VINÍCIUS MOREIRA MARQUES

RESPONSES OF A NATURAL ENEMY TO PEST-INDUCED PLANT VOLATILES IN COMPLEX ENVIRONMENTS

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

Orientador: Arnoldus Rudolf Maria Janssen

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A aqueles que tanto amei e já não estão mais aqui presentes

Dedico

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ABSTRACT

MARQUES, Vinícius Moreira, M.Sc., Universidade Federal de Viçosa, April, 2021. **Responses of a natural enemy to pest-induced plant volatiles in complex environments.** Adviser: Arnoldus Rudolf Maria Janssen.

Plants produce volatiles in response to attacks by arthropod herbivores, and these volatiles can be used as cues by natural enemies to find their prey. Because these volatiles are within a variety of other odour blends, here referred to as background volatiles, natural enemy response towards prey-associated plant volatiles may be affected. Thus, this research aimed to evaluate the effect of mixing blends of volatiles under controlled conditions on the foraging behaviour of the predatory mite *Phytoseiulus macropilis*. Moreover, we investigated whether background volatiles and other cues in a release-recapture experiment under semi-field conditions would affect the response of this predatory mite. Mixing the blends emitted by *Tetranychus urticae*-infested bean plants with uninfested coriander under controlled conditions, led to a preference of predatory mites towards blends emitted by infested-bean alone over the combination. Also, I found that the volatiles of an uninfested mint plant were attractive to the predatory mite when offered against no odour source. Besides, released-recapture experiments showed a low recapture percentage for every combination of plants tested, suggesting that background volatiles from both companion plants and the environment might have hindered *P. macropilis* in localizing the plants with prey. These results indicate that experiments under semi-field conditions have a higher accuracy in predicting natural enemy behaviour when compared to olfactometer experiments, that in spite of its importance, does not allow us to predict a behaviour under more complex conditions.

Keywords: *T. urticae*. Herbivore-induced plant volatiles. Companion plants. Semi-field experiments.

RESUMO

MARQUES, Vinícius Moreira, M.Sc., Universidade Federal de Viçosa, abril de 2021. **Respostas de um inimigo natural a voláteis de plantas induzidos por pragas em ambientes complexos.** Orientador: Arnoldus Rudolf Maria Janssen.

Plantas produzem voláteis em resposta a ataques de herbívoros artrópodes, e esses voláteis podem ser usados como pistas por inimigos naturais para encontrar suas presas. Como esses voláteis estão dentro de uma variedade de outros blends de odores, aqui referidos como voláteis de fundo, a resposta natural do inimigo aos voláteis das plantas associadas às presas pode ser afetada. Assim, esta pesquisa teve como objetivo avaliar o efeito da mistura de blends de voláteis sob condições controladas no comportamento de forrageamento do ácaro predador *Phytoseiulus macropilis*. Além disso, investigamos se os voláteis de fundo e outras pistas em um experimento de soltura-recaptura em condições de semi-campo afetariam a resposta desse ácaro predador. Misturando os blends emitidos por plantas de feijão infestadas com *Tetranychus urticae* com blends de coentro não infestados sob condições controladas, levou uma preferência dos ácaros predadores por blends emitidos apenas pelo feijão infestado ao invés da combinação. Além disso, descobri que os compostos voláteis de uma planta de menta não infestada foram atrativos para o ácaro predador. Além disso, experimentos de soltura-recaptura mostraram uma baixa porcentagem de recaptura para cada uma das combinações testadas, sugerindo que os voláteis de fundo de ambas as plantas companheiras e do ambiente podem ter impedido *P. macropilis* de localizar as plantas com a presa. Esses resultados indicam que experimentos em condições de semi-campo apresentam maior acurácia em predizer o comportamento de inimigos naturais quando comparados aos experimentos de olfatômetro, que apesar de sua importância, não nos permite predizer um comportamento em condições mais complexas.

Palavras-chave: *T. urticae*. Voláteis induzidos por herbívoros. Plantas companheiras. Experimentos de semi-campo.

SUMÁRIO

GENERAL INTRODUCTION

More than three decades have passed since the start of research on herbivore-induced plant volatiles (HIPVs) in the context of tritrophic interactions. Numerous studies have provided a basis for the current knowledge, mainly exploring the role of HIPVs in recruiting arthropod predators (Dicke and Sabelis 1988) and parasitoids (Turlings and Tumlinson 1990). This research has clearly shown that plants produce volatiles in response to attacks by arthropod herbivores, and these volatiles can be used as cues by natural enemies of the herbivores to locate their prey (Vinson 1981). Currently, it is well established that, besides herbivore damage, many other stimuli trigger volatile emissions in plants, such as insects walking over plant tissues (Hall et al. 2004), egg deposition by herbivores (Hilker and Fatouros 2015), or even vibrations caused by insect feeding (Appel and Cocroft 2014). These volatiles may help natural enemies to find their prey, because cues produced by herbivores (e.g. feces, cuticle, exuviae, honeydew) are often difficult to detect at long range due to their small body size in a complex environment (Turlings et al. 1991; Sabelis et al. 1984; Vet and Dicke 1992).

Because natural enemies have to search for prey within a complex environment, they struggle with the reliability-detectability issue: host/prey-derived stimuli are the most reliable in indicating host/prey presence, accessibility and suitability but they are generally hard to detect. Plant stimuli, on the other hand, are easier to detect but are generally less reliable indicators (Dicke et al. 1990; Wiskerke et al. 1993). Studies mostly done with parasitoids, showed that overcoming this issue relies on different strategies, but the main is learning the association of the host and its food source (Dicke et al. 1990). Besides, learning may aid carnivores to cope with different volatile blends, as host/prey-associated volatiles are always present with many other chemical stimuli under natural conditions.

Plants under attack emit different compounds, including terpenoids, fatty acid derivates, phenyl propanoids and benzenoids (Mumm and Dicke 2010; Dudareva et al. 2004), which can be emitted either at the site of damage or systemically from undamaged plant parts of affected plants (Heil and Ton 2008). There are complex blends, comprising hundreds of compounds, with both HIPVs and non-induced plant compounds (van Wijk et al. 2010; Pichersky and Gershenzon 2002; Das et al. 2013). The volatile blend emitted by plants under herbivore attack provides reliable information for natural enemies about which organism is feeding on the plant, but distinguishing these HIPVs from the background volatiles is required for optimal foraging efficiency (Schröder and Hilker 2008).

When a natural enemy entered a habitat and is foraging for a resource, the habitat odour becomes background odour with respect to the resource, thus, recognizing the HIPV *per se* may not be sufficient for their optimal attraction. Instead, they must be able to cope with the plethora of background volatiles (Schroder and Hilker 2008). Even though numerous studies about the response of natural enemies to host-associated plant volatiles carefully address the question of whether previous exposure to these volatiles affects the response by associative learning or sensitization, there is a great gap of information about the effects of exposure to natural background volatiles. Most studies investigating the behavioural responses of natural enemies were conducted in the laboratory without background volatiles. Thus, studies that target the response of mixing volatiles from prey-associated plants and background volatiles will help to unravel the complexity of factors that determine successful resource location by natural enemies. Such research may also contribute to improving strategies of biological pest and crop management.

Predatory mites are an example of natural enemies that can recognize cues from plants and use these to locate prey (Sabelis and van den Baan 1983; Janssen 1999). Mites of the family Phytoseiidae are important biological control agents of different pest species worldwide, especially in protected crops (Helle and Sabelis 1986; Moraes and Flechtmann 2008; Van Lenteren et al. 2018). The feeding habit of Phytoseiidae is quite diverse. There are specialist species that prey on phytophagous mites (Moraes et al. 2004) and generalist phytoseiids that feed on small insects on the aerial plant parts (McMurtry et al. 2013). Moreover, some phytoseiids feed on plant-derived sources, for instance, pollen, nectar and plant exudates (McMurtry and Croft 1997; van Rijn and Tanigoshi; Nomikou et al. 2003). Predatory mites of other families have been mostly used for the control of undesirable edaphic phytophagous mites and insects.

The predatory mite *Phytoseiulus macropilis* along with *Phytoseiulus persimilis* are outstanding control agents for one of the most polyphagous pests in the world, the two-spotted spider mite *Tetranychus urticae*, which causes huge losses in agriculture annually. Because pesticides are increasingly losing their effectiveness against *T. urticae,* these predatory mites are currently an effective management alternative. When *T. urticae* attacks a host plant, the emission of volatile blends is induced, and these are attractive for the predatory mites (Dicke et al. 1999; Oliveira et al. 2009; Fadini et al. 2010).

This research aimed to first evaluate the response of a natural enemy to mixtures of volatiles of different plant species in a controlled laboratory setting as an initial step towards the response to volatiles under more complex field conditions. Subsequently, I investigated whether the outcomes found under controlled conditions could be extrapolated to situations resembling natural conditions, where many other volatile and non-volatile cues were present.

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CHAPTER 1

THE RESPONSE OF *Phytoseiulus macropilis* (ACARI: PHYTOSEIIDAE) TOWARDS VOLATILE MIXTURES

Abstract

Plants emit volatiles upon herbivore attack that function as a signal for natural enemies. Several studies showed that these herbivore-induced plant volatiles (HIPV) mediate interactions in a tri-trophic context by affecting natural enemy behaviour. Most studies about the role of these volatiles on natural enemy behaviour have been conducted against an odour-free background, and little is known about the influence of mixing volatiles of prey-infested plant and background volatiles from a different plant species. Here, we investigated the effect of mixing two blends of volatiles on the foraging behaviour of the predatory mite *Phytoseiulus macropilis*. The experiment was done using an olfactometer under controlled conditions, aiming to ensure the mixing of the tested volatile blends. Firstly, we tested the volatiles of two bean plants infested with spider mites, the prey of *P. macropilis*, plus volatiles of a clean coriander plant against two similarly infested bean plants. Also, we used a mint plant against no odour source (air) to test the response of *P. macropilis*. Mixing the blends emitted by *Tetranychus urticae*- infested bean plants with uninfested coriander led to a preference of predatory mites towards blends emitted by infested-bean alone over the combination. Also, we found that the volatiles of an uninfested mint plant were attractive to the predatory mite. These results are an initial step towards a better understanding of the role of volatiles of companion plants on natural enemy behaviour.

Keywords: *T. urticae*. Herbivore-induced plant volatiles. Attraction. Companion plants. Predatory mite.

INTRODUCTION

There is abundant evidence that plants infested by herbivorous arthropods emit volatile compounds, and these volatiles attract natural enemies of herbivores (predators and parasitoids) (Dicke 1994; Turlings et al. 1993; Dicke and Sabelis 1988). These chemical cues, also referred to as herbivore-induced plant volatiles (HIPV) are blends of volatile compounds that vary with plant species and herbivore species (De Moraes et al. 1998; Van Den Boom et al. 2004; De Boer et al. 2004, 2005). Other factors, such as physiological state of the plant, environmental conditions (light intensity, time of year and water stress) (Takabayashi et al. 1994; Loreto and Schnitzler 2010) and the onset of infestation (Kant et al. 2008) may influence the composition of these compounds. In nature, pest-associated volatiles are present against a background of other plant volatiles (Schröder and Hilker 2008), thus understanding how the effects of these different volatiles interact in their perception and affect foraging behaviour of natural enemies is an important step towards a better use of these arthropods in biological control programs.

