LAILA CRISTINA REZENDE SILVA

BEHAVIOURAL RESPONSES OF A GENERALIST PREDATOR TO CUES OF PLANTS HARBOURING VARIOUS MIXTURES OF PREY

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Entomology Graduate Program, to obtain the title of *Magister Scientiae*.

Adviser: Arnoldus Rudolf Maria Janssen

Co-adviser: Renato de Almeida Sarmento

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Laila Cristina Rezende Silva

Laila Cristina Rezende Silva
Author

Arnoldus Rudolf Maria Janssen
Adviser

“Aguarda as surpresas do tempo, agindo sem precipitação.
Se cada noite é nova sombra, cada dia é nova luz.”

Chico Xavier

À minha mãe pelo apoio incondicional em todos os
momentos,
principalmente nos de incerteza.
Sem você nenhuma conquista valeria a pena.
Ao meu irmão, e aos meus amigos que me apoiaram
sempre com muito amor e carinho.
Aos meus mestres que sempre estiveram de prontidão.

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ABSTRACT

SILVA, Laila Cristina Rezende, Universidade Federal de Viçosa, July, 2019. **Behavioural responses of a generalist predator to cues of plants harbouring various mixtures of prey.** Adviser: Arnoldus Rudolf Maria Janssen. Co-adviser: Renato de Almeida Sarmento.

Foraging behaviour is fundamental in the lives of animals because finding food or hosts is essential for growth and reproduction. Many animals consume more than one type of resource mixed diets may increase their fitness. Consuming a mixed diet can redress nutritional imbalances of single resources and may dilute toxins present in food. However, resources often do not co-occur in time or space, hence, animals need to forage actively to obtain a mixed diet. To find food, animals use several cues, such as visual, vibrational, physical and chemical cues. Volatiles from prey and from plants under attack by the prey are important cues for many arthropods, including predatory mites. When attacked by more than one herbivore species, plants may release different volatiles compared to plants attacked by each herbivore separately. The generalist predatory mite *Iphiseiodes zuluagai* is associated with two main pests of *Jatropha curcas*: the broad mite *Polyphagotarsonemus latus* and the red spider mite *Tetranychus bastosi*. This predator feeds on both prey but has an increased performance when feeding on a mixture of both prey. Therefore, I investigated the foraging behaviour of *I. zuluagai* in a system composed of plants with both prey species or with either prey species separately. I found that this predator does not show a significant preference for plants with both prey, although it performs better on a diet consisting of both prey than on each prey alone. Furthermore, the predator does not show an innate olfactory preference. Thus, when this mite has no previous experience with these prey, it does not show a significant response to clean or attacked plants. Possible explanations for the apparent lack of preference of this mite are discussed, as well as the possible implications for biological control.

Keywords: Mixed diet. Volatiles. Multiple infestation. Olfactometer.

RESUMO

SILVA, Laila Cristina Rezende, Universidade Federal de Viçosa, julho de 2019. **Resposta comportamental de um predador generalista a pistas de plantas atacadas por várias misturas de presas.** Orientador: Arnoldus Rudolf Maria Janssen. Coorientador: Renato de Almeida Sarmento.

O forrageamento é fundamental para a vida dos animais, pois encontrar alimento ou hospedeiro é essencial para o desenvolvimento e reprodução destes. Muitos animais consomem mais de um tipo de recurso. O consumo de uma dieta mista pode corrigir os desequilíbrios nutricionais de uma dieta simples e diluir toxinas presentes nos alimentos, acarretando em um aumento no fitness do animal. No entanto, os recursos muitas vezes não co-ocorrem no tempo ou no espaço, portanto, os animais precisam forragear ativamente para obter uma dieta mista. E nesta procura eles utilizam pistas visuais, vibracionais, físicas e químicas. Os voláteis provindos de presas e plantas atacadas por herbívoros são pistas importantes utilizadas por vários artrópodes incluindo ácaros predadores. Quando atacadas por mais de uma espécie de herbívoro as plantas liberam voláteis diferentes de plantas atacadas por um único herbívoro. O ácaro predador generalista *Iphiseiodes zuluagai* está associado a duas pragas principais de *Jatropha curcas*: o ácaro branco *Polyphagotarsonemus latus* e o ácaro vermelho *Tetranychus bastosi*. Este ácaro se alimenta de ambas as presas, e aumenta seu desempenho ao consumir uma dieta mista. Sendo assim, eu investiguei o comportamento de forrageamento de *I. zuluagai* em um sistema composto de plantas atacas por ambas as espécies de presas, simultaneamente e separadamente. Foi mostrado que este ácaro predador não teve uma preferência significativa por plantas com ambas as presas, embora tenha um desempenho maior quando alimentado com dieta mista do que com uma dieta simples. Além disso, o predador não mostrou uma preferência olfativa inata. Portanto, quando este ácaro não tem experiência anterior com essas presas, ele não mostra uma resposta significativa a plantas limpas ou atacadas. Foi discutido as possíveis explicações para a aparente falta de preferência deste ácaro, bem como as possíveis implicações para o controle biológico.

