CLEIDE ROSA DIAS

FORAGING AND ANTIPREDATOR BEHAVIOUR IN AN ACARINE PREDATOR-PREY SYSTEM ON TOMATO

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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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.

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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, submetendo se à defesa da dissertação em fevereiro de 2013.

SUMÁRIO

RESUMO

DIAS, Cleide Rosa, M.Sc., Universidade Federal de Viçosa, fevereiro de 2013.**Forrageamento e comportamento antipredação no sistema ácaro predador-presa em tomateiro.** Orientador: Angelo Pallini Filho. Coorientadores: Arnoldus Rudolf Maria Janssen e Madelaine Venzon.

As plantas possuem mecanismos de defesa contra os ataques dos herbívoros. Estes mecanismos podem afetar diretamente os herbívoros, por exemplo, plantas podem produzir metabólitos secundários que reduzem ou param o desenvolvimento dos herbívoros, ou indiretamente por meio de interação com os inimigos naturais dos herbívoros. Plantas atacadas podem produzir compostos voláteis atrativos para inimigos naturais. Essas pistas voláteis são indicativas da presença das presas e são importantes para o sucesso do forrageamento de inimigos naturais. Por outro lado, herbívoros também são capazes de perceber pistas indicativas da presença de predadores e usá-las para evitar locais com risco de predação. Comportamentos antipredação aumentam a sobrevivência das presas, no entanto também podem gerar custos. No presente trabalho, foram estudados os comportamentos de forrageamento e antipredação no sistema do tomateiro com os ácaros fitófagos *Tetranychus urticae* e *Tetranychus evansi*, e os ácaros predadores *Phytoseiulus longipes* e *Phytoseiulus macropilis*. Ambos os predadores foram capazes de reconhecer pistas de tomateiros infestados mostrando preferência por plantas infestadas por *T. evansi* e *T. urticae* em relação a plantas limpas, mas não mostram preferência entre as presas. Estes predadores se alimentam de ambas as presas, no entanto *P. macropilis* não completa seu ciclo de vida se alimentando apenas de *T. evansi*. Assim, para *P. longipes* ambasas presas como fonte de alimento adequada, mas para *P. macropilis T. evansi* é um alimento de qualidade inferior. É possível que *P. macropilis* não seja capaz de distinguir pistas oriundas de tomateiros infestados por *T. evansi* ou *T. urticae*. Adicionalmente, *T. evansi* é capaz de perceber a presença de *P. longipes* e *P. macropilis*, e foi capaz de reconhecer pistas oriundas desses predadores e mudar seu comportamento de acordo com a espécie de predador e da escala espacial. *Tetranychus evansi* tentou escapar por mais vezes nos discos foliares com pistas de ambos predadores, tendo também redução no tempo de alimentação. No entanto, também há custos associados a estes comportamentos: *T. evansi* apresentou redução na taxa de oviposição nos discos foliares com pistas de *P. longipes*, mas não com pistas de

P. macropilis, provavelmente por que este predador não é perigoso para *T. evansi* como *P. longipes* é. Em uma escala espacial maior (hexágono de plantas) onde os ácaros caminhariam sobre substrato tendo de percorrer longas distâncias e perceber pistas com intensidade provavelmente mais fraca, *T. evansi* não evitou plantas com predadores. Todos os tomateiros estavam infestados com coespecíficos que produzem grande quantidade de teia, possivelmente *T. evansi* não evitou plantas com predadores pela possível proteção conferida pela teia. Concluindo, os predadores *P. longipes* e *P. macropilis* são capazes de usar pistas de plantas atacadas para localizar suas presas *T. evansi* e *T. urticae*, mas não as distinguem. *Tetranychus evansi* é capaz de perceber a presença de ambos predadores e mostrar comportamento antipredação de acordo com o perigo oferecido pelo predador e a escala espacial envolvida.

Palavras chave: teia alimentar, defesa induzida, *T. evansi*.

ABSTRACT

DIAS, Cleide Rosa, M.Sc., Universidade Federal de Viçosa, February, 2013. **Foraging and antipredator behaviour in an acarine predator-prey system on tomato.**Adviser: Angelo Pallini Filho. Co-advisers: Arnoldus Rudolf Maria Janssen and Madelaine Venzon.

Plants have mechanisms to defend themselves against herbivore attacks. These mechanisms may affect the herbivores directly, for example, plants can produce secondary metabolites that reduce or stop the growth of the herbivores, or indirectly by interacting with the natural enemies of the herbivores. Upon herbivore attack, plants are known to produce volatiles that are attractive to natural enemies. These volatile cues are indicative of the presence of prey, and are important for the foraging success of the natural enemies. In return, herbivores are able to recognize cues associated with the presence of predators and use these to avoid patches with predators. Such antipredator behaviour increases the survival of the prey; however, it may also have costs. Here, we studied the antipredator and foraging behaviour of the spider mites *Tetranychus urticae* and *T. evansi*, the predatory mites *Phytoseiulus longipes* and *P. macropilis* on tomato plants. Although the predators feed on both prey, *P. macropilis* can not complete its life cycle feeding only on *T. evansi*. Thus, for *P. longipes* both prey are adequate food sources, but *T. evansi* is a bad food source for *P. macropilis*. Both predators were able to recognize cues from infested or uninfested plants, showing preference for plants infested by *T. evansi* or *T. urticae* compared to uninfested plants, but they did not show a preference for plants with either of the two prey.It is possible that *P. macropilis* are not able to discriminate cues from tomato plants infested by these prey. Additionally, *T. evansi* can perceive the presence of *P. longipes* and *P. macropilis*. This herbivore was able to recognize cues from these predatory mites and to change its behaviour according to the species of predator and spatial scale. The spider mite tried to escape more often from leaf discs with predator cues, and also reduced its time spent feeding. However, there were also costs associated with this antipredator behaviour: *T. evansi* showed a decrease in oviposition rate on leaf discs with cues from *P. longipes*, but not with cues from *P. macropilis*, probably because this predator is not as dangerous to *T. evansi* as *P. longipes* is. At a larger spatial scale (hexagon of plants), where the spider mites walked on substrate soil and in all directions, *T. evansi* did not avoid plants with conspecific and predators; probably it perceived the presence of conspecific that produce high density of web which can protect it against predation. Concluding, the predatory mites *P. longipes* and *P. macropilis* can use cues from attacked plants to locate *T. evansi* and *T. urticae*, but do not discriminate between these two prey. In turn, *T. evansi* can perceive the presence of these predators, showing antipredator behaviour towards dangerous and harmless predators, according to the spatial scale.

Keywords: food web, induced defence, *T. evansi*, predators.

GENERAL INTRODUCTION

Communities of species are characterized by complex networks of interactions. Well-studied examples of interactions are those occurring in tritrophic systems where plants are the base of the food web. These plants do not just interact with their enemies, the herbivores, but also with the natural enemies of these herbivores (Dicke 1999a). Plants can activate their induced defence mechanisms when are attacked by herbivores (Karban and Baldwin 1997, Kaplan and Denno 2007, Kessler and Halitschke 2007, Ohgushi 2008), these mechanisms may have a direct or indirect negative effect on the herbivores (Baldwin 1997, Agrawal 2000,Traw and Dawson 2002, Poelman et al. 2008).

Plant defences with a direct effect on herbivores can be expressed, for example, as changes in the arrangement and density of trichomes, increases of production of secondary metabolites, thickening of the leaf cuticle (Walling 2000) or production of toxic compounds or defensive proteins (Walling 2000, Howe and Jander 2008). Direct plant defences can also affect herbivore eggs (Hilker and Meiners 2006) through changes of the leaf cuticle, thus lifting the eggs and increasing the probability of desiccation or predation of the eggs (Doss et al. 2000), or through necrosis of leaf cells surrounding eggs, causing its desiccation and death (Shapiro and Devay 1987, Balbyshev and Lorenzen 1997) or producing toxic substances, which kill the eggs (Seino et al. 1996).

With indirect plant defence, plants have many ways to attract and retain the natural enemies, such as providing refuges(Walter and O'Dowd 1992, O'Dowd and Pemberton 1994, Walter 1996, O'Dowd and Willson 1997, O'Dowd and Pemberton 1998, Matos et al. 2006, Ferreira et al. 2008) and alternative food such as nectar and pollen (Bentley 1977, Koptur 1992 , Pemberton and Lee 1996, Rogers 1985). Attacked plants can also produce volatile compounds which may be attractive to natural enemies (Dicke et al. 1990a, Dicke et al. 1990c, Turlings et al. 1990, Drukker et al. 1995, De Moraes et al. 1998, Thaler 1999, Dicke 1999b, Kessler and Baldwin 2001). Many studies have shown that plant volatiles attract carnivores such as parasitoids, predatory insects and mites (Sabelis and van de Baan 1983, Dicke et al. 1990b, Turlings et al. 1990, Steinberg et al. 1992, Scutareanu et al. 1997, Shimoda et al. 1997, van Loon et al. 2000).

Plants and natural enemies can both benefit from this interaction. The production of attractive volatiles by attacked plants can attract natural enemies helping them to find food that ensure their growth and persistence on the plant, thus reducing the population of herbivores and the damage they cause to the plant (Dicke and Sabelis 1990, Gomez and Zamora 1994, van Loon et al. 2000, Hoballah and Turlings 2001). The ability of natural enemies to perceive cues and locate patches containing prey, which ensures their development and reproduction, are fundamental for their foraging success (Vet and Dicke 1992, Venzon et al. 2000).

Predatory mites are an example of natural enemies that can recognize cues from attacked plants and use them to locate prey (Sabelis and van de Baan 1983,Jagers op Akkerhuis et al. 1985, Janssen 1999). Here we work with the predatory mites *Phytoseiulus longipes* and *Phytoseiulus macropilis*, bothcan discriminate volatile cues from infested and uninfested tomato plants (Sarmento et al. 2011) and show preference for odours of plants infested by *T. evansi* and *T. urticae* to odours of clean plants (Sarmento et al. 2011), but they do not show preference for odours of plants with either of the two prey (Sarmento et al. 2011, Lemos et al. unpublished data). These predators feed on both prey, but *P. macropilis* has a low rate of predation and oviposition and can not complete its cycle life when feeding only on *T. evansi* (de Moraes and McMurtry

1985). We therefore expected that that especially *P. macropilis* is able to discriminate between *T. urticae* and *T. evansi*. Besides we know that both predatory mites have preference for volatiles from infested plants than uninfested plants(Sarmento et al. 2011), here we wanted to know about their behaviour in a complex condition that in close to field conditions. Therefore we investigated the foraging behaviour of the predators *P. longipes* and *P. macropilis* on a large arena with entire plants when the mites could walk on the soil.

Additionally, prey are able to recognize cues from their predators, and use these to avoid predation. For prey, using such cues to access predation risk is important to ensure safety for themselves and their offspring. Traditionally, research has focused on lethal effects of predators on prey. However, non-lethal effects may be equally important because they cause changes in prey behaviour in order to hinder or avoid predation (Lima 1998a). These changes are called antipredator behaviour and can consist of seeking refuge (Ives and Dobson 1987, Wooster and Sih 1995), avoidance of patches with predators (Gore 1966, Brown et al. 1995), vigilance (Sweitzer and Berger 1992), changes in habitat (Lima and Dill 1990, Bolker et al. 2003), changes in oviposition sites (Hoffmeister and Roitberg 1997, Lemos et al. 2010) or oviposition frequency (Montserrat et al. 2007), and induction of diapause (Kroon et al. 2004, Kroon et al. 2005).