The emission of the volatiles can be adaptative to plants when attracted carnivores reduce herbivore damage, and it can also be beneficial to natural enemies when the volatiles increase their foraging efficiency (Takabayashi and Dicke 1996). Many olfactometer experiments under laboratory conditions show the preference of natural enemies to infested over clean plants (Turlings et al. 1990a; Dicke et al. 1990a; Reed et al. 1995; Oliveira et al. 2009; Janssen 1999). The ability to discriminate odours may be both innate or learned, and the response to volatiles may depend on previous foraging experiences (Vet and Dicke 1992; Takabayashi et al. 2006; Janssen et al. 2014). Therefore, plant volatiles are important cues for natural enemies that are searching for prey (Hilker and McNeil 2008; Dicke et al. 2003), since cues produced by herbivores are often difficult to detect at long range because of their small body size in a vast environment (Turlings et al. 1991; Sabelis et al. 1984; Vet and Dicke 1992).

Natural enemies are able to discriminate between volatiles of the same plant species that is infested by different herbivore species, and between volatiles of different plant species infested by the same herbivore species (Turlings et al. 1990b; Vet and Dicke 1992; Dicke 1999). As in other animals, it was shown that predatory mites perceive odour mixtures as a synthetic whole, and not as elemental objects (van Wijk et al. 2008, 2011). In other words, a mixture containing several compounds is perceived as a single signal, which is different from the perception of the individual components. For instance, methyl salicylate (MeSA) which is not a spider-mite specific volatile, is attractive to predatory mites in its pure form. Thus, if the predatory mite was able to detect this component in a complex mixture, all MeSA-containing mixtures would trigger attraction, which is not the case. Some mixtures of volatiles containing MeSA are not attractive to the predatory mite (van Wijk et al. 2011). Hence, predatory mites are attracted by volatile blends and not necessarily by the individual components of these blends, and this will affect how they respond to complexes of several volatile blends.

Because predators perceive mixtures as a synthetic representation, combining mixtures of volatiles may result in the perception of a different signal. Few studies consider the effect of mixing volatile blends of plants with prey and plants without prey on the response of natural enemies (Perfecto and Vet 2003; Gohole et al. 2005). Therefore, combining an attractive volatile blend produced by a plant with herbivores with another volatile blend from the surrounding may change the perception and response of the predators. Much information is still lacking about which outcomes could possibly arise when associating cultivated crops with companion plants, mixing those blends.

Companion plants are used worldwide in agriculture, they can prevent pest outbreaks by improving landscape complexity (Vandermeer 1989; Altieri and Nicholls 2004; Togni et al. 2009, 2010). Many studies showed that plants such as spurrey (Theunissen and den Ouden 1980), peas (Kostal and Finch 1994), rye-grass (Kostal and Finch 1994) and clover (Theunissen et al. 1992; Tukahirwa and Coaker 1982), mask volatiles of the target crops, hampering host location by the pest. Also, companion plants are known to be repellent (Franck 1983; Parker et al. 2013) or physical barriers to pests (Thresh 1982) obstructing host plant location. It is possible that companion plants have the same effects on the natural enemies as they have on the herbivores, however, there are few studies showing how volatiles of companion plants affect the response of natural enemies to the volatiles of target plants with their prey. Companion plants such as mint, basil and marigold work as attractants for enemies of herbivores (Beizhou et al. 2012; Song et al. 2013; Batista et al. 2017). For example, coriander attracts coccinellids (Togni et al. 2009, 2016) and mint plants attract both predatory mites and a generalist predatory insect (Togashi et al. 2019; Rim et al. 2020). Due to their attractive traits for natural enemies and repellence to pests (Mozaffari et al. 2013; Reddy and Dolma 2018), they seem profitable candidates to serve as companion plants in many systems. However, there is little research evaluating the effect of these plants as sources of background volatiles on natural enemy behaviour.

The predatory mite *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae), along with *Phytoseiulus persimilis*, is frequently used in biological control programs (Helle and Sabelis 1985; Lindquist et al. 1996; McMurtry et al. 2013). Both belong to the Phytoseiidae, an important family of predatory mites for control of the two-spotted spider mite *Tetranychus* *urticae* Koch (Acari: Tetranychidae). The latter is one of the most polyphagous pests known, attacking over 1100 plant species (Migeon and Dorkeld 2015). When this spider mite attacks a host plant, the emission of volatile blends is induced, and these are attractive for the predatory mites (Dicke et al. 1999; Oliveira et al. 2009; Fadini et al. 2010). It has been shown that *P. persimilis* is not attracted by lima bean leaves infested with *Spodoptera exigua,* which is known to emit very similar volatile compounds as lima bean leaves infested with *T. urticae* (Horiuchi et al. 2003). This response might be due to the difference between the blends emitted upon these pest attacks. Besides, *P. persimilis* is attracted by volatile blends of eggplant, grapevine, sweet pepper, soybean and other plants induced upon of *T. urticae* attack (van den Boom 2002). Similarly, testing *P. macropilis* towards volatiles of lima bean leaves induced by *T. urticae* resulted in attraction, but other Tetranychid species (*Oligonychus ilicis* on strawberry plants and *Oligonychus ununguis* on juniper leaves) do not trigger attraction (Fadini et al. 2010; Amin et al. 2009). Lastly, *P. macropilis* preferred volatiles of *T. evansi*-infested tomato plants over clean ones (Sarmento et al. 2011). Here, I investigated the response of *P. macropilis* to volatiles of bean plants and several companion plants.Thus, the aim of this study was to investigate the response of a predatory mite (*P. macropilis*) to mixtures of volatiles of different plant species in a controlled laboratory setting, as an initial step towards a better understanding of the response of predators to volatiles under more complex field conditions.

MATERIALS AND METHODS

Clean plants

Every week, coriander seeds (*Coriandrum sativum* cv. “Verdão”, Apiaceae), and jack bean seeds (*Canavalia ensiformis* (Fabaceae)) were planted individually in a commercial plant substrate enriched with macro and micronutrients (MecPlant®) in plastic pots (18 cm diameter x 15 cm high). Mint seedlings, *Mentha piperita* L. (2-3 weeks old) were bought from the floriculture Cantinho das Flores – Viçosa, Minas Gerais. After obtaining them, we thoroughly searched the leaves under a binocular microscope and every herbivore found (spider mites and aphids) was removed. The plants were used in experiments at least 15 days after herbivore removal. Coriander plants were not attacked by herbivores as they were seeded and kept in a greenhouse. All plants were kept in a greenhouse with natural light and a temperature ranging from approximately 12ºC (during colder nights in winter) to 25ºC (maintained by ventilation) and relative humidity ranging from 60-90%. One month old coriander and mint plants were fertilized with NPK (4-14-8) and superphosphate. Bean plants were not fertilized.

Tetranychus urticae rearing

Two-spotted spider mite (*Tetranychus urticae*) rearing were started with individuals obtained from cultures from Ecotrix Biodefensivos (Viçosa, MG, Brazil), where the they were in a greenhouse on jack bean plants. For the current work, spider mites were reared on 2-3-week-old jack bean plants. The pots were isolated inside plastic trays (53 x 37 x 9 cm) surrounded by water to prevent mite escapes. New plants were added to the rearing twice a week. The rearing was kept in a room in the laboratory under controlled conditions and with adequate light for plant growth (26 ± 2 ºC, 70 ± 10% RH, 12 hours of photophase).

Phytoseiulus macropilis rearing

Phytoseiulus macropilis was also obtained from Ecotrix Biodefensivos (Viçosa, MG, Brazil). Leaves from the spider mite culture (see above) were cut and brought to the laboratory where *P. macropilis* were reared on these detached leaves, which were kept inside plastic trays (53 x 37 x 9 cm) surrounded by water to prevent both predators and prey from escaping. The rearing unit was kept under controlled conditions (26 ± 2° C, 70 ± 10% RH and 12 hours of photophase). New bean leaves with *T. urticae* were added daily to the rearing.

Olfactometer experiments

To assess the response of adult females of *P. macropilis* to blends of plant volatiles, two-choice tests were done in a Y-tube olfactometer (Sabelis and Baan 1983; Janssen 1999). The olfactometer consisted of a Y-shaped glass tube (27 cm in length × 3.5 cm in diameter), with a black Y-shaped metal wire in the middle to guide the predators, with the base of the tube connected to a pump that caused an airflow from the arms of the tube to the base (Janssen 1999). Each arm was connected to a glass container (50 x 36 x 43 cm) in which the plants serving as volatile sources were kept (Figure 1). The wind speed in each arm of the Y tube was measured with a hot-wire anemometer and calibrated to 0.50 m/s (VelociCalc® Air Velocity Meter 9545-A). When wind speeds in both arms were equal, the air flows coming from the containers formed two separate fields in the base of the Y-tube, with the interface coinciding with the metal wire (Sabelis and van de Baan 1983).

Jack bean plants used in all experiments were 2 weeks old and infested with 200 spider mites per leaf one week before the experiment started (each plant had two leaves), as was done in earlier olfactometer experiments (M.M. Fonseca, pers. comm.). Using a pipette tip connected to a vacuum pump and sealed with a mesh at the wide end, spider mites were sucked up from rearing leaves and released on a clean bean plant. The spider mites were allowed to walk onto the new plant by hanging the pipette tip with a twine on the plant petiole (Figure 2). Both coriander and mint plants used in the experiments were about 6 to 8 weeks old.

Before the experiment, females of *P. macropilis* were collected from the rearing units and starved for about two hours, because this motivates the predators to walk upwind (Sabelis and van der Weel 1993). Each replicate consisted of twenty predatory mites that made a final choice, tested individually, each mite had five minutes to choose one side and reach the end of one of the arms of the tube. After each choice, the female was removed and another was introduced into the tube. Females that did not reach the end of the tube within five minutes were scored as having made no choice and were excluded from further analysis. To correct any unforeseen asymmetry in the experimental set-up, volatile sources were switched to the opposite arm of the olfactometer after each 5 females that made a choice. Four replicates were done per combination of volatile sources, each with a new set of plants and a new group of predators.

The following experiments were done:

(a) 2 jack bean plants uninfested vs 2 jack bean plants infested with spider mites; this experiment aimed to confirm the preference of predatory mites for infested plants over clean plants in our olfactometer set up;

(b) 2 jack bean plants infested with spider mites vs. 2 jack bean plants infested with spider mites plus 1 uninfested coriander plant;
after confirming that predatory mites are attracted by infested jack bean plants (experiment a) we aimed to understand their response towards a combination with increased volatile complexity;

(c) 1 Uninfested mint plant (*Mentha piperita*) vs. clean air;
to test the potential of mint plants to attract *P. macropilis* as was reported for other predators, including *P. persimilis* (Togashi et al. 2019; Rim et al. 2020)

Both olfactometer experiments (a) and (b) were part of M.M. Fonseca's unpublished data but used in this dissertation as a complement for the second chapter.

