 hours to lay eggs and produce web over the half leaf disc. Subsequently, the females were killed with a thin insect needle or removed with a thin painting brush depending on which test was carried out. We then counted the number of spider mite eggs on each half disc and removed the cotton yarn from the leaf midrib. Thereafter, we inserted an entomological needle at the center of the disc drilling the leaf midrib. The needle was used as a basis to release the predatory mites. We took adults females of *P. longipes* from the laboratory colonies. We had no control for age of female collected, but we took only mites with expanded opisthosoma, indicating they were ovipositing. With a fine brush, one adult female was placed on the “head” of the needle. In this way, the predatory mites could walk down the needle and choose freely between disc halves. After 24 hours, the numbers of spider mites eggs left and *P. longipes* eggs laid on each half side were recorded. Each replication consists of the response of one predatory mite on one disc. All the tests consisted of 20 replications.

In the first test, the preference of *P. longipes* for either *T. evansi* eggs without web or *T. evansi* eggs with web was evaluated. The experiment was set up as described above. In order to keep the web on one of the halves, we killed each spider mite by piercing it with a fine entomological needle. Killing the spider mites was the best procedure to remove the females while keeping the web, we observed that removing the females with a brush could substantially damaged the web. On the other disc half, the web was removed using a thin brush. Although the web had been removed, we also pierced and killed the spider mites on the half side without web, remaning only eggs.

The second test consisted on one half disc with web and no *T. evansi* eggs *versus* another half disc without web and with eggs of *T. evansi*. The initial set up was the same as described before. However, for this test, on the half disc with web, we used virgin adults female spider mites less than one day old. One day prior to setting up the experiment we collected females in the last quiescent stage (teliochrysalids) from the laboratory colonies. Males were not collected. We did this because one-day-old females have an oviposition rate close to zero (Bonato 1999) and unmated females oviposit less than mated females (personal observation). On the other half disc we used mated females taken randomly from the colonies. After 24h, we killed the spider mites on both sides and pierced the few eggs laid by young virgin females any eventual egg. From the side with adult female, we removed the web with a fine brush and counted the eggs.

The third experiment of this series was conducted to observe if the choices of *P. longipes* are based on infochemicals present on spider mite web or if they are product of preference behaviour for patches with complex structure. The experiment test consisted in evaluating the preference of *P. longipes* for disc half with eggs of *T. evansi* (only) *versus* a half disc with eggs of *T. evansi* and cotton fibers. We decided to use cotton fibers because this is a inert material without pheromones from spider mites. After 24 hours ovipositing and producing web the females were removed with a brush from both treatments. As in this test the web was not necessary important on both treatments, we decided just remove the spider mites with a brush also removing the web. After that, we placed some small amount of shredded cotton fibers on one half disc. The other half disc was left without cotton fibers and web.

Prey preference of P. longipes

Following the same procedure as described above for the first set of the dual-choice experiments, we tested the preference of *P. longipes* females for: (i) half leaf disc with eggs and web of *T. evansi versus* half leaf disc with eggs and web of *T. urticae* and (ii) half leaf disc with eggs of *T. evansi versus* half leaf disc with eggs of *T. urticae*, being both without web. As in the previous experiments, we killed the females after 24 hours of oviposition and web production on the leaves to preserve the web of spider mites. In the second test where the web was not necessary, the females were just removed from the discs using a fine brush. As on the previous experiment, one predatory female of *P. longipes* was released on upon a needle head each leaf discs and its preference was accessed 24 hours later by observing its oviposition and predation on each half disc.

Effect of spider mite web mediating intraguild predation

In this experiment we aimed to observing if the spider mite web can interfere with intraguild predation of *P. macropilis* on *P. longipes* eggs and juveniles. To this end, ten discs (1.5 cm of Ø) were cut from tomato leaflets and arranged on wet cotton wool on a Petri dish. Two Petri dishes were prepared to accommodate the treatments: a) leaf discs covered with spider mite web and b) leaf discs without spider mite web. Discs were infested with 50 adult female spider mites (shared prey) that were left for 24 hours to lay eggs and produce web.