Antipredator behaviour reduces the mortality of prey, but costs time or energy that could otherwise be spent on other activities related to fitness (Lima and Bednekoff 1999). Thus, prey may have many costs, because the energy spent on escape may result in less time spent on feeding, which can result in slower growth, reduced reproduction and delayed development (Spitze 1992, Barry 1994, Ylonen and Ronkainen 1994, Koskela and Ylonen 1995, Pallini et al. 1998), and those costs means a reduction on fitness (Lima and Dill 1990, Lima 1998b). For the prey, it is therefore important to be able to discriminate among dangerous and less dangerous situations in order to reduce the costs associated with antipredator behaviour. For example, prey may not avoid patches with natural enemies that do not feed on conspecific prey but avoid patches where they do (Venzon et al. 2000, Persons et al. 2001). Thus, prey should show appropriate antipredator behaviour according to the risk involved, while minimizing the costs of defence (Sih 1980, Lima and Dill 1990, Lima 1998a, Pallini et al. 1998). In spider mites, the most common antipredator behaviour is avoidance (Pallini et al. 1999, Choh and Takabayashi 2007) and increase in the production of web, which hinders predators (Sabelis and Bakker 1992, Shimoda et al. 2009). These behaviours can also come with costs, such as a reduction of oviposition as a result of decreased feeding (Oku et al. 2004, Choh et al. 2010).

Here, we studied the tritrophic interactions among tomato plants, the spider mites *Tetranychus urticae* and *T. evansi*, and the predatory mites *Phytoseiulus longipes* and *P. macropilis*. In the first chapter, we investigated whether *P. longipes* and *P. macropilis* are able to detect cues from infested plants, and use these cues to locate and discriminate prey from a distance. In the second chapter, we investigated the antipredator behaviour of *T. evansi* in response to the presence of cues from *P. longipes* and *P. macropilis* at several spatial scales.

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CHAPTER 1

Foraging behaviour of predatory mites in the presence of cues from tomato plants attacked by spider mites

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Abstract

Natural enemies can use cues from their herbivorous prey or from plants infested by prey to find food. Here, we investigate whether the predatory mites *P. longipes* and *P. macropilis* can find and discriminate between prey through perception of cues from infested plants. Females of the predators were released in the centre of a hexagon consisting of (a) 3 tomato plants infested by *T. evansi vs* 3 clean plants, (b) 3 plants infested by *T. urticae vs* 3 clean plants or (c) 3 plants infested by *T. evansi vs* 3 plants infested by *T. urticae*. The females that arrived on the plants were removed and counted every hour for six hours and after 24 and 30 hours. Females of both predators were recaptured significantly more often on plants infested by either of the two prey species than on clean plants, and showed no preference for either of the prey. Although these predators feed on both prey, *P. macropilis* can not complete its cycle life when feeding only on *T. evansi*. Recent studies showed that these predators are able to recognize volatiles cues from tomato plants infested by *T. evansi* or *T. urticae*, and that there are differences in these volatiles. Both predators can recognize cues from infested plants but did not discriminate between the two prey. It is possible that the predators can not identify the differences between the cues from plants infested with *T. evansi* or *T. urticae*.

Key-Word: *Phytoseiulus longipes*, *Phytoseiulus macropilis*, foraging, indirect plant defence, herbivore-induced plant volatiles.

Introduction

Plants are hypothesized to arrest and attract natural enemies in order to increase predation on herbivores, thus reducing the potential damage caused by these herbivores. They have several ways to do this, for example, providing refuges(Walter and O'Dowd 1992, O'Dowd and Pemberton 1994, Walter 1996, O'Dowd and Willson 1997, O'Dowd and Pemberton 1998, Matos et al. 2006, Ferreira et al. 2008, Matos et al. 2011) and alternative food such as nectar and pollen (Bentley 1977, Koptur 1992, Pemberton and Lee 1996, Rogers 1985, Amaral et al. 2013). Another way is through the production of volatile compounds. These volatiles are produced by attacked plants and may be attractive to natural enemies, such as parasitoids and predatory insects and mites(Sabelis and van de Baan 1983, Dicke and Sabelis 1988, 1990, Dicke et al. 1990a, 1990c, Turlings et al. 1990, Steinberg et al. 1992, Dicke 1994, Drukker et al. 1995a, Scutareanu et al. 1997, Shimoda et al. 1997, Sabelis et al. 1999, Dicke and van Loon 2000, van Loon et al. 2000a, Kessler and Baldwin 2001). The production of such attractive volatiles results in an increase in the number of natural enemies on the plant, and consequently a decrease of the number of herbivores (Drukker et al. 1995b, Shimoda et al. 1997, De Moraes et al. 1998, Janssen 1999). Thus, these interactions benefit the natural enemies, which find food or hosts more easily, and the plant, which suffers less from herbivory (Dicke and Sabelis 1990, Gomez and Zamora 1994, van Loon et al. 2000b). They are therefore referred to as indirect plant defence.

Induced plant volatiles consist of a blend of compounds that may vary quantitative or qualitatively with host plant species (de Moraes et al. 1998, Dicke et al. 1998, van den Boom et al. 2004), herbivore species (Sabelis and van de Baan 1983, de Moraes et al. 1998, Du et al. 1998), the degree of herbivore infestation (Du et al. 1998, Maeda and Takabayashi 2001, Nachappa et al. 2006) and other factors. In natural ecosystems, the blend of volatiles are mixed because of multiple infestations of plants (Schroeder and Hilker 2008), which may hamper the foraging of natural enemies. But even under such heterogeneous field conditions, many natural enemies are able to locate the host plant producing the volatiles (Shimoda et al. 1997). In acari, chemical cues are the most important cues used to explore the environment. For example, predatory mites can perceive chemical cues and use them to locate their prey (Hislop and Prokopy 1981, Sabelis and van de Baan 1983, Jagers op Akkerhuis et al. 1985, Janssen 1999).

For the natural enemy to succeed foraging, it is important that it is able to identify chemical cues to discriminate between odours induced by prey and non-prey herbivores, thus it may ensure its development and reproduction (Vet and Dicke 1992, Venzon et al. 2002). Many natural enemies are able to discriminate among plants infested with different prey species, showing preference for odours from high-quality prey relative to odours associated with inferior prey or other species (Sabelis and van de Baan 1983, Dicke et al. 1988, 1990, Bilde and Toft 1994, Lesna and Sabelis 1999). However, there are also natural enemies that do not discriminate between odours from plants with prey and plants with non-prey (Turlings et al. 1993, Geervliet et al.1996, Shimoda and Dicke 1999).

Using an olfactometer, Sarmento et al. (2011) showed that the predators *P. longipes* and *P. macropilis* prefer odours from plants infested by *T. evansi* or *T. urticae* compared to clean plants. Additionally, they demonstrate that both species do not show preference for odours emanating from plants with either of the two prey species (*T. evansi* or *T. urticae*, Lemos et al. unpublished data). Both predatory mite species feed on *T. evansi* and *T. urticae*, however, *P. macropilis* cannot complete its life cycle and has low predation and oviposition rates when feeding only on *T. evansi* (de Moraes and

McMurtry 1985). Thus, it was expected that *P. macropilis* would discriminate between these prey and choose the prey with high quality.

Here we investigated the foraging behaviour of these two species of predatory mites at a more natural, larger spatial scale than an olfactometer. A large arena (Ø= 80cm) contained a circle with entire tomato plants. The mites were released in the centre of this circle and could freely walk in all directions on a soil substrate until they entered a plant, where they were recaptured (Janssen 1999). Hence, the difference from the olfactometer experiments of Sarmento et al (2011) is that volatiles were emanating from many directions and and the mites had to cross the irregular surface of the soil. Here we wanted to know whether *P. macropilis* and *P. longipes* are able to perceive and discriminate between the cues induced by either of the two prey species from a distance. We expected that the predators would also be able to recognize cues from attacked plants and use them to locate their prey under these circumstances. Moreover, we wanted to know whether *P. macropilis* would be able to discriminate between plants with *T. evansi* and plants with *T. urticae*.

Materials and methods

Rearing Methods

Tomato plants (*Solanum lycopersicum*, variety Santa Clara I-5300) were grown in pots (2 and 5 L) using a commercial substrate composed of vermiculite plus organic fertilizer. The plants were kept inside a greenhouse, and fertilized with NPK (4-14-8) and superphosphate. The plants were watered one to two times a day, depending on the season.

The herbivorous spider mites *T. evansi* and *T. urticae* were obtained from naturally infested tomato plants, respectively, at the campus of the Federal University of

Viçosa, Brazil in 2002. The predatory mite *P. longipes* was provided by Dr. Gilberto de Moraes (University of São Paulo, Brazil) in 2006. The predatory mite *P. macropilis* was obtained from bean plants infested by *T. urticae* in a greenhouse at the campus of the Federal University of Viçosa, Brazil in 2006. The spider mite *T. evansi* and *T. urticae* were cultured on tomato leaves that were placed in a plastic tube with water to maintain leaf turgor. The tubes were kept in plastic trays filled with detergent and water, which served to prevent mite escapes and invasion of other arthropods. A clean tomato leaf was added to these cultures every two days. The predators *P. longipes* and *P. macropilis* were cultured on tomato leaves infested with *T. evansi* or *T. urticae* respectively. Rearing methods for the predators were otherwise similar to that of the spider mites. All cultures were maintained at controlled conditions in separate room (25 ± 2 °C, 80 ± 10% relative humidity and 12 h light).

Release and recapture experiments

Tomato plants that had four completely developed leaves were used for the experiment. The plants were maintained clean or were infested with around 400 adults females of *T. evansi* or *T. urticae*. Six days after infestation, the plants were allocated equidistantly in a hexagon (Ø = 80 cm) in a cage (160 x 160 x 120 m) covered with thin mesh and with a tarpaulin bottom (160 x 160 x 15 cm) filled with soil. The pots containing the plants were buried and soil was added to the pots so that soil levels inside and onside the pots were equal (Pallini et al. 1998, Janssen 1999). Each hexagon consisted of six plants containing two treatments: (a) 3 clean plants *vs* 3 plants infested by *T. evansi*; (b) 3 clean plants *vs* 3 plants infested by *T. urticae* and (c) 3 plants infested by *T. evansi vs* 3 plants infested by *T. urticae* (Fig. 1). Care was taken that each plant position was occupied by one treatment in half of the replicates, and other treatment in the other half.

This was done to control for any unforeseen directionality in the searching behaviour of the mites (Janssen 1999).