Figure 1. Y-tube olfactometer.

The data of each olfactometer experiment were analyzed with a log-linear model for contingency tables with a Generalized Linear Model (GLM) using a Poisson error distribution (Crawley 2013). We first tested whether there was a preference for one of the arms of the olfactometer, which would point at an asymmetry in the set-up. We found no evidence for this. We subsequently assessed whether the choice of the predators differed significantly among replicates, by constructing a GLM with volatile source, replicate and their interaction as factors. All statistical analyses were done using the software R version 1.3.959 (R Core Team 2020).

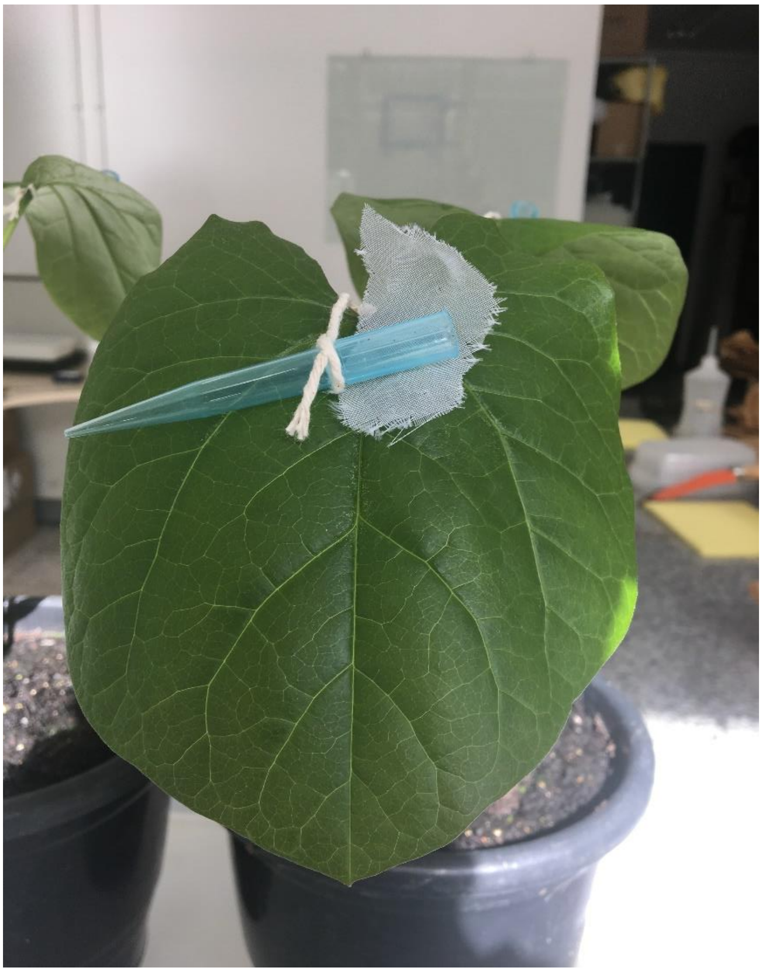

Figure 2. Pipette tip after removing the mesh to release *T. urticae*.

RESULTS

Olfactometer experiments

In the olfactometer, *Phytoseiulus macropilis* showed a significant preference for volatiles from jack bean plants infested with spider mites when volatiles of clean plants were given as alternative (Fig. 3, GLM, Chi² = 46.8, d.f. = 1, P < 0.001), confirming that the predator was capable of performing well in the olfactometer.

When the choice offered was between volatiles of infested jack bean plants versus infested jack bean plants plus one coriander plant, *P. macropilis* showed a significant preference for volatiles from jack bean plants only (Fig. 4, GLM, Chi² = 7.31, d.f. = 1, P = 0.007). This response was probably due to the masking effect of background volatiles from coriander thus reducing the attraction by spider mite infested plants.

Lastly *P. macropilis* was significantly attracted by volatiles of uninfested mint plants when offered versus clean air (Fig. 5, GLM, Chi² = 5.05, d.f. = 1, P = 0.025).

Both figure 4 and 5 show that replicate was not individually significant, however the result from four replicates were significant, indicating an overall preference for a specific treatment.

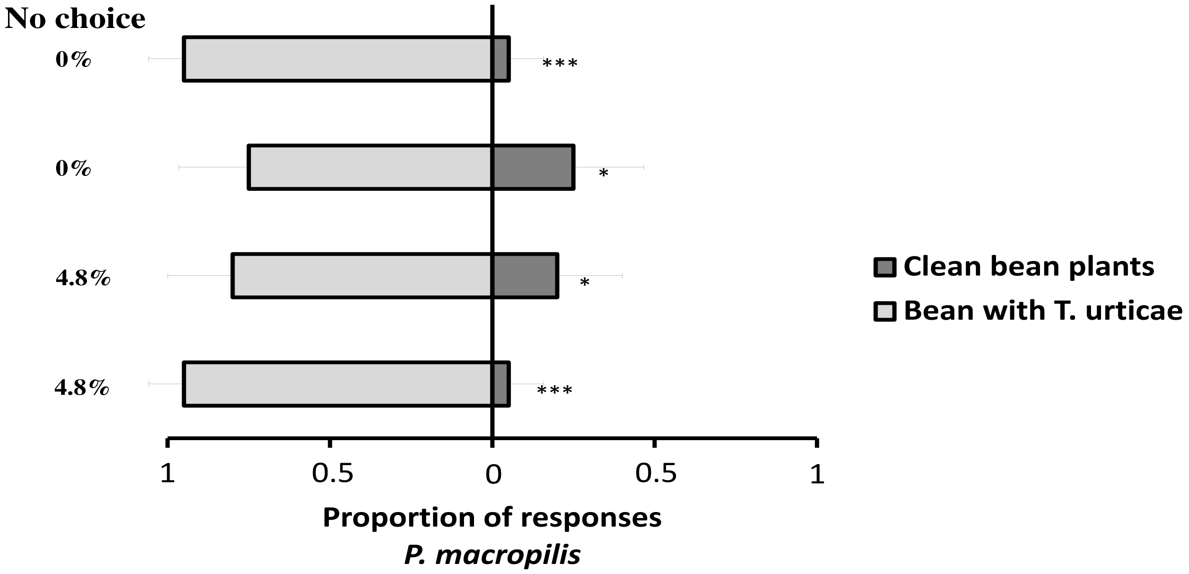

Figure 3. Response of *P. macropilis* to volatiles from clean and infested jack bean plants in an olfactometer. Each bar represents an independent replicate, each with a new set of plants and predatory mites. Right-side bars show proportion of predatory mites going to the arm with volatiles of uninfested plants, whereas left-side bars represent choice for infested plants. Asterisks indicate significance of results, ***: P < 0.001; *: P < 0.05 (Binomial Test). The choice of 20 predators were assessed per replicate. Results from MM Fonseca.

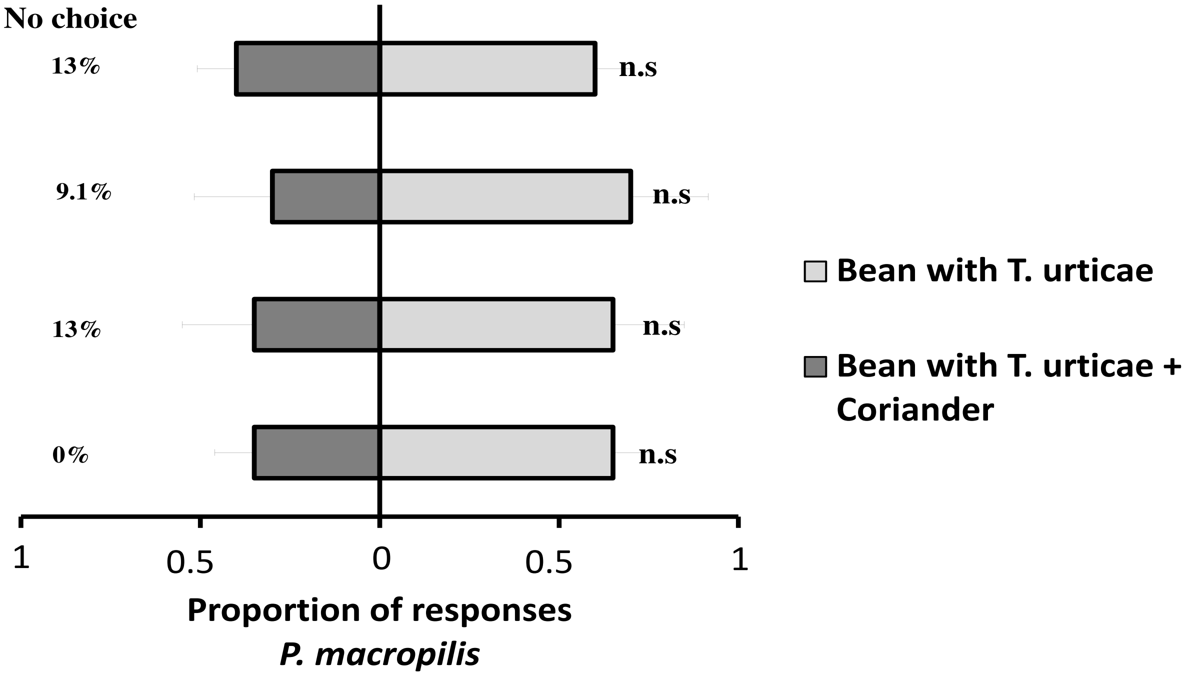

Figure 4. Response of *P. macropilis* in an olfactometer to volatiles from infested jack bean plants and infested jack bean plants plus one clean coriander plant. Each bar represents independent replicates, each with a new set of plants and predatory mites. Right-side bars show preference for infested bean plants, whereas left-side bars represent the combination of bean plus coriander. n.s indicate no statistical difference between treatments (Binomial test). The choice of 20 predators were assessed per replicate. Results from MM Fonseca.