After this period the spider mites were killed with an entomological needle on all discs. On ten discs, the web was removed with a fine brush while on the other ten discs the web was kept intact. The eggs laid by spider mites were left on all discs. Subsequently, ten eggs of *P. longipes* (intra-guild prey) were transferred from the laboratory colonies to each disc with a fine brush. The eggs of *P. longipes* were accommodated on along the main central vein. On discs with web, the eggs were consequently left on the web. Subsequently, three adult females of *P. macropilis* (intra-guild predator) were transferred from the laboratory colonies to each disc. The predation of *P. macropilis* on eggs of *P. longipes* was evaluated after 72 hours by counting the number of immature stages alive. Daily, the eggs of *P. macropilis* were carefully removed from the discs with a fine brush to not damaging the web of spider mites. When necessary, the dead or escaped *P. macropilis* individuals were replaced with new individuals from the laboratory colonies to keep the same initial density. Two experiments were carried out following the describe methodology, one with was *T. evansi* and another with *T. urticae* as shared prey.

Chaetotaxy study

To observe morphological differences on setae length among predatory mites, microscope slides with both species were prepared. Adult females from the laboratory colonies were taken and transferred to the glass slides. The females were mounted in medium Hoyer fixation (one dropt per slide) with five females per slide. After that, a coverslip was put over the mites. Then the slides were left to dry on an electric incubator at 60 ºC during one week. During the procedure each slide was properly indentified in terms of predator species with a paper label. After drying and fixing, the slides were observed with a polarized light microscope (Zeiss Axioskop 40®) and photos of the mites were taken with a digital camera (Canon PowerShot A640®). The images were stored on a computer for further analysis. The dorsal shield setae were measured using the software Image-Pro® Plus 4.5.0.29. We took measurements of the set of setae Z1, j6, j4, j3, Z5, z2, z4, s4 comprising the mid-dorsal setae (the 5 first) and margin-dorsal (the 5 last) according to the chaetotaxy classification adopted by Sabelis and Bakker (1992). The setae j3 and Z5 are present in both categories of chaetotaxy. We based our study on these setae because they are present on both predatory mites species and are the more easily observed under a microscope.

Statistical analysis

All experiments of preference where analyzed with Generalized Linear Models (G.L.M). The models with predation rate were performed with *Binomial* errors while with oviposition rate we used *Poisson* errors. In the predation models, the proportion of spider mites eggs preyed as function of the available eggs on the different treatments were analyzed. In the oviposition models, the number of *P. longipes* eggs in the different treatments were analyzed. The analysis were started with full models to observe if there was significant interaction between treatments and the half sides (left or right) of the treatments in each replication. When no significant interaction where observed, a step-wise procedure was conducted to remove the non-significant explanatory variables until a minimal model was reached (Crawley 2007). When overdispersion was observed the models, the error structures were changed from *Poisson* and *Binomial* to *Quasi-Poisson* and *Quasi-Binomial*, respectively (Crawley 2007). The final models were submitted to analysis of deviance or variance according with the error structure. Data from the intraguild experiment were analyzed as the preference experiment with Poisson errors. But in this case, the full model was in function of extra-guild prey specie (*T. evansi* or *T. urticae*) and treatments (with or without web) as explanatory variables. On the chaetotaxy study, the differences on the setae lengths between *P. longipes* and *P. macropilis* was analyzed with multivariate analysis of variance (MANOVA) followed by a series of univariate ANOVAs. All the statistical analyses were performed on the software RGui v. 2.11.1 (R-Development-Core-Team 2010).