Palavras-chave: Dieta mista. Voláteis. Infestação múltipla. Olfatômetro.

SUMMARY

GENERAL INTRODUCTION

Animals in general need to forage for a diet in order to gain enough energy and nutrients to support growth, development, and reproduction. Early foraging theory predicts that animals try to maximize energy intake by consuming more food with higher nutrient content per unit time, and this will consequently increase fitness (Stephens & Krebs 1986). However, this theory does not consider the importance of food nutritional composition for fitness. As a consequence, two theories have arisen to explain the consumption of a variety of food types. The first one is the *balanced diet theory* which predicts that different prey species have different nutritional compositions. By foraging for various prey species, the diet composed of more than one prey provides a rich nutritional food because the nutrients coming from different prey are complementary (Raubenheimer & Simpson 1997; Mayntz *et al*., 2005). The second one is the *toxin dilution theory* which suggests that each food type contains specific toxic compounds, and food mixing can limit the ingestion of the various toxic compounds (Freeland & Janzen 1974). Both theories on mixed diet suggest that animals could have a higher fitness when consuming a mixed diet than a single diet. Many studies have shown this pattern (Bernays *et al*., 1994; Toft & Wise 1999; Messelink *et al*., 2008; Lefcheck *et al*., 2013; Muñoz-Cárdenas *et al*., 2013; Marques *et al*., 2015).

Although predators can benefit from a mixed diet, they will not always find prey of different quality in the same place. Thus, to obtain a mixed diet in nature, they often need to forage for several food types that are patchily distributed and do not co-occur (Godin & Keenleyside 1984). Therefore, the positive effect of feeding on a mixed diet may be offset when the costs of foraging (time and energy) over long distances are not outweighed by the benefits of obtaining a mixed optimal diet.

In order to maximize their foraging efficiency, natural enemies in general use a variety of methods. Visual cues are important for host selection by parasitoids. For example, Michaud & Mackauer (1995) show that three aphid parasitoids use visual cues in host finding, the parasitoids showing innate preferences for green over pink colour morph aphids. Another cue utilized by natural enemies are vibrations. Van Alphen & Drijver (1982) showed that older host larvae of *Drosophila melanogaster* that are more active, and thus produce stronger vibrations in their feeding substrate, are found more easily by the larval parasitoid *Asobara tabida* than are smaller, younger host larvae. In addition, physical and chemical cues such as the presence of prey, products released by them such as, eggs, exuviae, web, faeces play a crucial role in predator searching behaviour (Hislop & Prokopy 1981; Dicke *et al*., 1998; Janssen *et al*., 1997 Collier *et al*., 2000; 2001; Hajek 2004; Hoy 2011).