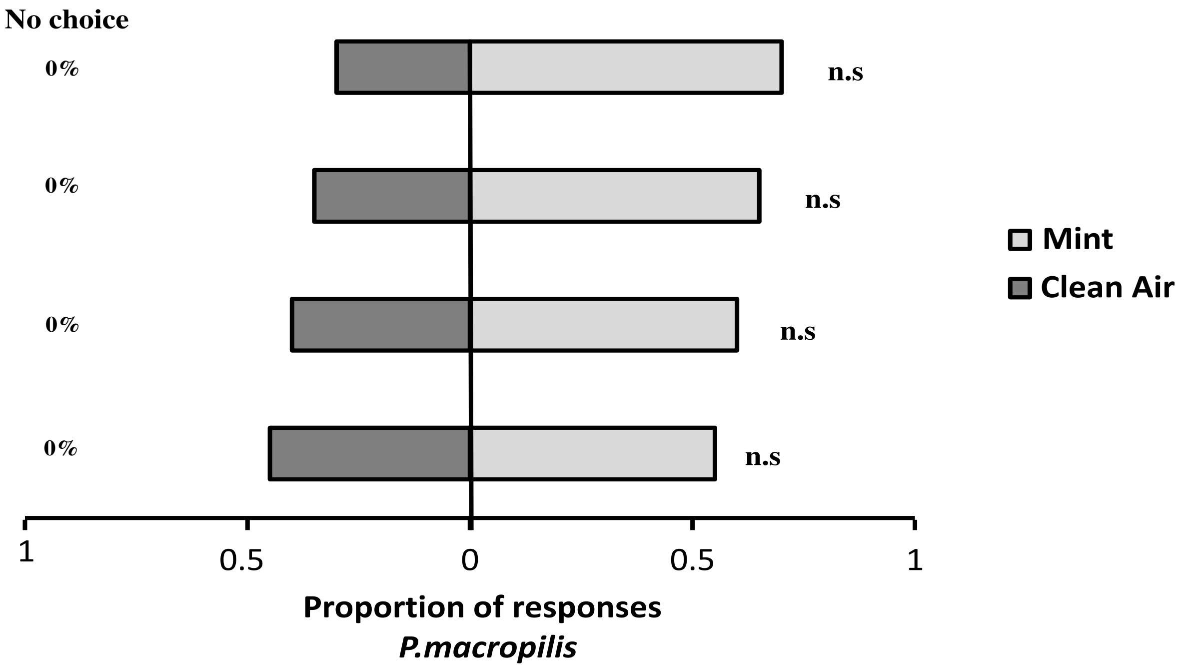

Figure 5. Response of *P. macropilis* in an olfactometer to volatiles from uninfested mint plant and no odour source (air). Each bar represents independent replicates, each with a new set of plants and predatory mites. Right-side bars show preference for mint plant, whereas left-side bars represent no odour source. n.s indicate no statistical difference between treatments (Binomial test). The choice of 20 predators were assessed per replicate.

DISCUSSION

The olfactometer experiments show that *P. macropilis* prefers volatiles from bean plants infested with spider mites over volatiles produced by clean bean plants (Fig 1). Hence, the predators were able to discriminate between volatiles from infested and uninfested plants, as has been found before (Dicke and Sabelis 1988; Dicke et al. 1990a; Janssen et al. 1997; Sabelis 1999; Oliveira et al. 2009). Yet, how *P. macropilis* females in our experiments were able to distinguish between volatile blends? It is known that the volatile blends of spider-mite-infested and uninfested conspecific plants share many compounds. For instance, spider-mite-infested tomato plants emit substantial amounts of 19 compounds, 5 of which change significantly in concentration after spider-mite infestation (van Wijk et al. 2010). If those compounds were perceived individually as attractants or repellents, the additive effect of the mixture would be a close estimation of mixture attractiveness. However, many compounds may compete for the same receptor site (Oka et al. 2004; Rospars et al. 2008), or may affect each other's representation through cross-glomerular circuit interactions in the olfactory system (Kay and Stopfer 2006; Riffell et al. 2009). Therefore, the possible outcome of odour perception is that the whole blend is perceived as a singular odour, different from the perception of the sum of its individual components (van Wijk et al. 2010). Thus, the blend produced by infested bean plants can be perceived by *P. macropilis* females in a different way as the blend emitted by uninfested ones.

An interesting response was found when a choice offered was between volatiles of infested jack bean plants versus infested jack bean plants plus one coriander plant. This combination was much less attractive (35%) than infested bean plants without coriander. Possibly, the mixture formed by bean plus coriander volatile blends is perceived by the predators as a novel blend, no longer recognized as a blend signalling the presence of prey on a plant. Because we know that infested bean is very attractive to predatory mites, we suggest that a masking effect of the combination caused the preference to infested bean without coriander. To our knowledge, there is no other study showing a masking or repellence effect of coriander plants on predatory mites. Togni et al. (2010) reported a masking effect of coriander in the herbivorous insect *Bemisia tabaci* biotype B, one of the most important pests of tomato worldwide. In this study, coriander plants were tested in a greenhouse and in laboratory olfactometer experiments, as whiteflies rely on both visual and volatile cues during foraging. In a four-arm olfactometer ('x' type) experiments, the authors tested the effect of mixing volatile blends from coriander plus tomato, against tomato constitutive volatiles, coriander and humidified air. Similar to our experiments, coriander used as a companion plant led to a lower preference towards this treatment, indicating that the novel blend formed by this mixture was unattractive to the herbivore insect. Besides, visual stimuli under greenhouse conditions were clearly an elicitor for avoidance of coriander plants by *B. tabaci,* as the majority of released individuals quickly colonised the monoculture plots (tomato only), over intercropped plots (tomato plus coriander). Differently from the insect, predatory mites do not rely on visual stimuli but rather on other non-volatile cues. To further investigate mite behaviour, one could test coriander alone versus air, aiming to unravel if there is a repellence to natural blends emitted constitutively.

The experiment using mint plants against clean air showed a preference of predatory mites towards mint odours. In total, mint plants attracted 62.5% of all predators. Togashi et al. (2019) identified the headspace volatiles from candy mint (*Mentha piperita*), in a series of experiments using *P. persimilis*, showing that none of the single mint compounds appears to attract the predatory mite, but instead, the precisely composed natural blend of mint is the elicitor for attraction. Interestingly, the response of *P. persimilis* used in his experiments was positive towards mint plants, even when tested against tested against *T. urticae*- infested beans. As we previously discussed, volatiles do not seem to be perceived as single compounds in the blend, so the attraction of *P. macropilis* in our work towards mint plants might be due to the natural blend emitted by mint plants and possibly, an innate attraction could be influencing it, but further experiments are required to elucidate this.

Lastly, abiotic factors such as temperature, air velocity and light intensity was standardized in the laboratory during experiments, thus care was taken so that these factors did not affect the choice of the predators. Some of the biotic factors were also standardized, such as experience of predatory mites and starvation time, as they may directly affect mite behaviour. Studies showed that attraction of some predatory mites towards a volatile blend depends on previous experience (Dicke et al. 1990b; Drukker et al. 2000; Maeda et al. 2006), for instance, *Neoseiulus womersleyi* reared on *Tetranychus kanzawai*-infested tea leaves showed significant preference for a synthetic blend mimicking infested tea leaves (Maeda et al. 2006), however, mixtures lacking some of the compounds that formed the mimicking blend were not attractive We therefore ensured that predatory mites used in our experiments were reared on infested bean leaves. Besides, we ensured that *P. macropilis* were kept with no food for a few hours prior to experiments, because it is known that starved *P. persimilis* females turn less and walk faster (Bernstein 1983) and are more likely to move upwind (Sabelis and van der Weel 1993) than satiated predators, even without upwind volatiles.

The results reported here are an initial step towards a better understanding of the role of companion plant volatiles on the behaviour of natural enemies. The behaviour of natural enemies in complex plant communities with different combinations of environmental cues has to be investigated in more detail, starting from semi-field setups and subsequently increasing the complexity of experimental conditions step by step. The present study is a first step towards understanding the behaviour of natural enemies in the field.

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CHAPTER 2

THE IMPACT OF BACKGROUND VOLATILES ON THE FORAGING BEHAVIOUR OF *Phytoseiulus macropilis* (ACARI: PHYTOSEIIDAE)

Abstract

Natural enemies of herbivores are able to find their prey using volatiles produced by plants under attack by this prey. These so-called herbivore-induced plant volatiles (HIPV) vary with plant and herbivore species as well as other biotic and abiotic factors. Discriminating HIPV from background volatiles under natural conditions is a hard task for natural enemies, because of the complex infochemical environment. Therefore, the aim of our study was to investigate how background volatiles would affect the response of the predatory mite *Phytoseiulus macropilis*, a natural enemy of *Tetranychus urticae*, an important pest of many plants, towards plants infested with this pest. Release-recapture experiments were done under semi-field conditions, which means that natural variation in other cues and parameters, such as light intensity, temperature and wind could also affect the foraging behaviour of predators. The experiments consisted of releasing the predators in the centre of a hexagon with six *T. urticae*-infested bean plants, half of which were accompanied by a companion plant, consisting of either coriander or mint plants were. The results show a low recapture percentage for every combination of plants, suggesting that background volatiles from both companion plants and the environment might have hindered *P. macropilis* in localizing the plants with prey. Besides, the preference of *P. macropilis* for infested bean plants either with or without companion plants differed from previous laboratory studies. For example, an olfactometer experiment showed that infested bean plants combined with a coriander plant was less attractive than infested bean plants without an accompanying coriander plant, but this was not found in the current experiments. This shows that laboratory results on foraging behaviour of these natural enemies cannot be extrapolated to field situations. Thus, semi-field experiments improve our understanding of how natural enemies behave under such complex environments, which is an important step towards improvement of the release efficiency in biological control programs.

Keywords: *T. urticae*. Herbivore-induced plant volatiles. Companion plants. Semi-field experiments.

INTRODUCTION

Predatory mites are known to be blind and thus rely mainly on volatiles to locate plants with prey at a distance (Sabelis and Van der Baan 1983; Sabelis et al. 1984). While foraging, they are attracted by plants under herbivore attack; these plants emit volatiles consisting of a complex mixture, with both herbivore-induced plant volatiles (HIPV) and non-induced plant compounds (van Wijk et al. 2010; Dicke and van Loon 2000; Kessler and Baldwin 2001; Pichersky and Gershenzon 2002; Das et al. 2013). Much laboratory research that explored the behavioural responses of herbivores and carnivores towards volatiles were conducted under controlled laboratory conditions without background volatiles (Dicke et al. 2003). Yet, in nature, volatiles of plants are present against a background of other plants and various biotic and abiotic volatile sources (Schröder and Hilker 2008).

Discriminating among volatiles is a hard task for predators. Van Wijk et al (2011) showed that predators perceive odour mixtures (blends) as a synthetic whole rather than a collection of individual components. This study with the predatory mite *Phytoseiulus persimilis* was performed using several herbivore-induced lima bean volatiles, (*E*)- and (*Z*)-β-ocimene, cis-3-hexenyl acetate, DMNT, TMTT and methyl salicylate, and only the last of these compounds was attractive when presented alone. Yet, the mixture of all five was more attractive than individual compounds or combinations of these compounds. The compound cis-3-hexenyl acetate was repellent when tested individually but was a key component to make the blend attractive. This shows that the response to blends of two or more compounds cannot be predicted from the response to the separate compounds (van Wijk et al. 2010). This may help the predators to cope with information with chemical overlapping compositions (van Wijk et al. 2008), but may also impede the proper identification of volatiles associated with the presence of prey, as a novel volatile blend can be formed when background volatiles are present, turning the prey-associated odour into a blend that is no longer recognizable. It is therefore important to investigate the searching behaviour of predators and parasitoids when searching for plants with their prey in the field, where the volatiles produced by the plants are mixed with volatiles from the environment, and where the natural enemies can also use other, non-volatile cues to detect plants with prey.