Results

Effect of environmental structure on preference behaviour of P. longipes

The predador *P. longipes* preferred half sides of leaf disc containing *T. evansi* eggs and web to half sides with only eggs Both predation rate (GLM with *Quasi-Binomial*: $F_{1,46}=36.852$, $P<0.0001$) and oviposition rate (GLM with *Poisson*: Dev=6.857, $P<0.0001$, d.f.=1) were much higher on half discs covered by *T. evansi* web (Figure 1). Of the overall predation, 88% (28.6±2.6, mean ± standard error) of it was on the half tomato discs with web of the spider mite while the oviposition was 100% on these webbed halves. The oviposition rate of *P. longipes* was 3.1±0.2 (mean ± standard error) eggs per disc. No significant interaction between half side (left or right) and the treatments for both predation (GLM with *Quasi-Binomial*: $F_{1,44}=0.0405$, $P=0.8414$) and oviposition (GLM with *Poisson*: Dev=6.727, $P=1.00$, d.f.=1) were observed.

When observed the preference of *P. longipes* between half disc with eggs and without web *versus* half disc with web and without eggs of *T. evansi*, the predatory mite chose the treatment with web for oviposition. All eggs of *P. longipes* were laid on tomato disc halves covered by web but without prey eggs (GLM with *Poisson*: Dev=10.733, $P<0.0001$, d.f.=1).

The mean oviposition rate of *P. longipes* was of 2.2±0.4 eggs per female. The predation of 19.7±3.6 *T. evansi* eggs per predatory female indicates that *P. longipes* were able to forage on both half sides of tomato discs.

On the third experiment we observed the predation and oviposition rate of *P. longipes* on half tomato discs containing either only eggs of *T. evansi* or eggs with cotton fibers. Both predation and oviposition were strongly higher on half discs covered with cotton fibers (GLM with *Quasi-Binomial*: $F_{1,38}$=19.942, P<0.0001; and GLM with *Poisson*: Dev=15.955, P<0.0001, d.f.=1, respectively). 74% of the eggs preyed and 98% of the eggs laid were on half tomato discs covered with cotton fibers (Figure 2). No significant interaction between half side (left or right) and the treatments for both, predation and oviposition (GLM with *Quasi-Binomial*: $F_{1,36}$=0.4061, P=0.5280 and GLM with *Poisson*: Dev=14.292, P=0.2192, d.f.=1, respectively) were found.

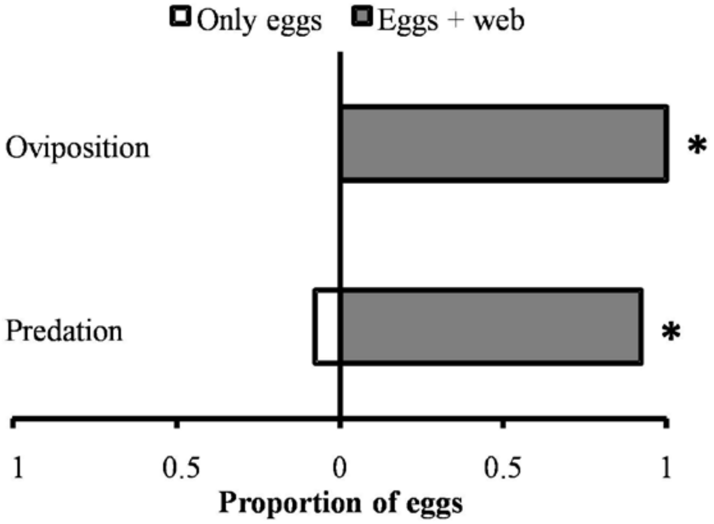

Figure 1.Proportion of eggs laid (oviposition) and preyed (predation) by *P. longipes* females on tomato leaflet half discs with eggs and web of *T. evansi* (grey bars) and with eggs without web of *T. evansi* (white bars). The asterisk after the bars represents a statistical significant difference of the proportion between the treatments.