Another source of cues important in prey/host finding are the volatiles produced by plants under attack by herbivores (so-called herbivore-induced plant volatiles, HIPV) (Dicke & Sabelis 1988; Turlings *et al*., 1990; Sabelis *et al*., 2007). The blend of volatiles released by an attacked plant can be highly specific, depending on the plant species (van den Boom *et al*., 2004), plant cultivar, (Takabayashi *et al*., 1994), herbivore species (Silva *et al*., 2017), and herbivore density (Horiuchi *et al*., 2003). Moreover, when a plant is attacked by more than one herbivore, which is common in nature, the induced plant volatiles can be qualitatively and quantitatively different from volatiles induced by a single pest (Shiojiri *et al.,* 2001; Magalhães *et al*., 2018). This difference in emitted plant volatiles can result in changes in behaviour response of the predators and parasitoids. Studies on predator and parasitoid responses to multiple herbivore attack showed different patterns of attraction. Plants attacked by more than one herbivore can be more attractive or less attractive to natural enemies (Shiojiri *et al*., 2001; Moyaeri *et al*., 2007; Dicke *et al*., 2009; Zhang *et al*., 2009; Lima *et al*., 2016). Nevertheless, if volatiles released by plants attacked by one or more herbivores differ, natural enemies could discriminate between them and may prefer plants infested by more than one herbivore, representing a mixed diet.

Predatory mites are blind, so they mainly rely on physical and chemical cues associated with the presence of their prey at short distances, and volatile chemical cues at longer distances, using sensory receptors on their legs and palps (Dicke *et al*. 1990; Hoy 2011). Hence, chemical and physical stimuli mediate the interaction of predatory mites and their prey. The predatory mite *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae) is a generalist that feeds on a variety of prey and pollen (Reis *et al*., 1997; Sarmento *et al*., 2011; McMurtry *et al*., 2013; Marques *et al*., 2014). *Iphiseiodes zuluagai* is widely distributed in the Americas; in Brazil, it occurs in many states (Moraes *et al*., 2004) and on many plant species (Sato *et al*., 1994; Reis *et al*., 2000; Pallini, 1992; Zacarias & Moraes, 2001; Albuquerque, 2006; Sarmento *et al*., 2011; Ferreira, 2017). Moreover, it is associated with many pest mites such as *O. ilicis* and *B. phoenicis*. On physic nut, *Jatropha curcas* L. (Euphorbiaceae), one of the plants on which it occurs in Brazil, it is associated with two main pests: the broad mite

Polyphagotarsonemus latus (Banks) (Acari: Tarsonemidae), which usually attacks the apical, young leaves, and the red spider mite *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae), which is mainly found on older leaves (Sarmento *et al*., 2011; Pedro Neto *et al*., 2013; Rosado *et al*., 2015). *Iphiseiodes zuluagai* can reproduce and develop by feeding on them. Moreover, *I. zuluagai* increased performance (i.e. oviposition rate) when consuming a mixed diet consisting of eggs of these prey under controlled laboratory conditions (Marques *et al*., 2015). However, it is not known whether it still actively searches for a mixed diet under more complex conditions, for example when searching for plants with prey, considering that there is an increase in distance compared to studies on leaf discs (Marques *et al*., 2015). Moreover, it is not known what cues this predatory mite uses to find a plant with prey. Many cues are involved in the predatory behaviour and many direct and indirect interactions occur among plants, prey and predators so these interactions can shape and drive food web dynamics. On *J. curcas* plants, many other predators are present (Sarmento *et al*., 2011; Fernandes *et al*., 2015; Marques *et al*., 2015; Cañarte *et al*., 2017). Hence, the interactions in the natural environment are not restricted to those between the plant, the predator *I. zuluagai* and the prey *T. bastosi* and *P. latus*. For example, the predatory mite *E. concordis* and *I. zuluagai* co-ocurr on *J. curcas* and can interact trough intraguild predation, which occurs when one specie feeds on another species that shares the same resources (Polis & Holt 1992; Marques *et al*., 2018). In this kind of interaction, the spatial structure can influence the coexistence of those two predators, but the diet of the two predators can also affect their interactions and co-occurrence (Daugherty *et al*., 2007; Holt and Huxel 2007; Marques *et al*., 2018). In addition, spatial structure can influence the interactions mediated by volatiles and other cues used for predatory mites to find prey. Therefore, this research aimed to study the foraging behaviour of *I. zuluagai* on *J. curcas* plants that were infested by *P. latus* and *T. bastosi* simultaneously or by either of them separately. In Chapter 1, I assessed the foraging behaviour of the predatory mite when searching for plants with either or both prey in release and recapture experiments. In Chapter 2, I assessed the role of volatiles in the searching behaviour of the predatory mites.