Background volatiles are usually thought to mask the perception of resource-indicating volatiles, reducing the attraction towards these attractants, however, some studies show that this is not always the case (Hilker and McNeil 2008). There are three distinct responses to be found in the interaction among volatiles from resources and background volatiles: (1) There is no effect on the response of predators to target volatiles (Dicke et al. 2003; Wäschke et al. 2014; Salamanca et al. 2015), because the olfactory system is fully adapted (habituated) to the background volatiles (Schröder and Hilker 2008), (2) it may enhance the response to target volatiles (Gebreziher and Nakamuta 2016; Hilker et al. 2002; Mumm et al. 2003) as they may provide a contrast that favours the detection of the resource volatiles (Hilker and McNeil 2008), or (3) it may mask the target volatiles (Schröder and Hilker 2008). Most of these studies were performed with parasitoids, herbivores, and predatory insects, frequently using simple background volatiles (one or a few volatile components) (Webster et al. 2010) but seldom with complex background volatiles (for review see Randlkofer et al. 2010). Olfactometer experiments are usually performed to understand how predators and parasitoids respond to specific combinations of volatiles. Thus, studies on the response to the same volatiles under semi-field conditions are needed to establish how responses in the laboratory translate to behaviour of predators and parasitoids under more natural conditions. Elucidating this response will show how behaviour under controlled conditions in the lab translates to behaviour under complex field conditions, and this is the aim of this chapter. This knowledge can aid in improving release methods of natural enemies in the field to increase efficiency of biological control.

Experiments under natural conditions are completely different from just combining odour sources because predators can freely explore the surrounding environment and are affected by many other stimuli, hence, not only volatiles, but also other cues may affect the response of predators and parasitoids. Here our aims were to elucidate if background volatiles would affect the predator behaviour, using a well-known acarine system.

The predatory mite *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) belongs to an important family of predatory mites, widely used in biological control programs (Helle and Sabelis 1985; Lindquist et al. 1996; McMurtry et al. 2013). The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is among the most polyphagous pests known, attacking over 1100 plant species (Migeon and Dorkeld 2015). It is frequently controlled by *P. persimilis*, *P. macropilis* and other Phytoseiid mites. When this spider mite attacks a host plant, the emission of volatile blends is induced, and these are attractive for *P. macropilis* (Oliveira et al. 2009, Fadini et al. 2010). However, it remains to be investigated whether the response to plant volatiles induced by their prey is affected by background volatiles.

In previous olfactometer experiments, the effects of the presence of another plant species on the attraction of *P. macropilis* towards volatiles of *T. urticae* was tested (M.M. Fonseca, pers. comm. and Chapter 1). An interesting response was found when bean plants infested with spider mites, known to emit volatiles that are attractive to the predators, were combined with coriander plants resulting in reduced attractiveness. Besides, we found mint (*Menta piperita*) to be attractive to predatory mites when offered against air (Chapter 1). Specifically, our aim was to investigate if the masking/attraction effect in the presence of coriander or mint plants respectively, would arise under semi-field conditions with complex background volatiles.

MATERIALS AND METHODS

Clean plants

Every week, coriander seeds (*Coriandrum sativum* cv. “Verdão”, Apiaceae), and jack bean seeds (*Canavalia ensiformis* (Fabaceae)) were planted individually in a commercial plant substrate enriched with macro and micronutrients (MecPlant®) in plastic pots (18 cm diameter x 15 cm high). Mint seedlings, *Mentha piperita* L. (2-3 weeks old) were bought from the floriculture Cantinho das Flores – Viçosa, Minas Gerais. After obtaining them, we thoroughly searched the leaves under a binocular microscope and every herbivore found (spider mites and aphids) was removed. The plants were used in experiments at least 15 days after herbivore removal. Coriander plants were not attacked by herbivores as they were seeded and kept in a greenhouse. All plants were kept in a greenhouse with natural light and a temperature ranging from approximately 12ºC (during colder nights in winter) to 25ºC (maintained by ventilation) and relative humidity ranging from 60-90%. One month old coriander and mint plants were fertilized with NPK (4-14-8) and superphosphate. Bean plants were not fertilized.

Tetranychus urticae rearing

Two-spotted spider mite (*Tetranychus urticae*) rearing were started with individuals obtained from cultures from Ecotrix Biodefensivos (Viçosa, MG, Brazil), where the they were in a greenhouse on jack bean plants. For the current work, spider mites were reared on 2-3-week-old jack bean plants. The pots were isolated inside plastic trays (53 x 37 x 9 cm) surrounded by water to prevent mite escapes. New plants were added to the rearing twice a week. The rearing was kept in a room in the laboratory under controlled conditions and with adequate light for plant growth (26 ± 2 ºC, 70 ± 10% RH, 12 hours of photophase).

Phytoseiulus macropilis rearing

Phytoseiulus macropilis was also obtained from Ecotrix Biodefensivos (Viçosa, MG, Brazil). Leaves from the spider mite culture (see above) were cut and brought to the laboratory where *P. macropilis* were reared on these detached leaves, which were kept inside plastic trays (53 x 37 x 9 cm) surrounded by water to prevent both predators and prey from escaping. The rearing unit was kept under controlled conditions (26 ± 2° C, 70 ± 10% RH and 12 hours of photophase). New bean leaves with *T. urticae* were added daily to the rearing.

Release-recapture experiments

To assess the response of *P. macropilis* to volatiles in a more complex environment, a release–recapture experiment was conducted where pots with plants (18 cm diameter x 15 cm high) were arranged in a hexagon in a wooden tray (2.50 x 2.50 m, 0.2 m high) supported by trestles (0.9 x 1 m). The tray was lined with an impermeable plastic sheet and filled with plant growing substrate (as above). Pots had their rims just below the soil surface (Pallini et al., 1997). The tray offered space to an inner and outer hexagon, with a radius of 50 cm and 83 cm respectively. Each position had a unique identification; those forming the inner circle of hexagon were A1, B1, C1, D1, E1, F1 matching with the compass directions SE, S, W, NW, NE, and E respectively, and those forming the outer circle were A2, B2, C2, D2, E2 and F2 matching with same positions as above (Figure 1). The experiments were conducted outdoors, in an area enclosed by a building on one side and a steep slope with native vegetation on the other side. A cover was built to protect the plants from rain and avoid direct sunlight and desiccation of the substrate (Figure 2).

We previously found that using one infested bean plant per pot lead to low recapture numbers, thus five infested bean plants per pot were used for all experiments. Moreover, to assess if there was an interaction between total recapture of predatory mites and spider mites per plant, plants were cut and taken to the laboratory after each replicate, and *T. urticae* adults were counted under a binocular microscope. Bean plants were infested one week before the experiment started, as was done in earlier olfactometer experiments (M.M. Fonseca, pers. comm. and Chapter 1). Each plant was supported by a wooden stick to prevent leaves from touching the substrate. Besides, in experiments where plants occupied the inner and outer circle, care was taken that plants did not touch each other. One-month-old jack bean plants and 6 to 8 weeks-old coriander and mint plants were used in this experiment.

To investigate the effect of coriander plants on the behaviour of the predatory mites, coriander plants occupied three positions of the outer circle, and *T. urticae* infested jack bean plants occupied all positions in the inner circle (Figure 3). Four replicates were done, each with a new set of plants, and changing the positions of coriander plants among replicates. This was done to control any unforeseen directionally in the searching behaviour of the mites (Janssen 1999). To verify the effect of mint plants on the behaviour of the predatory mites, four replicates were done following the same proceeding as above (Figure 4). Positions that were not occupied by plants in the outer circle were filled with substrate. Two different controls were performed: (1) alternated clean and infested bean plants in the inner positions and (2) infested bean plants occupying all inner positions. The first was performed aiming to evaluate the response to infested bean plants and uninfested bean plants under semi-field conditions. Comparing this response to our previous olfactometer experiments would allow us to state if background volatiles and other cues affect the predator behaviour. The second was performed aiming to compare the total recapture of the predatory mite with the experiments with coriander and mint, when no volatile sources from companion plants were present, so that we could state if companion plants were affecting predator behaviour.

Prior to experiments, 160 non-starved *P. macropilis* females were sucked up from the rearing units, using a pipette tip connected to a vacuum pump and sealed with a mesh at the wide end. Subsequently, they were carefully shaken from the pipette tip into a Petri dish placed in the centre of the hexagon, from where they could walk over the substrate towards the plants. The first evaluation was done one hour after release by carefully searching for the mites on both plant leaves and stems. Females were removed from the plants, aiming to reduce the effect of differential arrestment of mites on plants with different treatments. This evaluation was repeated 2, 3, 4, 5, 6, 24, 30 and 48 hours after the start. The data were analysed using Generalized Linear Models (GLM) with a Poisson or quasi-Poisson error distribution, in R Statistical Software (R Core Team 2020).

Figure 1. Set-up for the release–recapture experiments without cover.

Figure 2. Cover with shade net (side net was removed for experiments).

Figure 3. Set-up for coriander experiments. Inner circle occupied by jack bean plants infested with spider mites, outer circle occupied by interspersed coriander plants.

Figure 4. Set-up for mint experiments. Inner circle occupied by jack bean plants infested with spider mites, outer circle occupied by uninfested mint plants.

RESULTS

Infested jack bean plants versus uninfested jack bean plants

A significantly larger proportion of *P. macropilis* was recaptured on infested bean plants compared with uninfested plants (Fig. 5, GLM, F = 24.9, d.f = 1, P < 0.001). The effects of the interactions between treatment and position and between treatment and replicate on plant choice were not significant (Fig. 5, GLM, F = 0.13, d.f = 4, P = 0.96) (Fig. 5, GLM, F = 0.74, d.f = 2, P = 0.5), showing that the choice was consistent among replicates and among plant positions. Recapture numbers varied among replicates (Fig. 5, GLM, F = 3.4, d.f = 3, P < 0.05). Besides, plant position had significant effect on the number of recaptured mites (Fig.6, GLM, F = 6.32, d.f = 5, P < 0.01). Most of the predatory mites were recaptured on the plants E, NW and SE of the release point. In one of the four replicates we found no dead predatory mites in the release site (Petri dish), and in another we found five dead predators in the Petri dish, no data are available for the remaining replicates. This shows that by far most predators left the Petri dish, and that the low recapture rates were not caused by mortality due to handling or releasing the predators. Concluding, the predators showed a similar preference for infested bean plants as was found in olfactometer experiments (Chapter 1), despite the presence of background volatiles and other cues from the environment.