Two hundred female predatory mites of either of the two species, aged eight to ten days, were collected in a Petri dish, which was placed in the middle of the hexagon 24 hours after the plants. The cage containing the hexagon was situated outside in an area with limited airflow, thus ensuring that the predatory mites could reach the plants only by walking. The predators could disperse freely from the Petri dish to the plants by walking over the soil to the stem of the plants, from where they could walk onto the stem until reaching the leaves. The base of the stem of the tomato plants contains a high density of trichomes, which hinders walking of the predators, thus a wooden stick was placed near the base, touching the lowest leaf, thus forming a bridge for help the mites to climb onto the leaves. This is comparable to the commercial growing conditions of tomato, where tomato plants are supported with wooden sticks or are guided along wires suspended from above. During 6h, the predators that arrived on the plants were removed and counted every hour. This was further repeated twice the next day, after 24h and 30h. Each experiment was replicated four times, and data were analyzed using a GLM with a Poisson or quasi-Poisson error distribution.

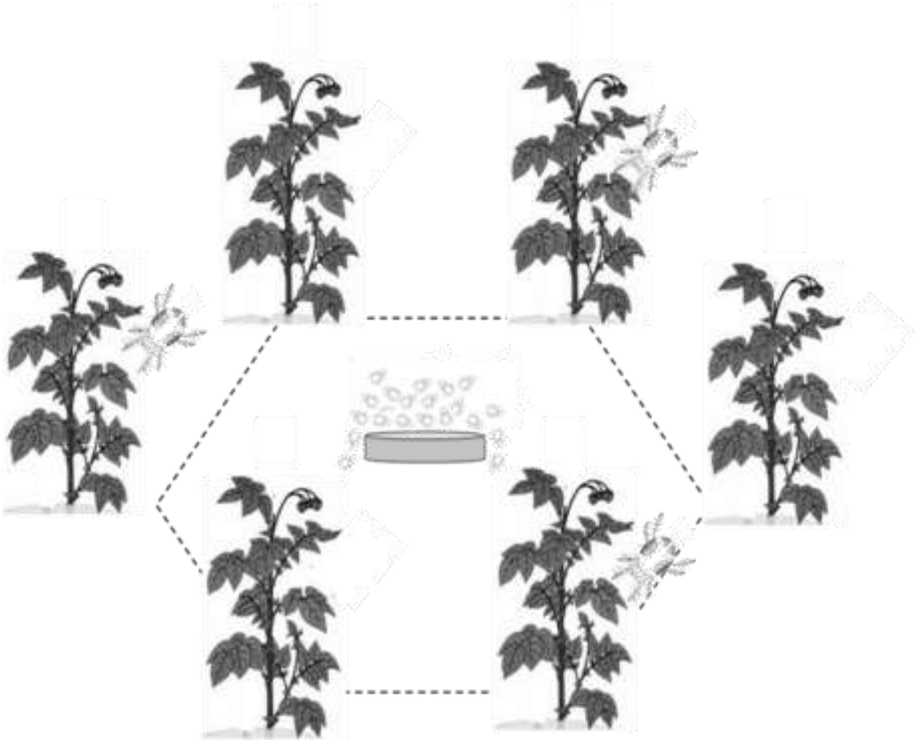

Figure 1 Illustration of the hexagon of tomato plants. Shown are infested plants (indicated by mites near the plants) *vs* clean plants (no mites). The predatory mites were released at the centre of the hexagon.

Results

There was no significant difference on the interaction between position and the treatments. The females of both predators were recaptured more often on plants infested by *T. evansi* (*P. longipes*: Fig. 2, $F_{(1,7)} = 7.38$: $P = 0.0087$) (*P. macropilis*: Fig. 3, $Chi^2_{(1,7)} = 8.21$: $P = 0.0041$) and by *T. urticae* (*P. longipes*: Fig. 4, $F_{(1,7)} = 7.51$; $P = 0.0081$)(*P. macropilis*: Fig. 5, $F_{(1,7)} = 18.78$: $P = 0.0001$) than on clean plants. When the predators were offered a choice between plants infested with *T. urticae* or *T. evansi*, there was no significant difference in the number of females recaptured on plants of either treatment (*P. longipes*: Fig. 6, $F_{(1,7)} = 0.15$: $P = 0.69$) (*P. macropilis*: Fig. 7, $F_{(1,7)} = 0.011$: $P = 0.91$).

There was a clear directionality in the searching behaviour of both predators (*P. longipes*: $F_{(1,23)}$= 6.27: P < 0.0001; *P. macropilis*: $F_{(1,23)}$= 14.25: P = 0.00033). Both predators were recaptured more often on the positions from southwest to northwest from the release point (Fig. 8).

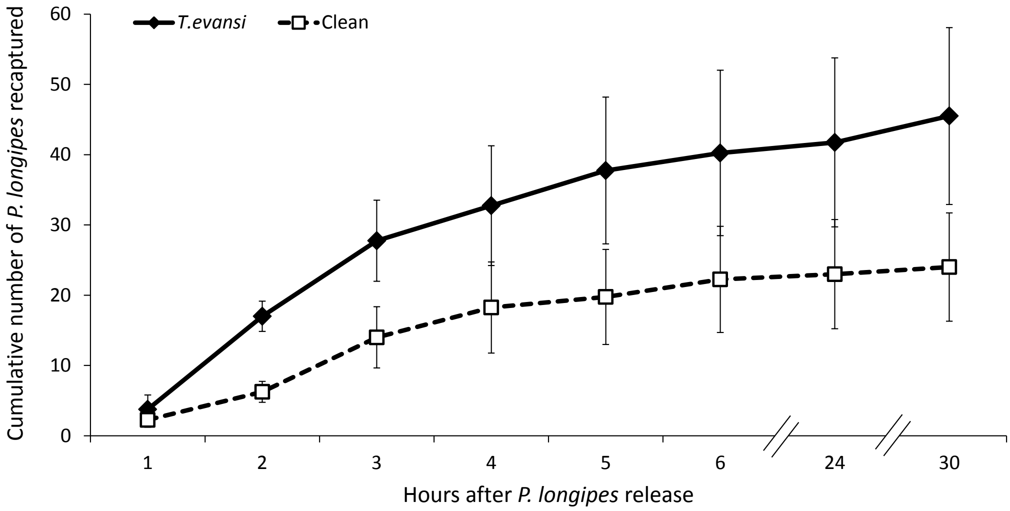

Figure 2 Cumulative number of *P. longipes* recaptured per plant through time on plants that either contained *T. evansi* (filled diamonds) or clean plants (empty squares). Error bars show the SEM of the mean cumulative number of predatory mites recaptured.

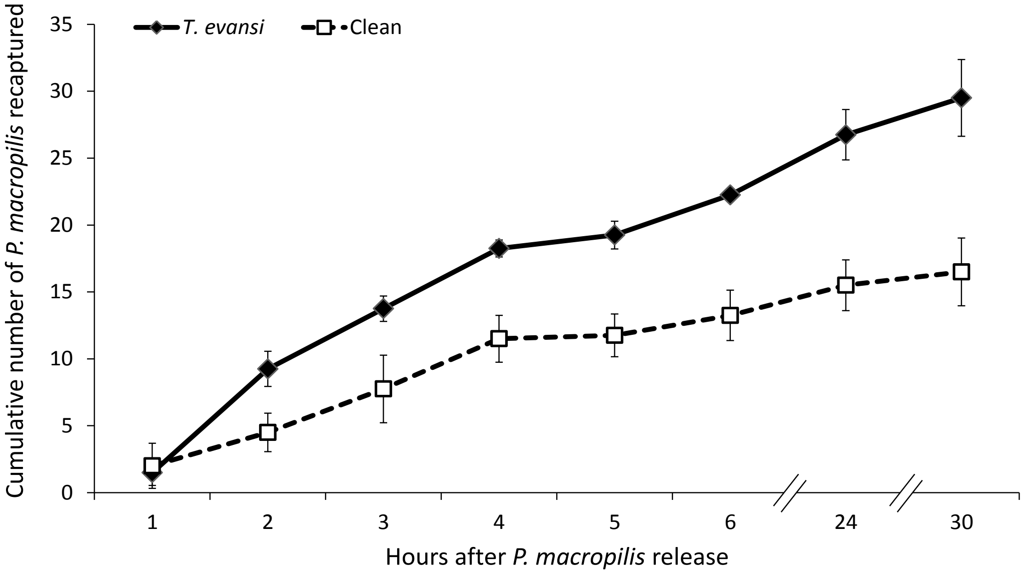

Figure 3 Cumulative number of *P. macropilis* recaptured per plant through time on plants that either contained *T. evansi* (filled diamonds) or clean plants (empty squares). Error bars show the SEM of the mean cumulative number of predatory mites recaptured.

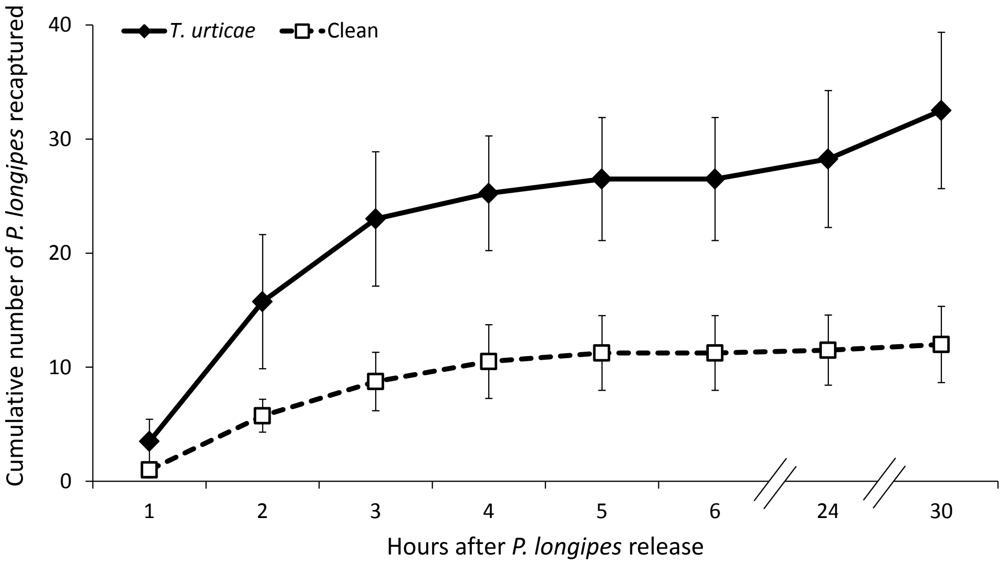

Figure 4 Cumulative number of *P. longipes* recaptured per plant through time on plants that either contained *T. urticae* (filled diamonds) or clean plants (empty squares).Error bars show the SEM of the mean cumulate number of predatory mites recaptured.