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CHAPTER I

FORAGING BEHAVIOUR OF A GENERALIST PREDATORY MITE FOR PLANTS WITH SINGLE AND MIXED DIETS

Laila Cristina Rezende Silva[1], Renato Almeida Sarmento[2] & Arne Janssen[3]

[1]Department of Entomology, Federal University of Viçosa (UFV), CEP 36570-000, Viçosa, MG, Brazil. Email: laila.rezende@ufv.br

[2]Federal University of Tocantins (UFT), PO Box 66, Gurupi, TO, Brazil. Email: rsarmento@mail.uft.edu.br

[3]IBED, Department of Evolutionary and Population Biology, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands. Email: arne.janssen@uva.nl

Abstract

Predators need to forage efficiently to enhance their fitness, and this efficiency depends on many factors. Because a balanced diet can positively affect reproduction and survival of predators, searching for a mixed diet may be essential for enhancing fitness. However, the different food types constituting a mixed diet do not necessarily co-occur, which makes searching behaviour more complex. Predatory mites locate prey patches through chemical cues released by prey and attacked plants, and through tactile stimuli. The predatory mite *Iphiseiodes zuluagai* is known to have a higher performance on a mixed diet composed of the two phytophagous mites *Tetranychus bastosi* and *Polyphagotarsonemus latus.* Here we investigated the searching behaviour of *I. zuluagai* for single and mixed diets in release-recapture experiments with *Jatropha curcas* plants infested by a single prey or both simultaneously. We show that the predators prefer plants infested by single and multiple prey over clean plants. However, they did not show a preference for plants with both herbivores to singly-infested plants, whereas the former would offer an optimal diet. Our results indicate that many cues are involved in this interaction and it is not just the quality of food that matters.

Keywords: foraging behaviour, volatiles, mixed diet, preference, predatory mite

Introduction

To ensure growth, development and reproduction, predators need to be efficient in finding prey patches to obtain food while spending a minimum of time and energy. Such foraging behaviour is known to be affected by many factors, such as the presence of competitors (Janssen *et al*., 1997), hyperpredators (Stephens 2008), prey density (Charnov 1976), interaction of prey species (Choh *et al*., 2017), plant volatiles (Dicke & van Loon 2000), experience with prey (Drukker *et al*., 2000), plant architecture, prey distribution (Gontijo *et al*., 2011). Moreover, many prey species differ in nutritional composition and predators therefore need to mix their diet to redress nutritional imbalances (Mayntz *et al*., 2005) and/or to dilute toxins present in the prey (Toft & Wise 1999). So foraging behaviour is also affected by the need for a balanced diet (Belovski 1978; Muñoz-Cardenas *et al*., 2013; Mayntz *et al*., 2005; Marques *et al*., 2015).

Thus, in order to find a balanced diet, predators need to search in a complex system, considering that different food types are patchily distributed (Godin & Keenleyside 1984). Hence, foraging predators need to identify patches with different prey species and evaluate if searching for a mixed diet is profitable. Predators can search using chemical stimuli (Janssen *et al*., 1997; Dicke *et al*., 1998; Hoy 2011), vision and movement (Hajek, 2004). When plants are attacked by herbivores, they release induced plant volatiles (HIPV), which are important cues for predators to find prey patches (Dicke & Sabelis 1988; Turlings *et al*., 1990; Sabelis *et al*., 2007; Dicke *et al*., 2009). Besides using HIPV, predatory mites can also locate prey at short distances by perceiving prey volatiles and other cues released by them, such as, eggs, exuviae, web, faeces (Hislop & Prokopy 1981; Collier *et al*., 2000; 2001). So, the combination of those chemical and physical cues can mediate attraction and arrestment of predators to a prey patch.