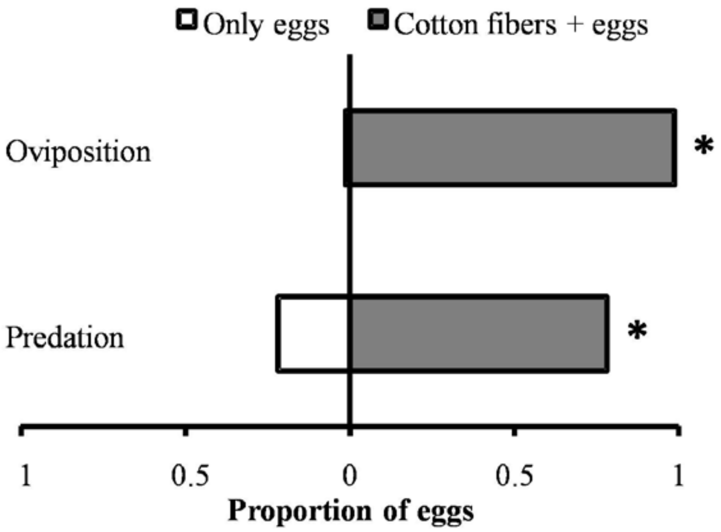

Figure 2. Proportion of eggs laid (oviposition) and preyed (predation) by *P. longipes* females on tomato leaflet half discs with eggs and no web of *T. evansi* (white bars) and half disc with eggs and covered by cotton fibers (grey bars). The asterisk after the bars represents a statistical significant difference of the proportion between the treatments.

Prey preference experiments

In the first test the two half sides contained eggs and web of both spider mites species. The predatory mite *P. longipes* showed a preference for discs with *T. evansi* to discs with *T. urticae* (Figure 5). Both the predation rate (GLM with *Quasi-Binomial*: $F_{1,28}$=29.12, P<0.0001) and oviposition rate (GLM with *Quasi-Poisson*: $F_{1,28}$=45.753, P<0.0001) were higher on half tomato discs with *T. evansi* eggs and web than with *T. urticae* eggs and web.

When testing the preference between patches with *T. evansi* eggs *versus T. urticae* eggs (both without web), *P. longipes* chose *T. evansi* again (Figure 6). Both the predation rate (GLM with *Quasi-Binomial*: $F_{1,40}$=371.13, P=0.0134) and oviposition rate (GLM with *Poisson*: $F_{1,40}$=48.244, P<0.0001) were higher on tomato half discs with *T. evansi* eggs.

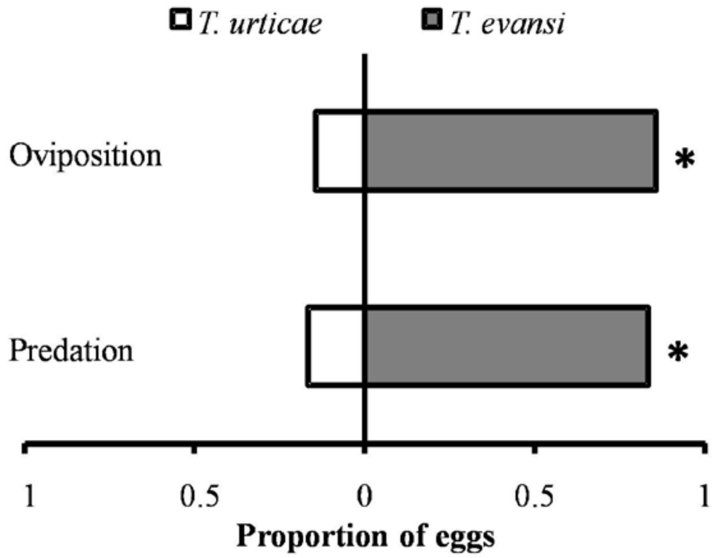

Figure 5. Proportion of eggs laid (oviposition) and preyed (predation) by *P. longipes* females on tomato leaflet half discs with eggs and web of *T. urticae* (white bars) and half disc with eggs and web of *T. evansi* (grey bars). The asterisk after the bars represents a statistical significant difference of the proportion between the treatments.