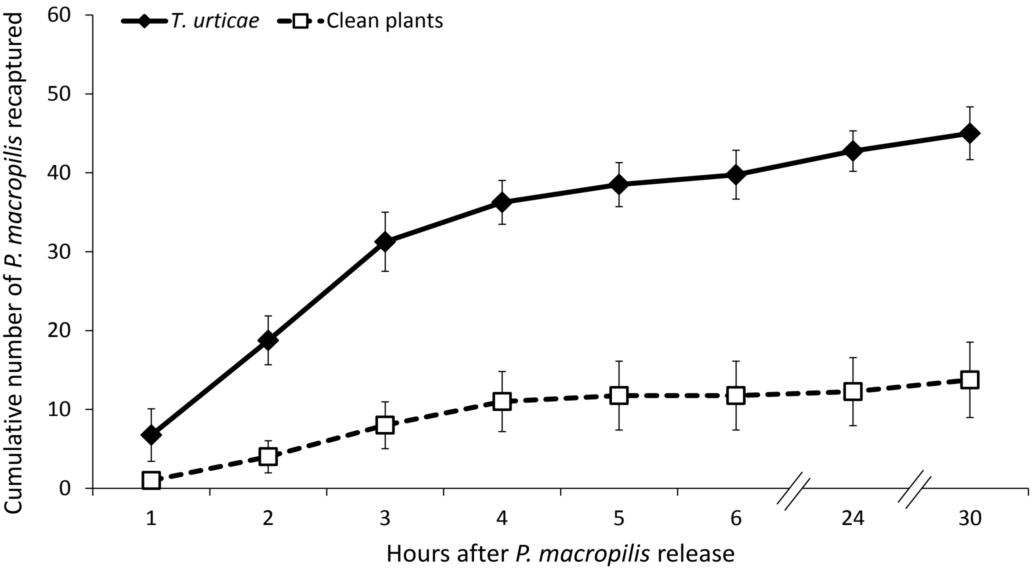

Figure 5 Cumulative number of *P. macropilis* recaptured per plant through time on plants that either contained *T. urticae* (filled diamonds) or clean plants (empty squares). Error bars show the SEM of the mean cumulate number of predatory mites recaptured.

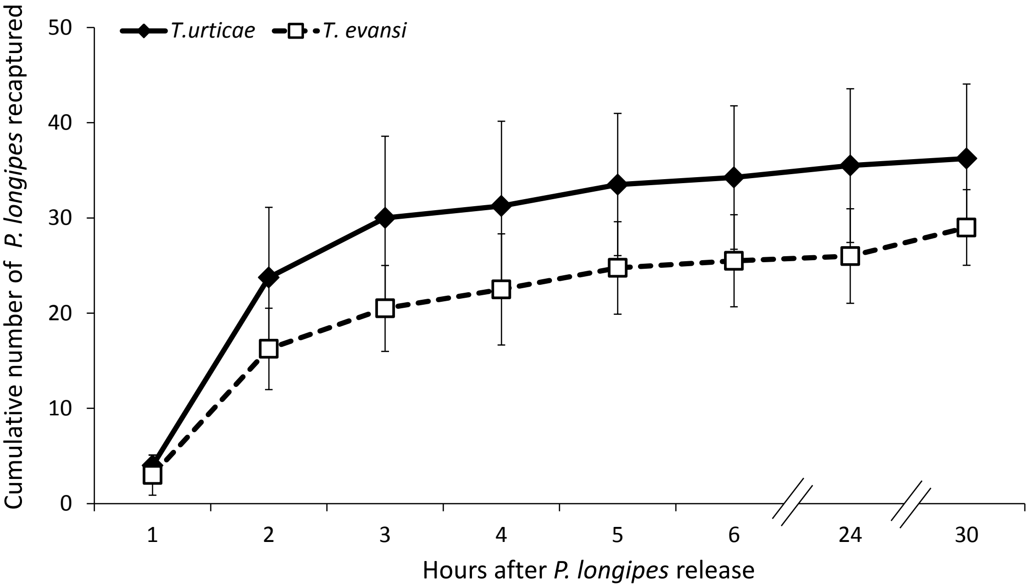

Figure 6 Cumulative number of *P. longipes* recaptured per plant through time on plants that either contained *T. urticae* (filled diamonds) or *T. evansi* (empty squares). Error bars show the SEM of the mean cumulate number of predatory mites recaptured.

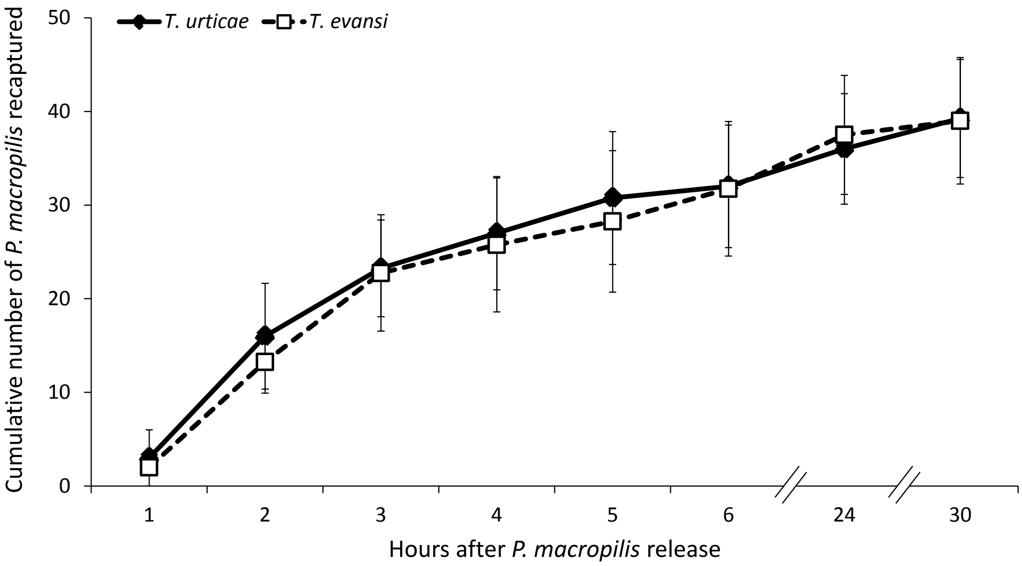

Figure 7 Cumulative number of *P. macropilis* recaptured per plant through time on plants that either contained *T. urticae* (filled diamonds) or *T. evansi* (empty squares).Error bars show the SEM of the mean cumulate number of predatory mites recaptured.

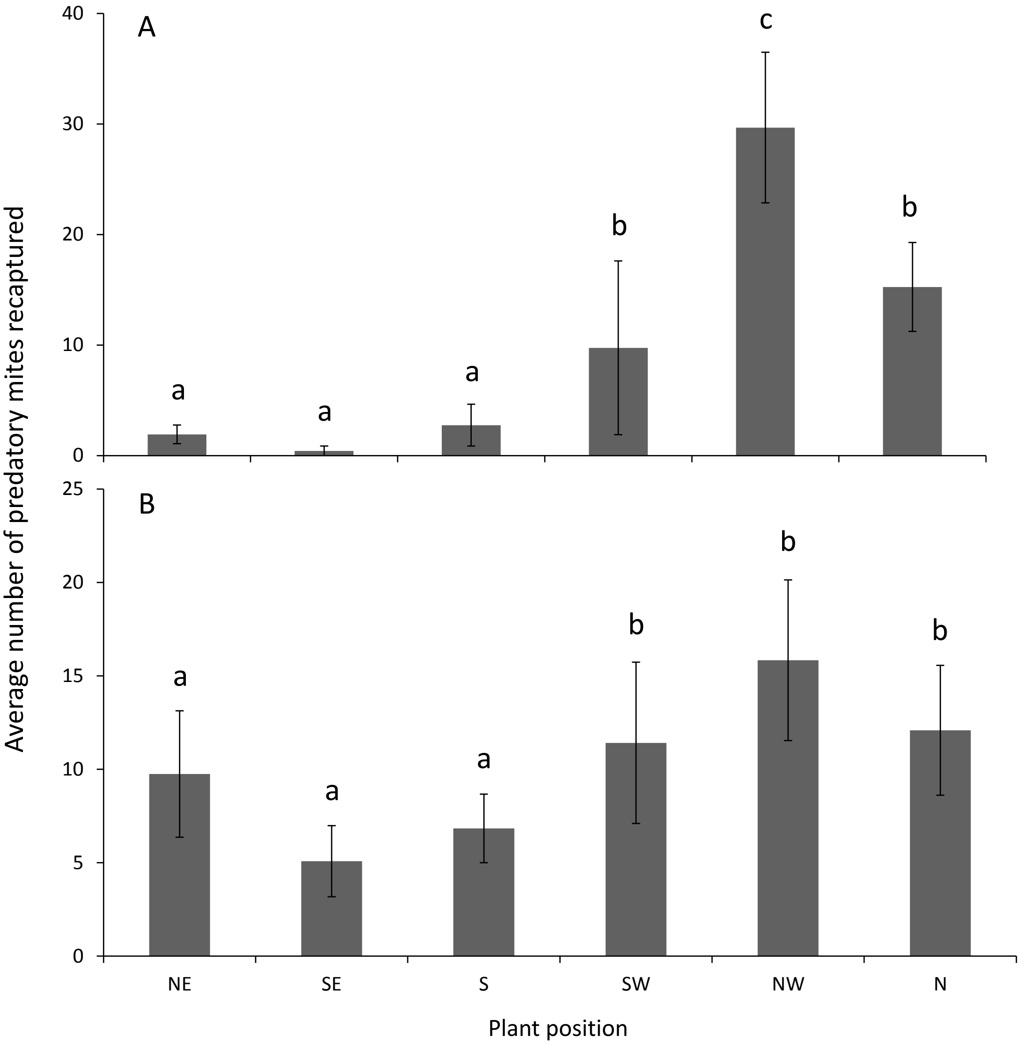

Figure 8 Average numbers of *P. longipes* (A) and *P. macropilis* (B) recaptured on the plants per position. Labels along the horizontal axis indicate the position of the plants relative to the release point: NE = northeast, SE= southeast, S= south, SW= southwest, NW= northwest and N= north. Bars with different letters indicate a significant difference between the positions. Error bars show the SEM of the mean number of predatory mites recaptured.

Discussion

Both predators showed a preference for plants with prey compared to clean plants, and both did not show preference for one of the two prey species. It is known that predatory mites are able to discriminate between odours of plants infested with their prey and uninfested plants (Dicke and Sabelis 1988, Dicke et al. 1990c, Janssen et al. 1997, Sabelis 1999). Previously, it has been shown that *P. macropilis* is attracted to volatiles produced by strawberry plants infested with *T. urticae* (Oliveira et al. 2009, Fadini et al. 2010). This also resulted in higher numbers of predators recaptured on plants with prey than on plants without prey in a similar experiment as carried out here (Oliveira et al. 2009). Recently, it was shown that *P. longipes* and *P. macropilis* prefer volatiles of tomato plants infested by *T. evansi* or *T. urticae* to those of uninfested plants (Sarmento et al. 2011). In agreement with this, we show that *P. longipes* and *P. macropilis* are able to discriminate between clean and infested tomato plants at the spatial scale presented here, and use them to find their prey.

Both predators can feed, develop and reproduce on *T. urticae* (Watanabe et al. 1994, Furtado et al. 2007b), but only *P. longipes* can complete its life cycle when feeding on *T. evansi*, whereas *P. macropilis* can not(de Moraes and McMurtry 1985). It was therefore expected that *P. macropilis* would prefer plants with *T. urticae* to plants with *T. evansi*, but it did not show any preference. It is known that predators can show preference for prey species of higher prey quality as food source (Sabelis and van de Baan 1983, Dicke et al. 1988, 1990, Bilde and Toft 1994, Lesna and Sabelis 1999). Additionally, it is known that volatile cues from tomato plants infested by *T. evansi* and *T. urticae* are different (Sarmento et al. 2011). However, *P. macropilis* also did not show any preference for volatiles from plants infested by *T. evansi* or *T. urticae* in an olfatometer(Lemos et al. unpublished data). It is possible that *P. macropilis* is not able to identify the differences in volatiles from plants infested by these prey. Perhaps the predators need some experience with the volatiles and both prey in order to discriminate between plants with either of the two species.