Many studies show that predators benefit from a mixed diet. However, most of them use an artificial diet and set-up (Mayntz *et al*., 2005; Messelink *et al*., 2008; Jensen *et al*., 2012; Marques *et al*., 2015), and studies on the foraging behaviour of predators for a natural mixed diet under natural conditions are scarce. This research is a first step in this direction: we studied the foraging behaviour of a predatory mite searching for two natural prey species that occur on the same plants, but on different areas of the plant. The generalist predatory mite *Iphiseiodes zuluagai* Denmark & Muma reaches a significantly higher oviposition rate on a mixed diet of the two herbivorous prey species *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) when foraging on small arenas in the laboratory, where it actively forages to obtain a mixed diet (Marques *et al.,* 2015). Whereas it prefers leaves with either of the prey species to clean leaves (Sarmento *et al.*, 2011), it is unknown whether it forages for a mixed diet under more natural conditions, and this is what we tested here.

The predator and the herbivorous mites studied here co-occur on *Jatropha curcas* plants in Brazil (Sarmento *et al*., 2011), where *Polyphagotarsonemus latus* attacks the apical leaves and *T. bastosi* is found on older leaves (Rosado *et al*., 2014). We investigated the foraging behavior of *I. zuluagai* through release and recapture experiments where we assessed the preference of the predatory mites to plants attacked by *T. bastosi*, *P. latus* or both together. We expected these predators to prefer plants infested by both herbivores simultaneously in order to obtain a mixed diet, considering that such a diet is beneficial for them (Marques *et al.*, 2015). Because the two prey mites differ in the position on the host plant, we also assessed the distribution of predatory mites over plants with different species of prey mites.

Material and Methods

Plant material and mites rearing

The experiments were conducted at the Federal University of Tocantins, Gurupi, Tocantins, Brazil. Plants were cultivated in plastic pots (2.8 L) and irrigated once per day. They were kept in a fine-meshed cage to prevent any pest infestation. The initial laboratory stock colonies of *I. zuluagai*, *T. bastosi* and *P. latus* originate from mites collected from *J. curcas* in an experimental field in Gurupi, state of Tocantins, Brazil. They were kept in a fine-meshed cage to prevent any pest infestation. The initial laboratory stock colonies of *Iphiseiodes zuluagai* were fed with castor bean pollen (*Ricinus communis* L.) and honey. *Tetranychus bastosi* were reared on uninfested plants of *J. curcas* that were renewed when necessary. *Polyphagotarsonemus latus* were reared on leaves of *J. curcas* placed on a foam layer surrounded by hydrophilic cotton to prevent mites to escape. All cultures were maintained in a climate-controlled room (25 ± 2 °C, 65 –70% relative humidity, and a 12 hr L/12 hr D photoperiod.)

Infestation of J. curcas plants

Jatropha curcas plants of 30 days old with four leaves were used in the following experiments. There were four treatments: Uninfested plants, *T. bastosi* infested plants, *P. latus* infested plants and mixed infested plants (*T. bastosi* + *P. latus*). The apical leaf (leaf 1) and the leaf below (leaf 2) received 100 adult females of herbivorous mites, according to the treatment. When a plant was infested by a single (*P. latus* or *T. bastosi*) prey, we released them on both leaves and when it was simultaneously infested with both species, the two species were placed on different leaves: *P. latus* on the apical leaf and *T. bastosi* on the leaf below. To prevent phytophagous mites from moving to other leaves, moistened cotton wool was placed on the petioles of the infested leaf. Three days later, we removed the moistened cotton wool to carry out the experiments.

Release-recapture experiments

Release-recapture experiments were carried out to test the attractiveness and arrestment of the predatory mites on plants infested with herbivorous mites as explained above, following the methodology described by Zhang *et al.* (2018) with slight modifications. The plants were placed side by side in a plastic tray filled with water. A pot was placed between these two plants, on top of which a Petri dish was placed. The plants were connected with a string which served as bridge between the plants and the Petri dish (50 cm). A string was tied between the 2nd and 3rd leaf of each plant and fixed to the rim of the Petri dish with a paperclip. Forty female predatory mites were starved for 2 hours. Subsequently, they were released in the centre of the Petri dish from where they were allowed to walk to the plants. Thus, these forty predators could move within the plant, between plants, return to the Petri dish during 24 hours. After 24 h, we counted the predatory mites on each leaf of each plant. Four pairs of plants per combination were tested. The number of predatory mites recaptured on plants were analysed using a GLM with a Poisson error distribution. The distribution of predatory mites over the plant was analysed with a GLM with negative binomial error distribution. Contrasts among treatments and leaves were assessed using a Tukey test. All statistical analyses were performed in R.