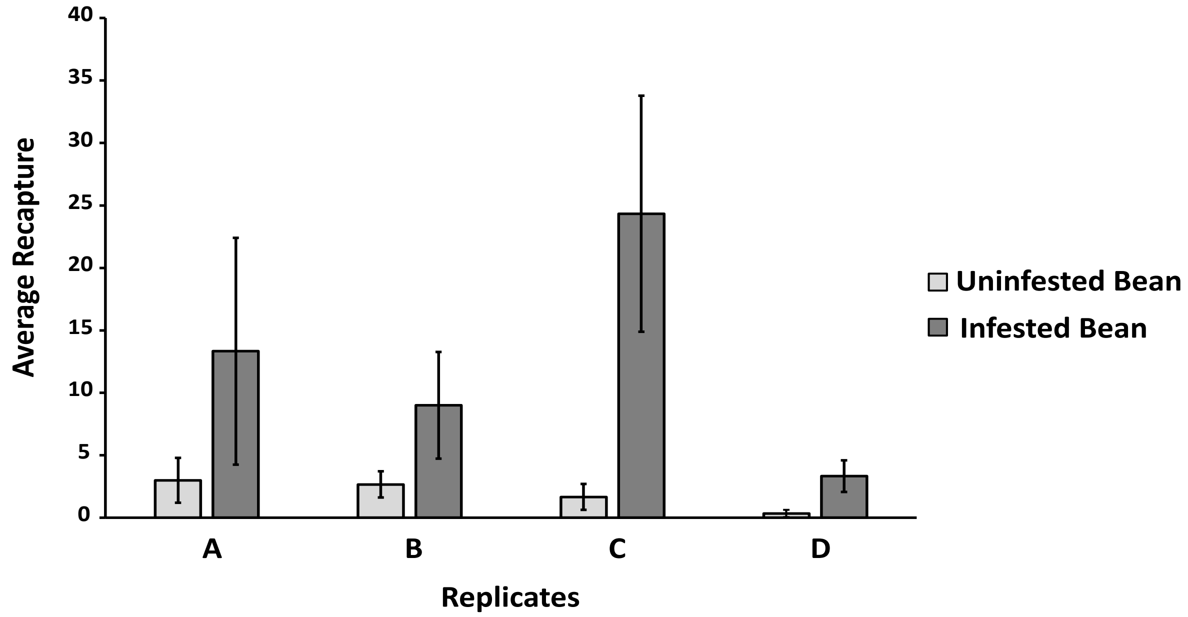

Figure 5. Average number of *P. macropilis* recaptured on three plants of each treatment per replicate. Each replicate had two treatments (uninfested bean vs infested bean). Error bars show the SEM of the mean number of predatory mites recaptured.

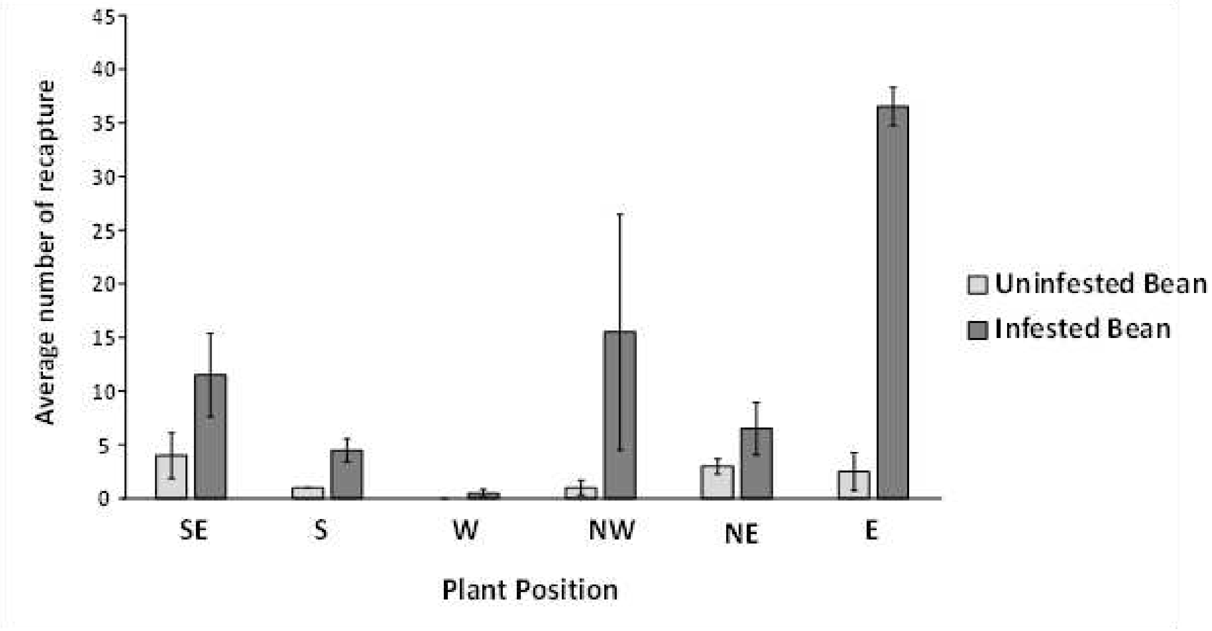

Figure 6. Average number of *P. macropilis* recaptured on the plants per position. Abbreviations under X-axis refer to position of the plants relative to the release point: SE = southeast, S = south, W = west, NW = northwest, NE = northeast and E = east. Error bars show the SEM of the mean number of predatory mites recaptured.

Infested bean plants versus infested bean plants plus coriander

There was no significant difference in the proportion of *P. macropilis* recaptured on infested bean plants compared with infested bean plants plus coriander (Fig. 7, GLM, F = 0.81, d.f = 1, P = 0.38). The effects of the interactions between treatment and position and between treatment and replicate on plant choice were not significant (Fig. 7, GLM, F = 1.13, d.f = 4, P = 0.41) (Fig. 7, GLM, F = 2.39, d.f = 2, P = 0.13), showing that the choice was consistent among replicates and among plant positions. The number recaptured was similar among replicates (Fig. 7, GLM, F = 0.57, d.f = 3, $P < 0.05$). However, plant position had significant effect on the number of recaptured mites (Fig.8, GLM, F = 9.08, d.f = 5, $P < 0.001$). Most of the predatory mites were recaptured in E, S and SE positions. The numbers of dead predators found in the Petri dishes after the experiment were again low: four in one replicate and five in another, no data are available for the remaining replicates. Concluding, the response of the predators was not impaired by the presence of coriander plants when background volatiles and other environment cues were present, which means that coriander does not affect the predator attraction towards infested plants in this context.

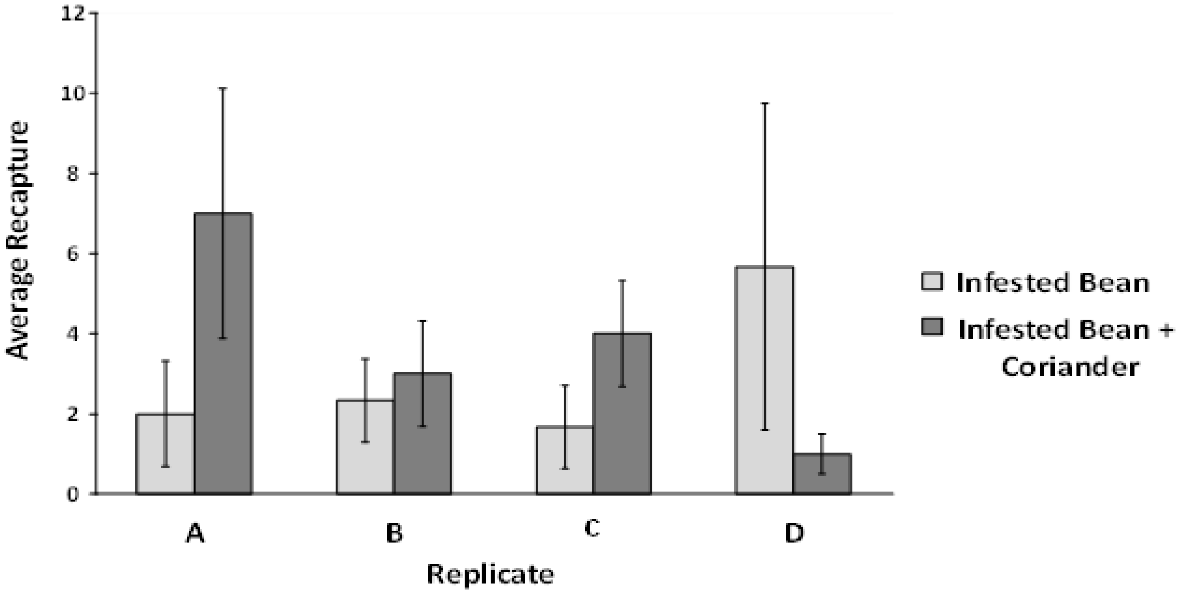

Figure 7. Average number of *P. macropilis* recaptured on three plants of each treatment per replicate. Each replicate had two treatments (infested bean vs infested bean plus a clean coriander plant). Error bars show the SEM of the mean number of predatory mites recaptured.

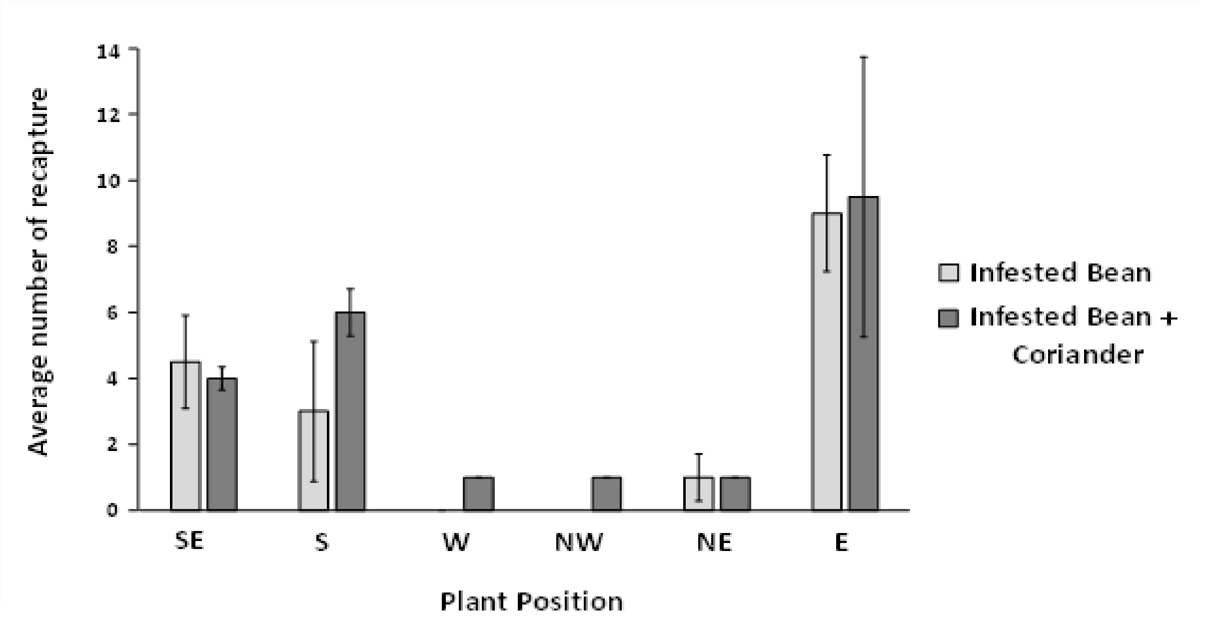

Figure 8. Average number of *P. macropilis* recaptured on the plants per position. Abbreviations under X-axis refer to position of the plants relative to the release point: SE = southeast, S = south, W = west, NW = northwest, NE = northeast and E = east. Error bars show the SEM of the mean number of predatory mites recaptured.