Although care was taken to avoid any external influence on the choice of the predatory mites, both predator species were recaptured more often on the plants situated northwest of the release site. The plants were placed in a cage with fine mesh, and there was limited airflow. Moreover, there was no influence of the treatment of the plants on the directionality of the mite dispersal, because each position was occupied by plants of one treatment in half of the replicates, and the other treatment in the other half. Curiously, such directionality was observed earlier for a closely related predatory mite species (*P. persimilis*) in greenhouses in the Netherlands (Janssen 1999) and Denmark (Zemek and Nachman 1999). As of now, there seems to be no explanation for this directionality.

The ability of the predatory mites *P. longipes* and *P. macropilis* to find attacked plants to locate their prey is an important issue for biological control of these pests. When the natural enemy is able to find its prey easily, it has more chances to survive and reproduce under field conditions. Thus, the ability to use cues to locate prey increases the chances of the natural enemy to control a pest. However, if the predatory mite can not discriminate between cues from plants infested by prey and non-prey herbivores, such as was shown here to *P. macropilis,* it may be unsuccessful on its foraging. On these cases, the predator will have difficulties to find food and could easily die on its dispersal, so they probably will not be successful on biological control.

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CHAPTER 2

Responses of the spider mite *Tetranychus evansi* towards different predators cues at various spatial scales

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Abstract

Prey use cues of all sensorial modalities to detect and avoid predation. Although this avoidance plays an essential role in survival and reproduction, it comes with costs. The aim of this study was to investigate if the spider mite *Tetranychus evansi* can detect cues from predators that pose different predation risks, and subsequently changes its behaviour to avoid predation at different spatial scales. In small scale experiments, one *T. evansi* female was released on a leaf disc (Ø= 2.4cm), half of which contained cues of the predatory mites *Phytoseiulus longipes* or *P. macropilis*, whereas the other disc half was clean. The spider mites did not spend significantly less time feeding and did not attempt to escape more often from the disc half with predator cues, but oviposited less on the side with predator cues than on the clean side. The spider mites did not spend less time on the disc half with cues of *P. longipes* than on the clean half, but spent more time on the clean side compared to the side with *P. macropilis* cues. In experiments where the spider mites were offered an entire leaf disc either with or without predator cues, *T. evansi* spent significant less time feeding, attempted to escape more often and laid fewer eggs on discs with *P. longipes* cues than on clean discs. The spider mites did not spend significantly less time feeding and also did not oviposit less on discs with predator cues, but attempted to escape more often from the discs with *P. macropilis* cues than from clean discs. At a large spatial scale, six plants infested by *T. evansi* were placed in a hexagon (Ø= 80 cm); three plants also contained the predator *P. longipes* or *P. macropilis*. There was no difference in the number of mites arriving on plants with or without either of the predators. We suggest that the response of *T. evansi* to the presence of cues associated with predators varies according to the spatial scale and to the species of predator.

Key words: predation risk, *Tetranychus evansi*, *Phytoseiulus longipes,* predator-prey interaction

Introduction

Predator-prey interactions are important for population dynamics, species composition (Paine 1966) and species distributions (Sih 1980, Kats and Dill 1998, Lima 1998a). Predation results in removal of the prey from the population, and this can affect the entire ecosystem (Taylor 1984). Often, only lethal effects of predation are considered, but non-lethal effects of the presence of predators on prey can be as important, and can affect species composition and ecosystem processes (Schmitz 1998).

Many prey can detect chemical cues that are associated with the presence of predators, and may exhibit defensive behaviour even in the absence of predators but in the presence of these cues (Chivers and Smith 1998, Kats and Dill 1998). Thus, prey change their behaviour upon perceiving predators to reduce predation risk, and these behavioural changes can go at the expense of population growth (Lima 1998a, Pallini et al. 1998, 1999).

Prey may display many different forms of antipredator behaviour, such as seeking refuge (Ives and Dobson 1987, Wooster and Sih 1995, Venzon et al. 2000, Faraji et al. 2001, 2002), avoidance of areas with predators (Gore 1966, Brown et al. 1995, Magalhaes et al. 2002, Nomikou et al. 2003, Meng et al. 2006), increased vigilance (Sweitzer and Berger 1992), changes in habitat or diet (Lima and Dill 1990, Bolker et al. 2003), delayed oviposition (Hoffmeister and Roitberg 1997, Montserrat et al. 2007), counterattack (Janssen et al. 2002, Magalhaes et al. 2005) and entering into diapause (Kroon et al. 2004, Kroon et al. 2005).

Besides having obvious benefits, antipredator behaviour usually carries costs, for example, trying to escape from a predator may result in more energy spent on running and less time spent on feeding, which may ultimately result in slower growth, reduced reproduction or delayed development (Spitze 1992, Barry 1994, Ylonen and Ronkainen

1994, Koskela and Ylonen 1995, Pallini et al. 1998), and those costs cause fitness reduction (Lima and Dill 1990, Lima 1998b). There are also cases when the predator induces egg retention in prey, which obviously results in a lower oviposition rate (Montserrat et al. 2007).

In spider mites, antipredator behaviour manifests itself as avoidance behaviour (Grostal and Dicke 1999, Pallini et al. 1999, Magalhaes et al. 2002, Choh and Takabayashi 2007), the induction of diapause (Kroon et al. 2008), changes in oviposition sites (Lemos et al. 2010) and the production of web to reduce predation (Sabelis and Bakker 1992, Horita et al. 2003, Oku et al. 2003, Shimoda et al. 2009). Costs associated with antipredator behaviour in spider mites are a reduced oviposition rate of spider mites that had previous experience with predators cues (Oku et al. 2004, Skaloudova et al. 2007, Choh et al. 2010).

Here, we investigate the antipredator behaviour of the spider mite *T. evansi* and its predators *P. longipes* and *P. macropilis*. This spider mite has become a pest of tomato in Brazil and other countries in South America, Africa and Europe (Bolland and Vala 2000, Knapp et al. 2003a, Saunyama and Knapp 2003, Escudero and Ferragut 2005, Wekesa et al. 2005) and stands out among the Tetranychidae because it is a highly destructive pest, its high population growth rate and large temperature range (Bonato 1999). Several studies have been devoted to finding an efficient biological control agent for *T. evansi*, many of them reporting negative results, including for the predator *P. macropilis*, which has been found associated with *T. evansi* in the field (de Moraes and McMurtry 1985). Recently, a promising species has been found; the predatory mite *P. longipes* has been indicated as potential biological control agent of *T. evansi* on tomato (Furtado et al. 2007a, Silva et al. 2010).

Previous studies showed that the predator *P. macropilis* has a low predation rate and cannot complete its life cycle when feeding on *T. evansi*. In contrast, *P. longipes* develops well and has a high population growth rate when feeding on *T. evansi* (de Moraes and McMurtry 1985). Considering the costs of the antipredator behaviour, the capacity of accurately identifying predation risk is important in order to minimize those costs. Besides, the spatial scale is also important, because the risks for prey might be different depending on the distance from the predator, the distance of safe sites and the presence of conspecific prey. On small leaf discs, spider mites can probably detect cues from predators and try avoid predation by antipredator behaviour such as escape. However, if the spatial scale is too large, it may be too costly for the spider mites to move to a plant without predators, thus, dispersal could result in death. Thus, the objective of this study was to investigate if the spider mite *T. evansi* can detect cues from predators that pose different predation risks and if it subsequently changes its behaviour to avoid predation at different spatial scales.

Materials and methods

Rearing Methods

Tomato plants (*Solanum lycopersicum*, variety Santa Clara I-5300) were grown in pots (2 and 5 L) using a commercial substrate, composed of vermiculite plus organic fertilizer. The plants were kept inside a greenhouse and fertilized with NPK (4-14-8) and superphosphate. The plants were watered one or twice per day.

The herbivorous spider mites *T. evansi* were obtained in 2002 from naturally infested tomato plants of the same variety as mentioned before, at the campus of the Federal University of Viçosa, Brazil. The spider mite was cultured on tomato leaves with the petioles in a plastic tube with water to maintain the leaf turgid. The tubes were kept in plastic trays filled with detergent and water, which served to prevent mite escapes and invasion of other arthropods. A clean tomato leaf was added to the *T. evansi* culture very two days.

The predatory mite *P. longipes* was provided by Dr. Gilberto de Moraes (University of São Paulo, Brazil) in 2006. The predatory mite *P. macropilis* was obtained from bean plants infested by *T. urticae* that were cultured in a greenhouse on the campus of the Federal University of Viçosa, Brazil. Both predators were cultured as mentioned before, but the cultures of *P. longipes* and *P. macropilis* received a leaf infested by *T. evansi* or *T. urticae* respectively every two days. Both cultures were maintained at controlled conditions in a room (25 ± 2 °C, 80 ± 10% relative humidity and 12 h light).

Small-scale experiments

Leaf discs (Ø = 2.4 cm) were cut from tomato leaves, taking care to keep the central vein exactly in the middle of the disc. The discs were placed individually in a Petri dish (Ø = 4.0 cm) on top of a hydrophilic sponge soaked in water. That water served to maintain the turgidity of the leaf. A thin line of hydrophilic cotton wool was placed on the vein, touching the water at both ends. Predatory mites cannot cross such humid cotton wool (Pallini et al. 1998).

Twenty five adult female predators were placed on one of disc halves. Hence, the two disc halves received different treatments, one half remained clean, whereas the other half received cues from the predators. The predators and their eggs were carefully removed after four hours. Subsequently the cotton wool was removed and the vein was dried with tissue paper. One female spider mite, aged 12 days, was released in the centre of each disc and its behaviour was observed for ten minutes (Fig. 1). For these experiments, two different predators were used to provide cues of predation risk, *P. longipes* and *P. macropilis.* These cues consisted of everything left by the predatory mites, such as remains of prey eggs on which they had fed, feces, exuvia and other chemical cues. Hence, the cues could be easily perceived by the spider mites because they were concentrated. The behaviour assessed was the time spent on each disc half, the time spent feeding on each disc half, and the number of times that *T. evansi* tried to escape. We considered the spider mite touching its first pair of legs in the water or moving to the other disc as escape attempts. Spider mites were considered feeding when they stopped walking and movement of fluids in the intestines could be observed. Oviposition at each side of the disc was evaluated 24 hours after the release. The data were analyzed using an ANOVA and a GLM with Poisson error distribution, respectively. Ten replicates were done.