Results

The numbers of *I. zuluagai* on clean plants were higher than on plants infested with *T. bastosi* (Fig. 1, GLM: Chi^2 = 9.87, d.f. = 1, P = 0.0017). In contrast, significantly higher numbers of *I. zuluagai* were encountered in plants infested with *P. latus* or both prey than on clean plants (Fig. 1, GLM: Chi^2 = 12.5, d.f. = 1, P < 0.001 and GLM: Chi^2 = 14. 9, d.f. = 1, P = 0.0001). When offered plants infested with *T. bastosi vs.* plants infested with *P. latus*, more *I. zuluagai* were recaptured on plants infested with *P. latus* (Fig. 2, GLM: Chi^2 = 26.5, d.f. = 1, P <0.0001). There was no significant difference when offered plants infested with *T. bastosi* or *P. latus vs.* plants with a mixed infestation (Fig. 2, GLM: Chi^2 = 2.28, d.f. = 1, P = 0.13 and GLM: Chi^2 = 0.20, d.f. = 1, P = 0.65). There was no significant difference in the proportions of mites that did not make a choice among experiments (GLM: Chi^2 = 26.98, d.f. = 5, P = 0.17).

There was no significant interaction between position and treatment in either of these experiments, but there was a significant interaction of replicate with treatment for the experiments with mixed infested plants *vs.* clean plants, (Chi^2 = 21.8, d.f. = 1, P = 0.0005) and plants with *P. latus vs.* plants infested with *T. bastosi* (Chi^2 = 18.9, d.f. = 1, P = 0.0002). These significant interactions mean that the preference of the predators was not consistent among replicates. We have no explanation for this at present. There was a significant interaction between the leaf on which the predators were found and the treatment that the plant had received (Fig. 2, d.f. = 6, Chi^2 = 21.3, p = 0.0016). On clean plants and plants infested with *T. bastosi*, predatory mites were distributed equally over the two infested leaves and the rest of the plant (Fig. 3, 1st and 2nd bar). On plants infested with *P. latus* or by both herbivores, the predators were found more often on the first leaf than on the other leaves (Fig. 3, 3rd and 4thbar), which was the leaf infested by *P. latus*.