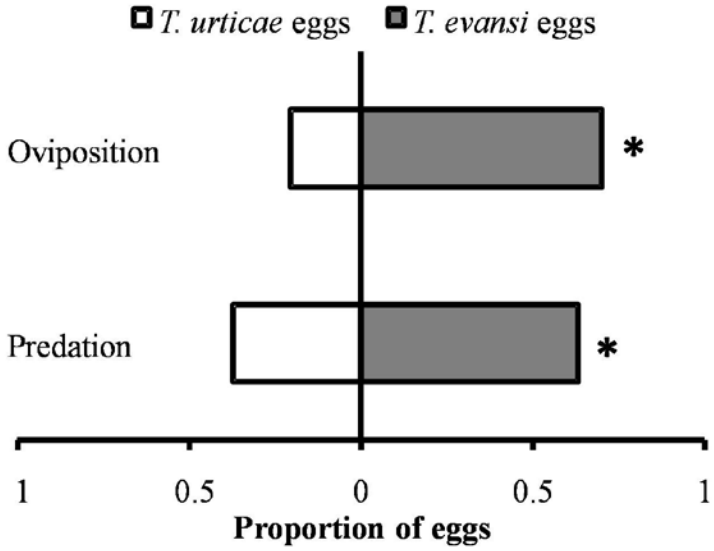

Figure 6. Proportion of eggs laid (oviposition) and preyed (predation) by *P. longipes* females on tomato leaflet half discs with eggs and no web of *T. urticae* (white bars) and half disc with eggs and no web of *T. evansi* (grey bars). The asterisk after the bars represents a statistical significant difference of the proportion between the treatments.

Intraguild predation experiment

The intraguild predation rate of *P. macropilis* on juvenile stages of *P. longipes* was reduced by the presence of web of *T. evansi* and *T. urticae* (GLM with *Quasi-Poisson*: $F_{1,38}$=12.859, P=0.0009). It was not observed difference on the intraguild predation between discs with web of *T. evansi* and *T. urticae* (GLM with *Quasi-Poisson*: $F_{1,36}$=0.8423, P=0.3649). The rate of intraguild predation was two-fold less on discs covered by web of spider mites than on discs without web (Figure 3).

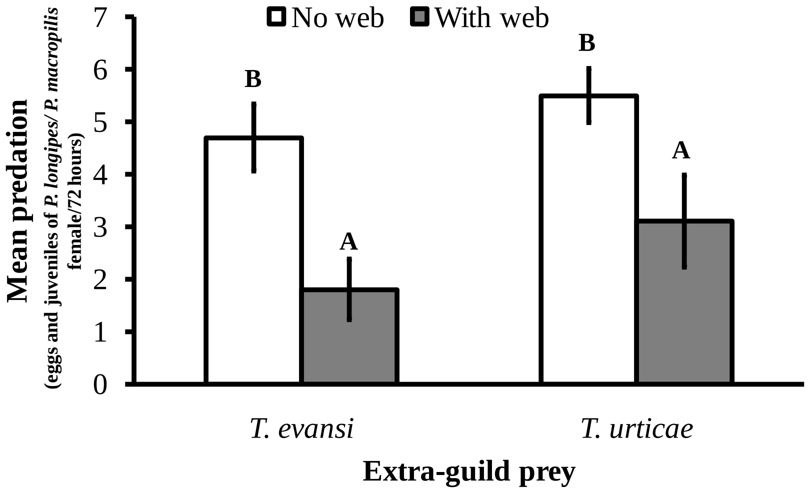

Figure 3. Intraguild predation rate of *P. macropilis* on *P. longipes* on discs without web and with web produced by two different spider mite species (shared prey). The intraguild predation is represented in terms of mean (±SE) number of juveniles *P. longipes* preyed. Different letters above the bars represent statistical significant different means.