At a slightly larger scale, entire discs of the same size as above received one treatment. A disc either received 25 female predators for four hours or was left clean. Subsequently, the predators and their eggs were removed from the discs and one adult female spider mite was released at the centre of each disc (Fig. 1). Behaviour and oviposition were observed as above. In this case, an escape attempt was inferred when the mite put its first pair of legs in the water surrounding of the disc. Behavioural data were analyzed using an ANOVA and oviposition using a GLM with Poisson error distribution. Ten replicates were done. We also compared the results of this experiment with the previous experiment using an ANOVA.

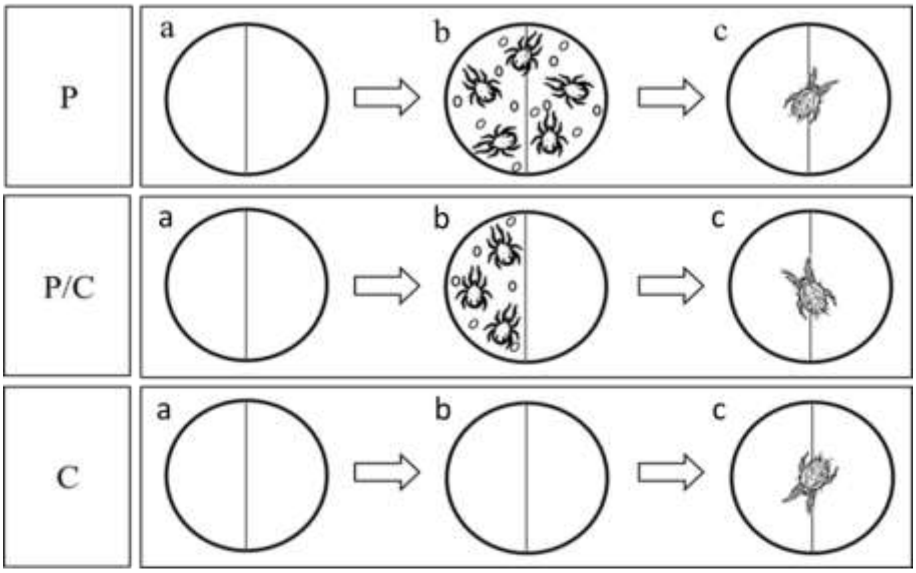

Figure 1 Illustration of the leaf disc experiments. Leaf discs were cut from tomato leaflets with the main vein kept on the centre of the leaf disc (a). Twenty five females of the predators were released on the discs according to the treatments (b). The predators and their eggs were carefully removed after four hours, subsequently one female of *T. evansi* was released in the centre of each disc (c).

Release-recapture experiments

This large scale test was done using six tomato plants that had four completely developed leaves. The 3 highest leaves of all plants were infested with *T. evansi*, around 400 spider mites per plant, for seven days. To prevent spider mites moving from the upper leaves to the lowest leaf, a ring of glue was applied to the main stem of the plants immediately above the first leaf, isolating it from the rest of the plant. Six days after infestation, the plants were allocated equidistantly in a hexagon (Ø = 80 cm), in a cage covered with thin screen (160 x 160 x 120 m) with a tarpaulin bottom (160 x 160 x 15 cm) filled with soil. The cage was situated outside, in a place with limited airflow, thus ensuring that the mites could not reach the plants through dispersal on air currents, but only by walking. The pots containing the plants were buried and soil was added to the pots so that soil levels inside and outside the pots were equal (Pallini et al. 1998, Janssen 1999). To provide cues of predation risk, 100 adult females of *P. longipes* or *P. macropilis* were added to the highest leaves with spider mites on three of the plants. Plants without predators were alternated with plants with predators in the hexagon. To control for any unforeseen directionality in the searching behaviour of the mites, care was taken that each plant position was occupied by plants without predators in half of the replicates, and by plants with predators in the other half (Janssen 1999).

Two hundred adult female spider mites, aged eight to ten days since they borne, were collected in a Petri dish, which was placed in the middle of the hexagon 24 hours after the predators were added to the plants. The spider mites could disperse freely from the Petri dish to the plants by walking over the soil to the stem of the plants, from where they could walk onto the stem until reaching the lowest leaf. The base of the stem of the tomato plants, contains a high density of trichomes, thus a wooden stick was placed near the base, touching the lowest leaf, thus forming a bridge and helping the mites to climb onto the leaves.

During 6h, the mites that arrived on the lowest leaf of the plants were counted and removed every hour. This was repeated twice after 24h and 30h. A similar experiment was done using three plants infested by *T. evansi* and three clean plants to assess that *T. evansi* was able to discriminate between plants that received different treatments in the set-up used here. The experiments were replicated four times, and data were analyzed using an ANOVA or GLM with Poisson error distribution respectively.

Here the cues provided by the predators were mainly volatile cues. Here, the spider mites were far away from the plants and probably had more difficulties to perceive the cues compared with the cues on leaf discs because they are not in direct contact with the cues. Thereby, here the predation risk may is smaller than on a leaf disc, because predator-prey ratio is smaller than on the disc leaf experiment, because the plants were previously infested with 400 females of *T. evansi* four days before. In the experiment on leaf disc we had twenty five predators on a disc with 2.4 cm of diameter, and here we had one hundred predator distributed on an entire plant.

Results

Small-scale experiments

There was no significant difference in the time spent on a disc side with or without predator cues (W= 22: df = 1: p= 0.61), time spent feeding on each disc side (W= 9: df = 1: p= 0.44) and escape attempts (F= 2.64: df = 1: P= 0.12) in the experiment where mites had a choice between half a leaf disc with cues of *P. longipes* and half a leaf disc without. Spider mites spent 41.28% of their time on the disc side with *P. longipes* cues and 49.1% on the clean side. They spent on average 28.6 seconds feeding on the disc side with *P. longipes* cues and 52.8 seconds on the clean side and tried to escape on average 1.4 times from the disc half with *P. longipes* cues and 1.1 times from the clean side. However, *T. evansi* laid more eggs on the half without *P. longipes* cues than on the clean half (F= 4.54: df = 1: P = 0.04) (Fig. 2).

With respect to the response to the other predator species, there was no significant difference in the time spent feeding (W = 13.0: df= 1: P = 0.68) and in the escape attempts (T = 2.56: df = 1: P = 0.050) on the disc half with or without *P. macropilis* cues. The spider mites spent on average 17.9 seconds feeding on the clean side and 16s on the side with *P. macropilis* cues. They tried to escape on average 3.5 times from the clean side and 5.5 times from the side with *P. macropilis* cues. The spider mites spent more time (66.7% of the time on the clean side, T= 3.99: P= 0.003), and laid more eggs (F= 4.54: df= 1: P= 0.04) (Fig. 3) on the clean side than on the side with *P. macropilis* cues.

At a slightly larger spatial scale, where entire discs received one treatment, *T. evansi* oviposited significantly less (Chi^2= 12.89: df = 1: P= 0.001; Fig. 2), attempted to escape more often (F= 10.1: df = 1: P= 0.0036; Fig. 4), and spent less time feeding (F= 5.05: df = 1: P= 0.033; Fig. 5) on the disc with cues of *P. longipes* than on the clean discs. The spider mites showed no significant difference in the oviposition rate (Chi^2= 25.0: df = 1: P= 0.55; Fig. 3), on the disc with or without *P. macropilis* cues. They tried to escape more often (F= 9.02: df = 1: P= 0.005; Fig. 6), but not showed significant difference in the time spent feeding (F= 1.27: df = 1: P= 0.29; Fig. 7) when there were *P. macropilis* cues present on either or both sides of the disc than from discs without cues.

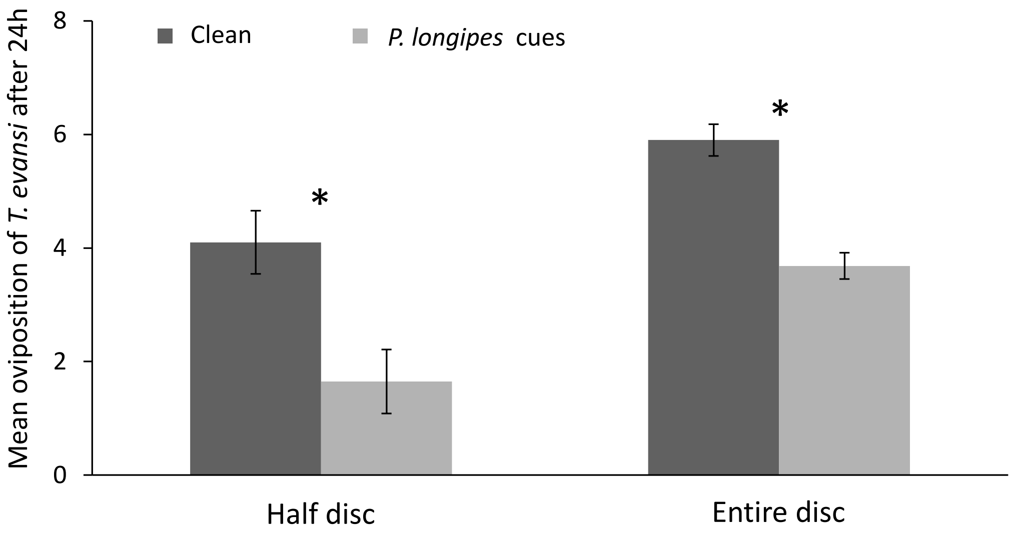

Figure 2 Mean (+ SEM, n = 10 females) oviposition of *T. evansi* after 24 hours. Entire disc refer to discs that were completely clean or with cues of *P. longipes*; half disc refer to discs that had cues of predators on half of its surface, whereas the other half was clean. Bars with asterisk indicate a significant difference between the treatments.

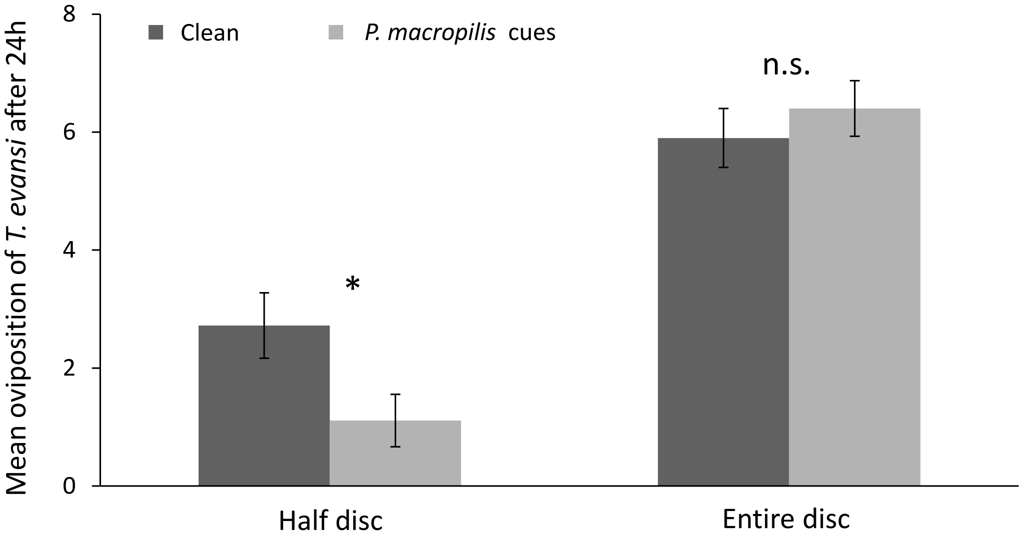

Figure 3 Mean (+ SEM, n = 10 females) oviposition of *T. evansi* after 24 hours. Entire disc refer to discs that were completely clean or with cues of *P. macropilis*; half disc refer to discs that had cues of predators on half of its surface, whereas the other half was clean. Bars with asterisk indicate a significant difference between the treatments.