Infested bean plants versus infested bean plants plus mint

A significantly larger proportion of *P. macropilis* was recaptured on infested bean plants compared with infested plants plus mint (Fig. 9, GLM, F = 6.85, d.f = 1, P = 0.02). The effects of the interactions between treatment and position and between treatment and replicate on plant choice were not significant (Fig. 9, GLM, F = 1.15, d.f = 4, P = 0.40) (Fig. 9, GLM, F = 1.31, d.f = 2, P = 0.31), showing that the choice was consistent among replicates and among plant positions. Recapture number varied among replicates (Fig. 9, GLM, F = 12.32, d.f = 3, P < 0.0001). Besides, plant position had significant effect on the number of recaptured mites (Fig.10, GLM, F = 8.88, d.f = 5, P < 0.001). Most of the predatory mites were recaptured in E, SE, N and NE positions. In one of the four replicates we found no dead predatory mites in the release site (Petri dish), and in another we found one dead predator in the Petri dish, no data are available for the remaining replicates. Concluding, the predators showed preference for infested bean plants without background volatiles of mint.

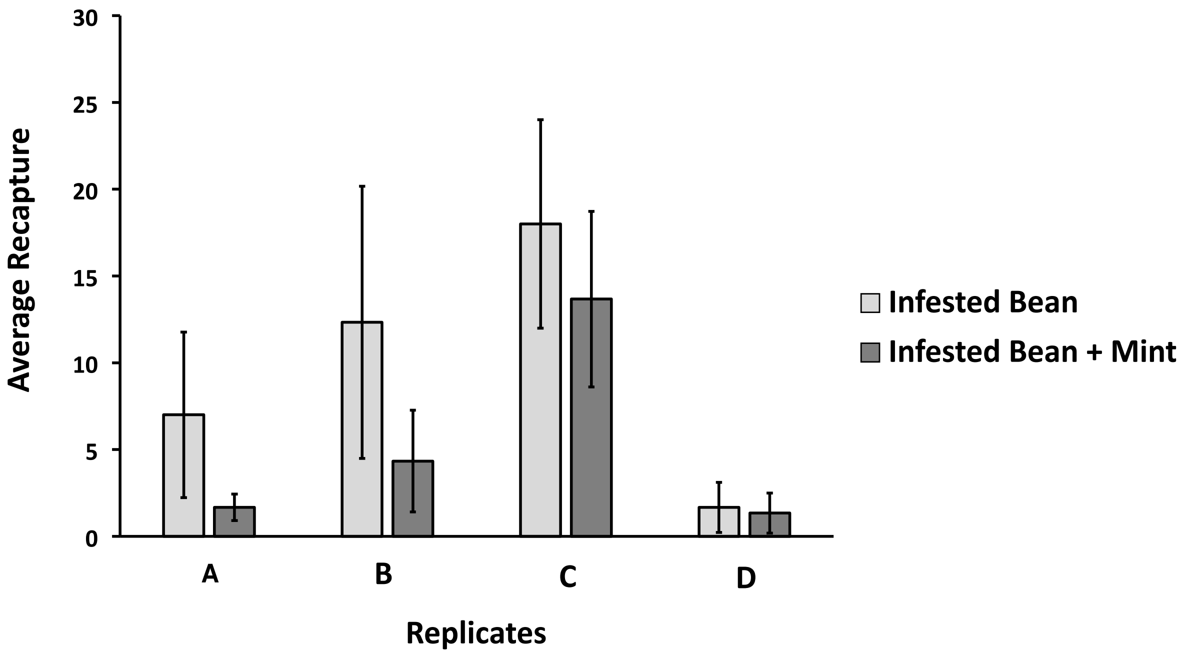

Figure 9. Average number of *P. macropilis* recaptured on three plants of each treatment per replicate. Each replicate had two treatments (infested bean vs infested bean plus mint plant). Error bars show the SEM of the mean number of predatory mites recaptured**.**

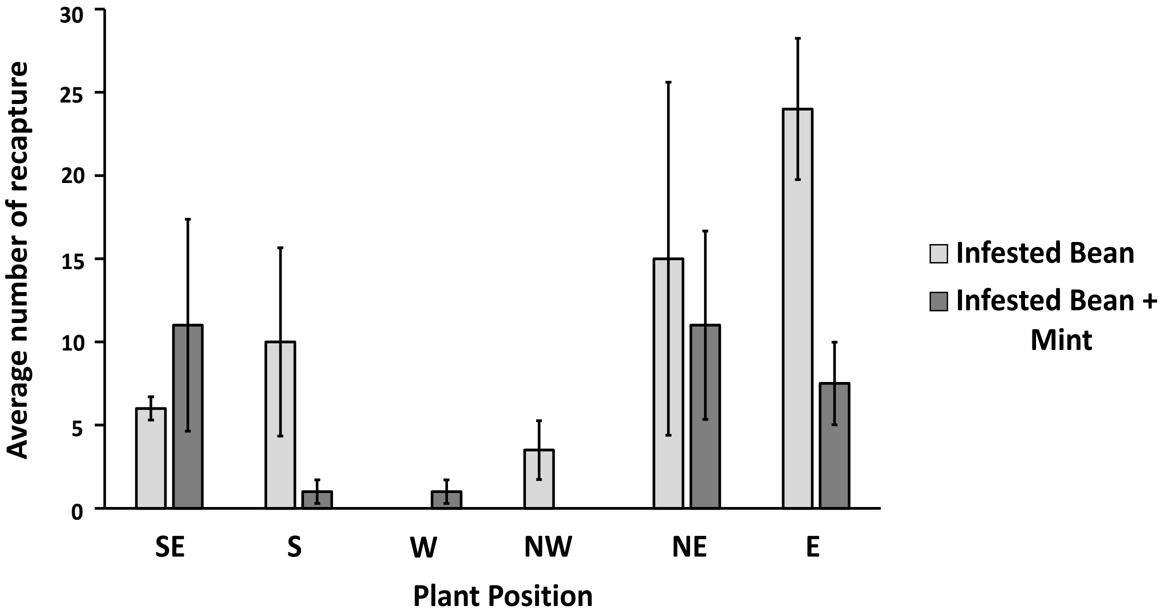

Figure 10. Average number of *P. macropilis* recaptured on the plants per position. Abbreviations under X-axis refer to position of the plants relative to the release point: SE = southeast, S = south, W = west, NW = northwest, NE = northeast and E = east. Error bars show the SEM of the mean number of predatory mites recaptured.

Infested bean plants

In the experiment with six infested bean plants without companion plants, plant position had significant effect on the number of recaptured mites (Fig.11, GLM, Chi² = 152, d.f. = 5, P < 0.0001). Most of the predatory mites were recaptured SE, S, NE and E from the release point. In one of the four replicates we found one dead predatory mite in the release site (Petri dish), and in another we found four dead predators in the Petri dish, no data were collected in the remaining replicates. Concluding, we expected that predators would not show preference for any specific position, however, more mites were recaptured on these four positions.

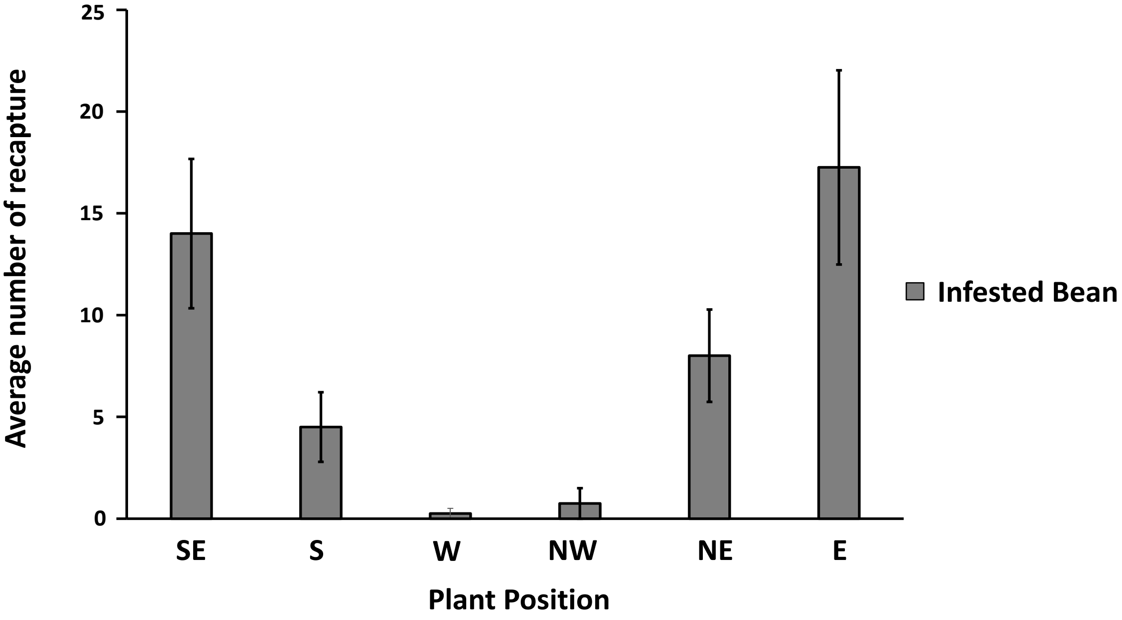

Figure 11. Average number of *P. macropilis* recaptured on the plants per position. Individual bars represent the union of treatments means (Infested Bean and Infested Bean+). Abbreviations under X-axis refer to position of the plants relative to the release point: SE = southeast, S = south, W = west, NW = northwest, NE = northeast and E = east. Error bars show the SEM of the mean number of predatory mites recaptured.

Effect of companion plants on total predator recapture

There was no significant effect of the total numbers of *T. urticae* per plant in the number of predatory mites recaptured (GLM, F = 0.69, d.f = 1, P = 0.41). There was no significant difference among combinations (Fig. 12, GLM, F = 2.81, d.f = 2, P = 0.08). In general, fewer predatory mites were recaptured when infested bean plants were combined with coriander than the combination of infested bean plants with mint and with infested bean plants only, but the differences were not statistically significant. In total 12.5%, 28.1% and 27.9% of all predatory mites released were recaptured with coriander, mint and infested bean only respectively.