Chaetotaxy study

The length of the setae of *P. macropilis* and *P. longipes* are differed significantly (MANOVA: Wilks' λ = 0.996, $F_{1,17}$=304.68, P<0.0001) (Figure 4). The setae Z1 was the only one that did not differ in length between *P. longipes* and *P. macropilis* (ANOVA: $F_{1,17}$=2.095, P=0.1660). The setae j6, j4, Z5 and s4 were longer on *P. macropilis* than *P. longipes*

(ANOVA: $F_{1,17}=185.27$, $P<0.0001$; $F_{1,17}=176.31$, $P<0.0001$; $F_{1,17}=51.89$, $P<0.0001$; $F_{1,17}=74.461$, $P<0.0001$ respectively). Meanwhile the setae j3, z2 and z4 were longer on *P. longipes* than *P. macropilis* (ANOVA: $F_{1,17}=248.72$, $P<0.0001$; $F_{1,17}=14.64$, $P=0.0014$; $F_{1,17}=113.57$, $P<0.0001$ respectively).

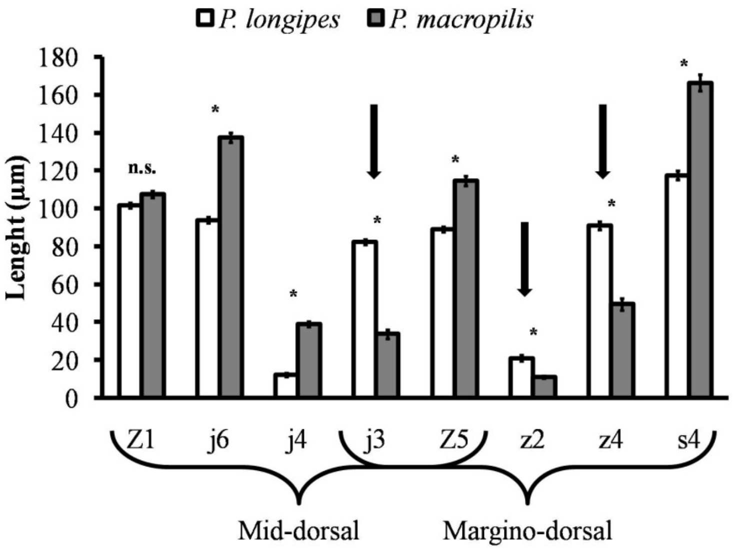

Figure 4. Length (µm) of eight dorsal-shield setae of the predatory mites *P. longipes* (white bars) and *P. macropilis* (grey bars). The brackets indicates the setae that comprises the two classification adopted by Sabelis and Bakker (1992). Asterisks represent significant statistical differences while “n.s.” indicates statistical similarities.

Discussion

We observed that the predatory mite *P. longipes* innately prefers environments with a complex structure (either spider mite webbing or cotton fibers), which ensures its protection against intraguild predation. When not under risk of intraguild predation, *P. longipes* foraged and laid eggs on patches covered by web of *T. evansi* instead of patches without web. The change of oviposition behavior due to avoidance of intraguild predation is usually stimulated by cues of the competitor (Faraji et al. 2001). However, in our experiments the preference by patches with complex web occurs without cues or previous contact with intraguild predators. Furthermore, *P. longipes* foraged and oviposited on patches covered with web and without food in opposite to un-webbed patches with eggs of *T. evansi*. This reinforces the idea that the preference behaviour of *P. longipes* for spider mite web is strongly modulated by intraguild predation suffered from *P. macropilis*. It is also interesting to mention that in our experiments the predation rate of *P. macropilis* upon eggs and juveniles of *P. longipes* did not differed between discs with *T. evansi* and *T. urticae* as shared prey. The predatory mite *P. macropilis* feeds well on *T. urticae* but on *T. evansi* its performance is very poor (F. Lemos personal observation). Usually, the provision of suitable food for intraguild predator could reduce its predation rate on intraguild prey (Roda et al. 2000). However our results indicate that in this case, the shared food is less important in determine the incidence of intraguild predation between the two competitors than the web.