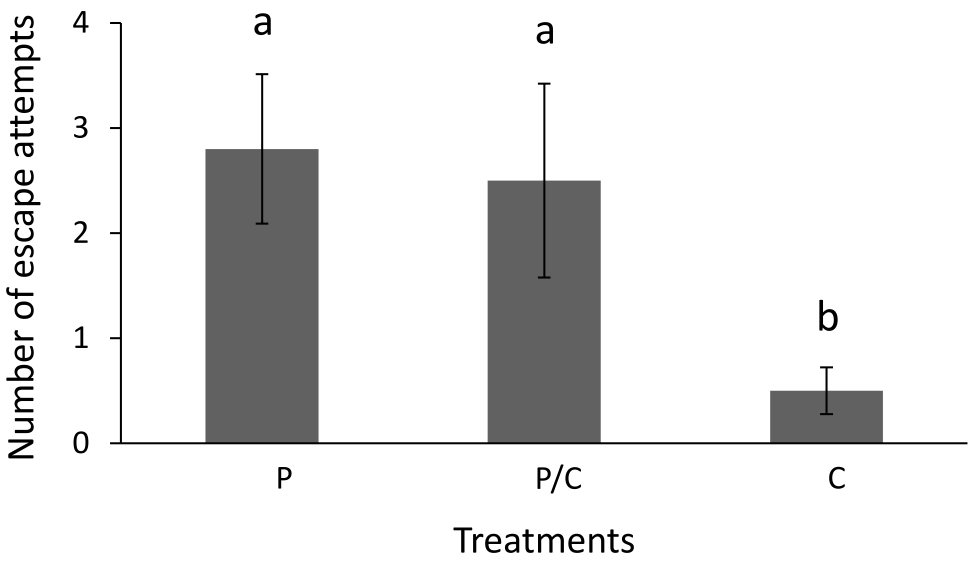

Figure 4 Mean (+ SEM, n = 10 females) frequency of escape attempts by *T. evansi*. The treatment P/C refers to experiment on discs that had cues of *P. longipes* on half of its surface, whereas the other half was clean. The treatments C and P refer to an experiment on entire leaf discs which were clean (C) or contained *P. longipes* cues (P) respectively. The vertical axis shows the mean frequency of escape attempts by *T. evansi*. Bars with different letters indicate a significant difference between the treatments.

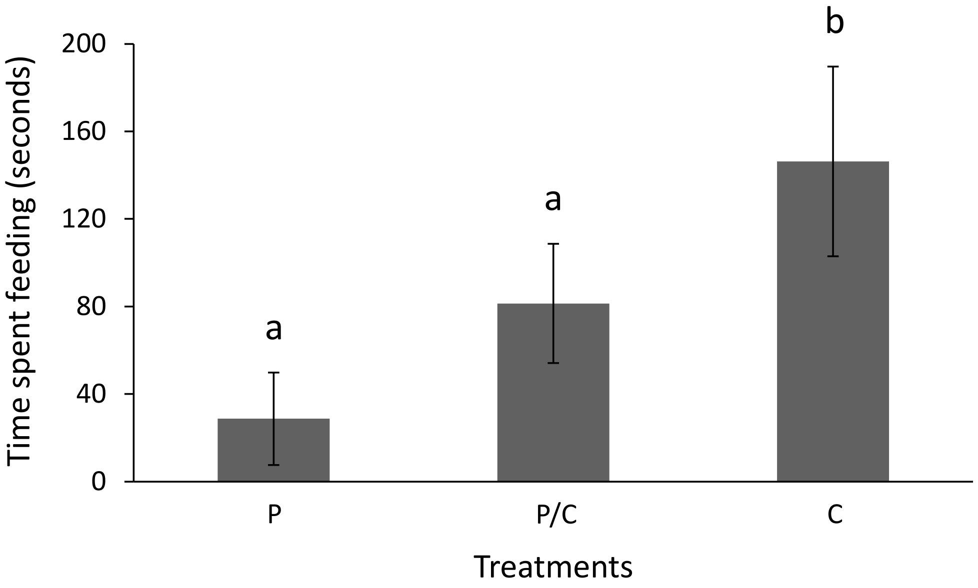

Figure 5 Mean (+ SEM, n = 10 females) time that *T. evansi* spent feeding. The treatment P/C refers to experiment on discs that had cues of *P. longipes* on half of its surface, whereas the other half was clean. The treatments C and P refer to an experiment on entire leaf discs which were clean (C) or contained *P. longipes* cues (P) respectively. The vertical axis shows the mean amount of time that *T. evansi* spent feeding. Bars with different letters indicate a significant difference between the treatments.

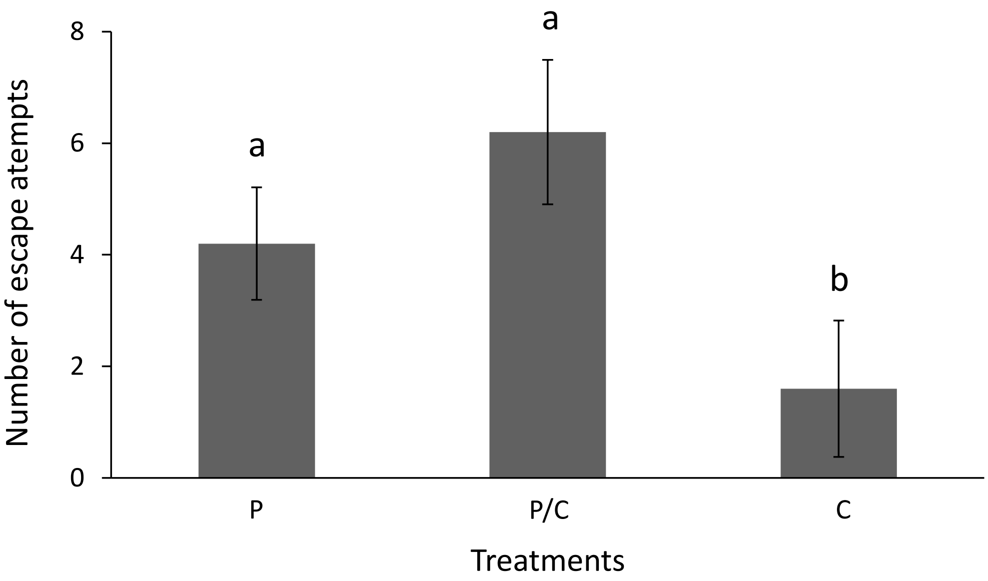

Figure 6 Mean (+ SEM, n = 10 females) frequency of escape attempts by *T. evansi*. The treatment P/C refers to experiment on discs that had cues of *P. macropilis* on half of its surface, whereas the other half was clean. The treatments C and P refer to an experiment on entire leaf discs which were clean (C) or contained *P. macropilis* cues (P) respectively. The vertical axis shows the mean frequency of escape attempts by *T. evansi*. Bars with different letters indicate a significant difference between the treatments.

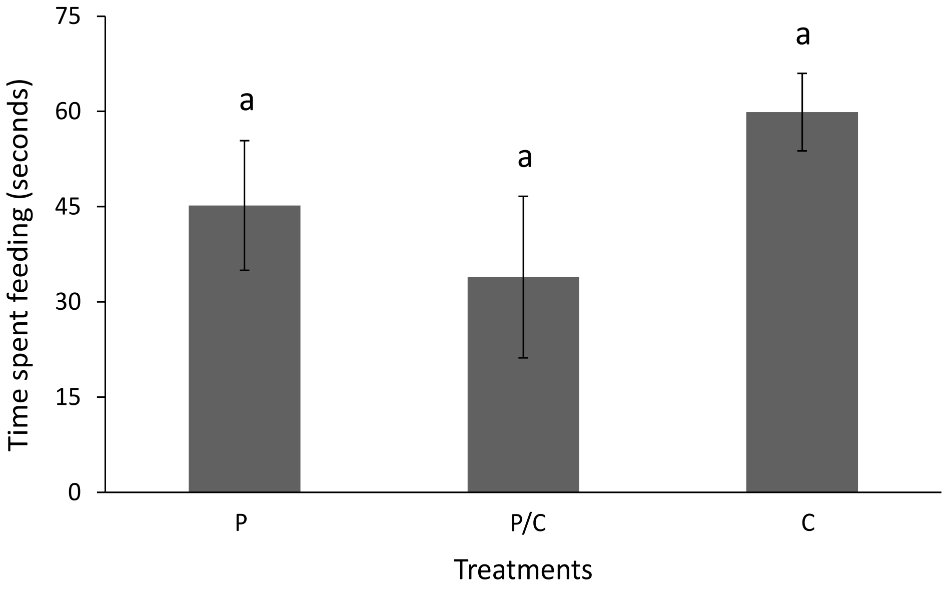

Figure 7 Mean (+ SEM, n = 10 females) time that *T. evansi* spent feeding. The treatment P/C refers to an experiment on discs that had cues of *P. macropilis* on half of its surface, whereas the other half was clean. The treatments C and P refer to an experiment on entire leaf discs which were clean (C) or contained *P. macropilis* cues (P) respectively. The vertical axis shows the mean amount of time that *T. evansi* spent feeding. Bars with different letters indicate a significant difference between the treatments.

Release-recapture experiments

In the release-recapture experiments, there was no significant difference in the numbers of *T. evansi* recaptured on plants with or without *P. longipes* cues (Chi^2 = 0.043: df = 1: P= 0.83; Fig. 8) or *P. macropilis* cues (F= 0.45: df = 1: P= 0.59; Fig. 9). However, *T. evansi* was recaptured more often on plants infested with conspecifics than on clean plants (F=10.36: df = 1: P= 0.0015; Fig. 10).