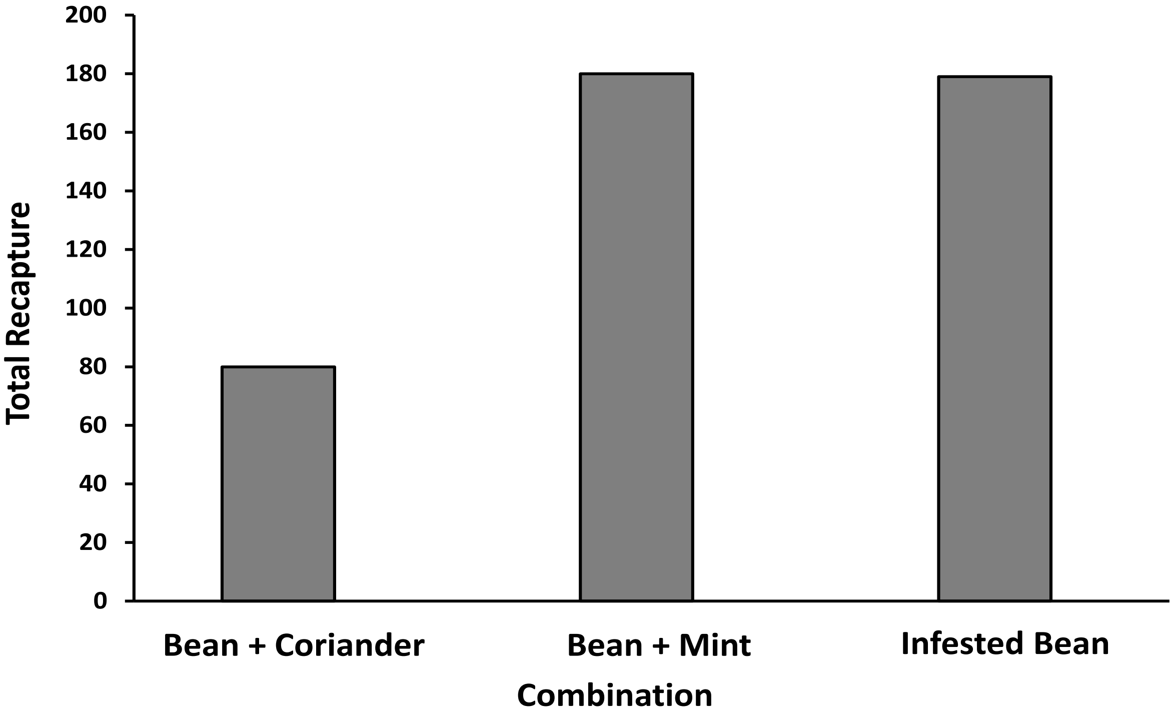

Figure 12. Total recapture of *P. macropilis* out of 640 released in the different experiments. Combinations with different letters were significantly different. Error bars show the SEM of the mean number of predatory mites recaptured.

DISCUSSION

In the semi-field experiments presented here, we found a clear preference of predatory mites for *T. urticae*-infested bean plants when compared to clean plants (Fig. 5). A large fraction of the recaptured predatory mites (86.7%) was found on infested plants. One of the cues that the predators could have used to detect and localize the plants with prey are the volatiles emitted by these plants, but other cues may also have played a role, such as the wind and possibly the magnetic field of the earth. In my experiment, the wind direction was mostly from the SE and NW, and least frequently from the W. Besides, we recorded the presence or absence of light for every position during each experiment interval. Despite of the preference towards infested plants, the recapture number among positions was highly variable, with a clear a general preference for direction: 40.35% of all recaptured mites were found on plants located in the East of the release site (Figs 6, 8, 10 and 11). This suggests that not all infested plants are equally attractive for the predatory mites, so that cues other than volatiles are important for predator choice. Because predatory mites are blind and rely mainly on chemical stimuli to locate their prey (Sabelis and Van der Baan 1983; Sabelis et al. 1984), wind plays an important role on predator's behaviour as an odour plume carrier (Murlis 1992), thus the influences by wind can be huge during searching. However, light intensity seems to play a minor role in predator preference, as we noticed a uniform light distribution, with each position receiving similar amounts of sunlight during the whole experiment. Curiously, in studies under greenhouse conditions, where there is virtually no wind, a preference for a general direction was also found, as observed for *P. persimilis* in greenhouse experiments in the Netherlands (Janssen 1999) and Denmark (Zemek and Nachman 1999), but in this case, the preferred positions were located from the NNW to the East of the release site. The authors suggest light intensity as a possible candidate for this preference.

In my experiment I show that regardless of the presence of other cues, including background volatiles, *P. macropilis* was able to discriminate between plants infested with their prey and uninfested plants. However, a large fraction of the released predatory mites (77%) was not recaptured. This low recapture may have been caused by difficulties in finding the target plant in the presence of other volatile and non-volatiles cues, in other words, they are able to differentiate infested from uninfested plants but it is much harder to find any plant under such conditions (Schröder and Hilker 2008; Hilker and McNeil 2008).

Despite their ability to find infested plants under semi-field conditions, a large percentage of the predators were not recaptured (87.5%, 71.9% and 72.1% from the coriander, mint and infested bean experiments respectively), confirming that it is a difficult task to perform as we suggested in the experiment (above-mentioned). In some experiments, we removed every plant after the last evaluation (48 hours) and put *T. urticae*-infested bean leaves, with abaxial surface on the substrate during one night, aiming to recapture the remaining predators. Very few *P. macropilis* were recaptured on these leaves, suggesting that mites could have died or left the experimental set-up. In some replicates we found a few dead predatory mites on the Petri dish, showing that they did not leave the release site, but instead, died before the experiment started, probably due to the sucking procedure with the vacuum pump. However, the low recapture rates were certainly not caused by high mortality rates of the predators during and before release. Also, in release-recapture experiments, the tiny mites had to cross relatively long stretches of barren substrate before reaching a plant (Pallini et al. 1997), and had to deal with many other abiotic factors such high/low temperature and humidity. It is known that *P. macropilis* is a plant-inhabiting mite dispersing mainly by wind. In my experiment, the predators had to walk on substrate, until they find a plant stem, and despite the substrate being humidified before each experiment, the substrate dried on the sunnier days. Croft and Jung (2001) showed that *Neoseiulus fallacis* suffered a 90% mortality on soil surface under dry conditions, and Sabelis (1981) noted that large losses of predatory mites occurred due to high temperatures (above 30 ºC). Such conditions were frequently found in my experiment; thus, it may have influenced total recaptures. Moreover, many studies that had similar setups but under greenhouse conditions showed a higher total recapture rate of predators, 35.8%, 41.9% and 34.1% respectively when compared to my experiment (Oliveira et al. 2009; Janssen 1999; Venzon 1999). Here, the total recapture was 23% of all released predatory mites, suggesting that abiotic factors may have affected the searching behaviour of the predatory mites in my experiments. In our previous study (Chapter one), we used the same treatments as we did here, but under controlled conditions, i.e., only volatile cues differed between treatments, and just like we found here, they were clearly able to distinguish infested from uninfested plants. In those experiments, virtually every released mite was able to reach the end of the Y-tube and make a choice, showing that localizing plants under more natural conditions was much more difficult than under controlled lab conditions. The response towards infested plants under olfactometer and greenhouse conditions was expected as reported for other species in previous studies (Janssen 1999, Dicke 1999, Oliveira et al. 2009). Comparing the outcomes from olfactometer and semi-field experiments leads us to an important question: how valuable are olfactometer experiments in predicting mite behaviour? As we discuss here, most of the outcomes found in this chapter were different from what we found in experiments under controlled conditions, thus, olfactometer experiments are essential under specific situations, but the results cannot be extrapolated to situations in field. Instead, olfactometer experiments provide basic knowledge that supports the design of relevant manipulative experiments under more complex conditions.

Very few studies report similar experiments considering the effects of background volatiles and other cues on the localization of plants by predators and parasitoids. One exception is the study by Shimoda et al. (1997), where the predatory thrips *Scolothrips takahashii* responded positively towards *T. urticae*-infested Lima bean plants in a satsuma mandarin orchard, despite the presence of highly complex background volatiles and other cues. Using two traps with infested bean plants, the authors evaluated the total number of captured thrips comparing with two control traps (uninfested bean plants), the traps did not allow the predator to contact the plant, ensuring attraction by volatiles. As a result, no predatory thrips was attracted by control traps, suggesting that volatiles from infested Lima bean plants were efficient in luring the predators under field conditions.

The experiments with coriander as companion plants showed that *P. macropilis* was equally attracted to bean plants associated with coriander as to bean plants without companion plants (Fig. 7). A large fraction of the released predatory mites (87.5%) was not recaptured, similar to the previous experiments. Possibly, the distance between the coriander and infested bean plants may have caused incomplete volatile mixing. In our previous experiments under controlled conditions (Chapter one), we showed that the presence of coriander volatiles reduced the attractiveness of volatiles of bean plants with spider mites. Differently from experiments under field conditions, the volatile sources in the olfactometer were fully enclosed by glass containers which might guarantee proper odour mixing. A study by Murlis et al. (1992), demonstrates that the strength of a signal (odour plume) decreases systematically as the distance from the source increases. Therefore, in my experiment, it is most likely that the coriander blends mixed with bean blends formed a weak signal due to their distance, and were no longer recognizable by the predators. Aiming to elucidate this gap, future experiments could be done to test coriander plus bean volatiles at a closer distance in a similar setup, and evaluate the response of predators.

In the mint experiment, the association of infested bean plants with uninfested mint plants led to lower recapture of predatory mites compared to infested bean plants alone (Fig. 9). A large fraction of the released predatory mites (71.9%) was not recaptured, similar to the previous experiments. We cannot assume that preference for infested bean over infested bean plus mint plant was due to environmental background volatiles. Unfortunately, we did not test the effect of mixing the blends of *T. urticae*-infested plants plus mint on predator response under controlled conditions (as we did for coriander). Instead, we tested volatiles of clean mint plants against air, and we found mint to be attractive for predatory mites. Thus, we cannot predict a response for the semi-field experiment, as predatory mites could react in many ways to this combination. van Wijk et al. (2010) showed that responses to mixtures cannot be predicted from the response to the individual compounds, in other words, although we found mint to be attractive in the olfactometer, we cannot assume that this outcome will be the same for mint and bean blend mixtures under field conditions.

Comparing the total recapture rates of the three experiments (coriander, mint and infested bean plants: 80, 180 and 179 individuals respectively) shows that similar numbers of predators were recaptured in the control experiment (infested bean plants) and the experiments with mint as companion plants. If we take the control experiment as expected recapture number, because no volatiles from companion plants were present in this setup, any recapture that is significantly different from this control may have been caused by the volatiles of the companion plants. When comparing total recapture among experiments, we found the lowest numbers of recaptured predators with coriander as companion plant (although it was not significantly different from the other experiments), suggesting that predatory mites are somewhat hindered in finding any bean plants with their prey when coriander plants are close by, even the bean plants that had no coriander next to them. Aiming to elucidate if coriander does really interfere with finding bean plants, one could perform experiments with mint and coriander interspersed as companion plants, and evaluate the recapture between the treatments.

Knowledge of the interactions of background volatiles with pest-associated volatiles is essential for understanding the foraging behaviour of natural enemies and for improving the efficiency of biological control in the field. Therefore, this is an initial step towards a better understanding of how volatiles of companion plants can interfere with the behaviour of natural enemies used for biological pest control.

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GENERAL CONCLUSIONS

In this dissertation, I show that the effects of companion plant volatiles on predatory mite foraging cannot be predicted by a response towards these volatiles, but instead, need to be assessed by testing mixed blends of plants producing prey-associated volatiles and of companion plants. A specific volatile blend that causes no response in a natural enemy could trigger a different response when added to another volatile source.

Furthermore, I show that responses of a predator in laboratory olfactometer experiments cannot be extrapolated to responses in the field, and that the responses of predators in environments with increasing levels of complexity (decreasing levels of control of environmental factors) need to be studied to understand the role of volatile cues in complex field situations, where other volatile and non-volatile cues are present. Thus, to comprehend the real impact of volatile mixing on the behaviour of a natural enemy, I suggest that one could go deeper and carry out experiments under field conditions.