The mediation of intraguild predation among Phytoseiidae mites by spider mite web has been hypothesized on other studies. As observed by Montserrat et al. (2008), *N. californicus* mainly co-occurs with *Euseius stipulatus* (Athias-Henriot) (Acari: Phytoseiidae) and share the same Tetranychidae species as prey (the persea mite *Oligonychus perseae* Tuttle, Baker & Abbatiello (Acari: Tetranychidae). The former is able to penetrate on the web nest of the prey while the second is hindered by the web and mostly forages on mobile stages wandering outside nests. Additionally, given that both phytoseiid mites are potentially engaged in intraguild predation, the nests of the persea mite may act as refuges for *N. californicus* eggs and juveniles (Montserrat et al. 2008). On apple plants, the intraguild predation by western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) on eggs of *P. persimilis* was reduced by the presence of *T. urticae* web (Roda et al.

2000). Moreover, the authors observed that the complex leaf topography promoted by addition of cotton fibers also reduced the intraguild predation. Unfortunately in our study we did not test if the cotton fibers could reduce intraguild predation by *P. macropilis* on *P. longipes*.

The lower intraguild predation of *P. macropilis* on *P. longipes* on discs with web of spider mite could be the result of lower ability of this predator to cope with spider mite web and consequently to locate intraguild prey. The ability of Phytoseiidae mites to cope with complex webs can be formulated on basis of behavioral, as well as morphological features (Sabelis and Bakker 1992; Shimoda et al. 2009, 2010). The main morphological feature is the length and distribuition of dorsal shield setae (Sabelis and Bakker 1992; Shimoda et al 2009). Predatory mites with more and longer dorsal setae are expected to be more able in foraging on complex environments. Our study pointed out differences in chaetotaxy of *P. longipes* and *P. macropilis* based on the length of some dorsal-shield setae. From the eight setae measured, only one was not different in length on both predatory mite species. Four were longer on *P. macropilis* and three on *P. longipes*. If the lower intraguild predation in presence of web is due to the lower efficiency of *P. macropilis* to forage in environments with *T. evansi* web, the chaetotaxy hypothesis can be reinforced. However, due to be well adapted to feed and suppress colonies of *T. urticae* (Oliveira et al. 2007), we expected that the web of this spider mite could not reduce the intraguild predation of *P. macropilis* on *P. longipes*. Moreover, *T. urticae* produces an inferior amount of web in comparison to *T. evansi*. But in this case, this lower quantity seems to be enough to reduce the forage capacity of *P. macropilis*.

Predatory mites can also behaviorally cope with spider mites webs. Phytoseiidae mites can use their chelicerae and palps to cut the treads of spider mite web (Shimoda et al. 2009, 2010). On *P. longipes* the cheliceral lobe is exceptionally large compared to other

Phytoseiulus species (Flechtmann et al. 1993). Another feature is the presence of only two subapical teeth on the fixed digit that is unique within the genus *Phytoseiulus* (Flechtmann et al. 1993). *Phytoseiulus longipes* is also unusual among the Phytoseiidae in having a larger number (10-12) of transverse rows of denticles in the subcapitular groove situated on the anterior margin of a scale-like structure (Flechtmann et al. 1993). Unfortunately in our work we did not study these other kind of defense. But the morphological differences on the chelicerae of *P. longipes* in relation to others *Phytoseiulus* mites, associated with its capacity to cope with spider mite web indicate that this predator can have a great ability to cut the tread-silk of spider mites. However, this still needs to be tested.

The preference of predatory mites for patches with spider mite web could be hypothesized as a food-searching behavior. The web of spider mites has pheromones that are used by conspecific males to find females to mate (Helle and Sabelis 1985). Hence, predators could be adapted to recognize the chemical cues on the web and indentify the presence and prey species (Schmidt 1976). However, in our experiments we observed that *P. longipes* prefer patches covered with cotton fibers in opposite to patches without cotton, which are inert and are expected to be without any chemical cues related with prey. These manipulations demonstrate that the simple presence or absence of web is not the only factor influencing the *P. longipes* response to different surfaces, but most important is the multi-dimensional habitat formed. Similar results were found by Roda et al. (2001) for two Phytoseiidae species, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) and *P. persimilis*. These two predatory mites also spend more time and laid more eggs on patches with cotton fibers.