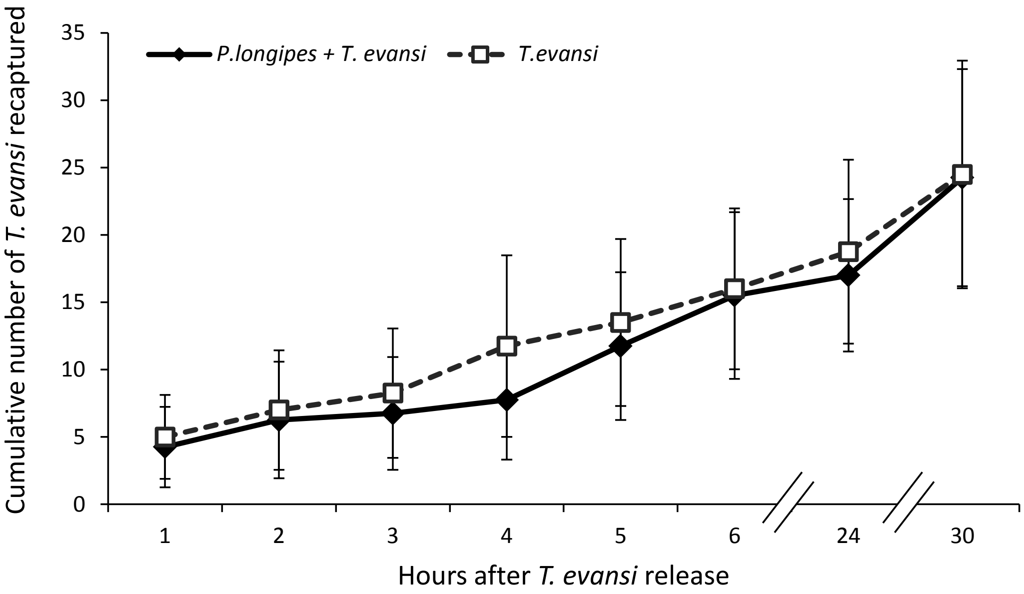

Figure 8 Cumulative number of *T. evansi* recaptured per plant through time on plants that either contained *T. evansi* (empty squares) or *T. evansi* plus the predatory mite *P. longipes* (filled diamonds).

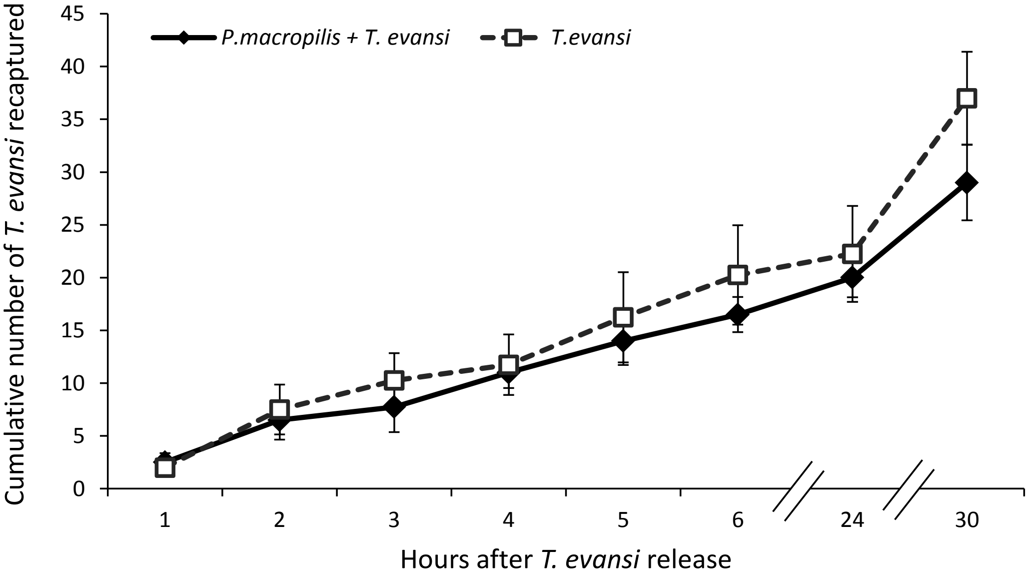

Figure 9 Cumulative number of *T. evansi* recaptured per plant through time on plants that either contained *T. evansi* (empty squares) or *T. evansi* plus the predatory mite *P. macropilis* (filled diamonds).

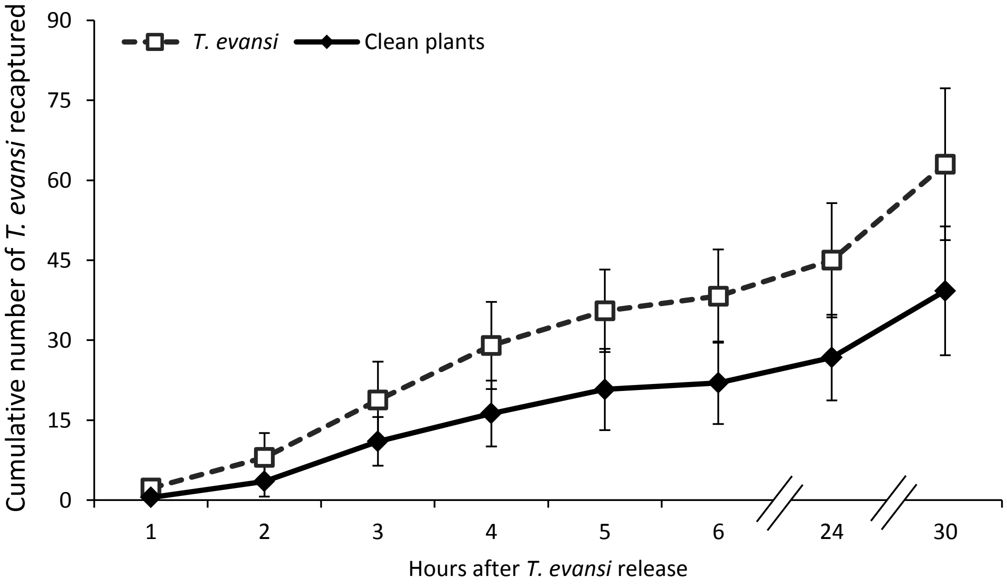

Figure 10 Cumulative number of *T. evansi* recaptured per plant through time on plants that contained *T. evansi* (empty squares) or clean plants (filled diamonds).

Discussion

The spider mite *T. evansi* did not show changes in the number of escape attempts, the time spent on each side of a leaf disc or the time spent feeding on leaf disc halves with cues of predators compared to disc halves without cues. However, the mites tried to escape more often from leaf discs with predator cues (on the entire disc or only on half of the disc) than from clean discs, and preferred to oviposit on disc halves without predator cues. It is possible that the spider mites could perceive predator cues also on the clean disc half, thus they estimate the predation risk being similar on both leaf discs halves and consequently show the same antipredator behaviour on both disc halves. This was confirmed by the higher number of escape attempts from discs with cues of predators (on the entire disc or only on half of the disc) compared with clean discs. Thus, if the spatial scale is too small, such as might have been the case with the two leaf-disc halves that received different treatments, prey perhaps do not attempt to escape by moving to the clean disc half because a predator could easily follow them.

Many prey evolved the capacity to detect cues associated with predators and change their behaviour in response to those cues even in the absence of predators (Chivers and Smith 1998, Kats and Dill 1998).Spider mites can also detect predator cues, which enable them to avoid patches with increased predation risk (Grostal and Dicke 1999, Pallini et al. 1999). However, those changes carry some costs, the spider mites may spent much time trying to escape that have a reduction on oviposition rate(Oku et al. 2004, Choh et al. 2010).Likewise, our results suggest that there are also costs involved in the behavioural changes in *T. evansi*; the oviposition on discs with *P. longipes* cues was lower than on discs without cues. However, such a response was not observed in the presence of cues of *P. macropilis*. Although *T. evansi* tried to escape less often and preferred to lay eggs on clean patches rather than on patches with cues from both predators, it showed a reduction of the time spent feeding and oviposition rate only in the presence of *P. longipes* cues. Apparently, *T. evansi* strives more to avoid predation by *P. longipes*. Possibly, avoidance of cues of *P. macropilis* occurs less because it is not an efficient predator of *T. evansi*(de Moraes and McMurtry 1985).

From a distance, *T. evansi* did not avoid plants with predators, although it is known that spider mites are able to respond to volatiles from alerted conspecifics(alarm pheromone) and odours of conspecifics and predators (Janssen et al. 1997, Pallini et al. 1999).Moreover, we know that *T. evansi* is able to recognize cues at the spatial scale studied here, because it did show a preference for plants infested by conspecifics than clean plants (Fig. 10), as was also found in olfactometer experiments (Sarmento et al. 2011). In the release-recapture experiment, we recaptured approximately 36% of the spider mites that had been released, suggesting a high mortality risk for dispersing mites. Hence, the costs of searching for plants may be higher than trying to escape from leaf discs and this may cause the spider mites to accept plants with predators, whereas they do seem to change their behaviour on leaf discs with predator cues. It is possible that *T. evansi* did not avoid plants with predators at this spatial scale in order not to run the risk of not finding plants without predators and dying during dispersal.

Additionally, we recaptured 47.1% of the spider mites from all experiment when we offered plants infested with *T. evansi* and clean plants, and only 22.4% and 30.4 % in experiments with plants infested with *T. evansi* and with *P. longipes* or *P. macropilis* respectively (F= 3.39,df=2, P= 0.06). These results indicate that the spider mites did not end up on plants more often in the presence of cues from predators than without such cues. Probably the spider mites spent more time walking trying to move far away from predators cues, thereby they not arrive on the plants. Perhaps it is difficult to discriminate between plants with and without predators from a distance, and the spider mites therefore chose to disperse away from the entire experimental arena.

Additionally, it is know that the web produced by *T. evansi* can protect them against predation (Lemos et al, 2010). It is possible that *T. evansi* did not avoid plants with predators, because on all plants tested also had *T. evansi* which can confer it a protection by the web. However, this protection does not work against *P. longipes* as works to *P. macropilis*.

Our results show that *T. evansi* is able to perceive predation risk through predator cues. However, the spider mites apparently avoid predators at a small spatial scale but not at a large scale. Perhaps the per capita predation risk on the entire plants is much lower than on the leaf discs. On the entire plants, there were much more prey, so the probability that the predator will attack one particular prey is perhaps perceived as lower than that on the leaf disc, where there was a high concentration of predator cues and killed prey eggs, but no other prey. Future work should assess the balance between the risks that *P. longipes* represents for the fitness of *T. evansi* and the cost from the antipredator behaviour.

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GENERAL CONCLUSIONS

The predatory mites *P. longipes* and *P. macropilis* are able to recognize cues from attacked plants and use them to find food. Both predators show preference for plants infested with *T. evansi* and *T. urticae* compared to uninfested plants, but they do not show preference when offered a choice between plants infested with one or the other prey species. These results suggest that these predatory mites use cues to forage, and do not discriminate between cues from plants infested by *T. evansi* or *T. urticae*.

In turn, on a small spatial scale (leaf discs), *T. evansi* is able to recognize cues from the predatory mites *P. longipes* and *P. macropilis*. On entire leaf discs with or without predators cues, the spider mites try to escape more often and spend less time feeding in the presence of cues of *P. longipes*. This is accompanied by a decrease of their oviposition rate, hence, there seem to be costs associated with this behaviour. Although *T. evansi* also try to escape more often in the presence of cues from *P. macropilis*, it does not show a reduction in the time spent feeding or in its oviposition rate. A possible explanation for this is because *P. macropilis* is not as dangerous to *T. evansi* as *P. longipes* is.

On a leaf disc of which only half contained predator cues (the other half was clean), the spider mites did not show significant differences in their behaviour on both disc halves. However, escape attempts were more frequent in the presence of predator cues, even when present on half of the disc only, than from clean discs, suggesting that the spider mites can perceive cues from short distances. Additionally, *T. evansi* preferred to oviposit and stay on the side of the leaf disc without predator cues, possibly trying to move away from sites with a high risk of predation. At a larger spatial scale (hexagon of tomato plants), *T. evansi* did not avoid plants with predators.