Dicke et al. (1989) studied the prey preference of the predatory mite *Typhlodromus pyri* for two prey species based only on food quality. In our experiments we observed also that *P. longipes* chose patches with *T. evansi* eggs to patches with *T. urticae* eggs. This preference was observed both when the patches were covered by web as well as when the web was not present. But in the experiments without spider mite web, the preference was not as strong as in the other tests with web. This could indicate that the web is not the only factor affecting the prey preference of *P. longipes*. Nonetheless, the web can have an important weight to determine predator preference. As observed by Furuichi et al. (2005), the prey preference of predatory mite *Neoseiulus womersleyi* Schicha depends not only on the species that produced the eggs, but rather on the species that produced the webs. This supports the hypothesis that predators can use environmental context cues in prey discrimination. Food and habitat are closely linked for small herbivores that live on plants, hence food choice in the field may be constrained by the need to choose patches that provide safer living sites (Duffy and Hay 1991). To *P. longipes,* the preference of *T. evansi* as main prey can be in part related with the protection ensured by its webbing against competitors.

Apparently, *P. longipes* has an innate preference of the red spider mite as the performance of this predatory mite is slight higher when fed with *T. evansi* than on *T. urticae* (Furtado et al. 2007). Additionally, learning behaviour could not be excluded from our experiments. Because the predatory mites tested were reared on *T. evansi* diet previously to the experiment, treir preference could be a conditioned response to previous food (Coyle et al. 2011). However, Furtado et al. (2007) tested the preference of two strains of *P. longipes* and yet predatory mites fed with *T. urticae* continued preferring patches with *T. evansi*. But in their experiment, the effect of web was not excluded since the tomato leaflets were offered with 24 hours of previous infestation and the web was not removed.

Plant structures has a direct positive effect on abundances of natural enemies on plants (Agrawal et al. 2000, Matos et al. 2006). We observed that complex structures provided by herbivores on plants, such as spider mite web, can benefit the presence of predators and protect them against intraguild predation. This result has important relevance for integrated pest management. As observed by Agrawal et al. (2000) in field experiments, the predation was enhanced by adding artificial leaf domatia to plants. So, we can hypothesized that artificial structure as cotton fibers could be used to benefit natural enemies such as Phytoseiidae mites, (Kawashima et al. 2006) by decreasing intraguild predation. Furthermore, the preference of *P. longipes* for *T. evansi* indicates that it will not fail to control the pest when released on crops with multiple infestations.

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GENERAL CONCLUSIONS

The spider mite *T. evansi* is able to recognize odours indicating presence of *P. longipes*. Predation risk cues stimulates *T. evansi* to change its oviposition behaviour. The spider mite laid part of its eggs suspended on the web instead of on the leaf surface. This changes in behaviour ensure protection to *T. evansi* offspring, considering the preference of *P. longipes* to feed on prey eggs laid on the leaf surface. Moreover it was not observed a increase on web production of *T. evansi* induced by predation risk which indicates that silk web *per si* could not be a good defence mechanism against specialized Phytoseiidae predators.

The predation efficiency and oviposition of *P. longipes* were not hampered by the web produced by *T. evansi*. The predatory mite seems to have morphological adaptations, as longer dorsal setae, which permit it to walk through the webbing. This caracteristics reinforce the idea that *P. longipes* is a excellent candidate as biological control agente of *T. evansi*.

Among all Phytoseiidae tested as control agent of *T. evansi*, *P. longipes* is the one that has shown better results. Besides not being hampered by the web, *P. longipes* is yet attracted to webbed patches. This behaviour guarantees to *P. longipes* protection against predation from its competitors (intraguild predation) as the predatory mite *P. macropilis*.

The predatory mite *P. longipes* has a preference for *T. evansi* over *T. urticae* as prey, in the presence or absence of spider mite webs. However, the observed preference could be the results of a condicioning feeding with *T. evansi*.