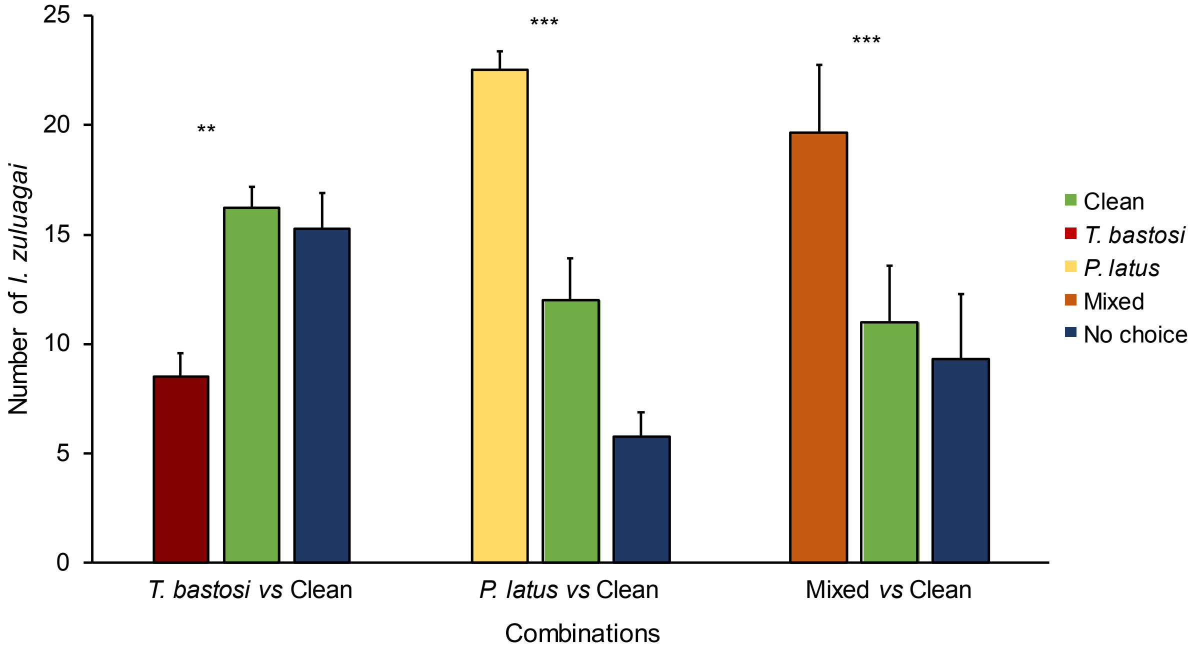

Fig 1. Average number of *I. zuluagai* captured on plants after 24 hours when offered a choice between clean plants versus plants infested with *T. bastosi*, with *P. latus* or with a mixed infestation. Significant differences are indicated by asterisks (***P* < 0.01, ****P* < 0.001).

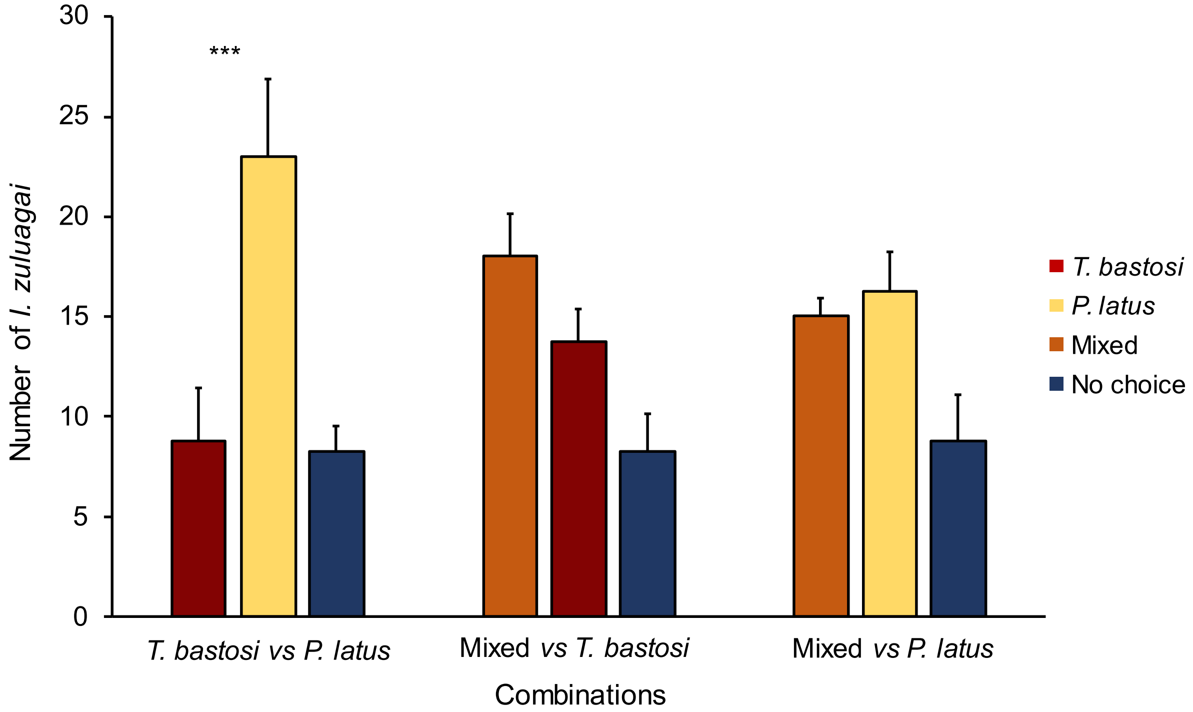

Fig 2. Average number of *I. zuluagai* captured after 24 hours on plants when offered a choice between plants infested with *T. bastosi* versus plants infested with *P. latus* or a mixed infestation versus plants with only *T. bastosi* or *P. latus*. Significant differences are indicated by asterisks (****P* < 0.001).

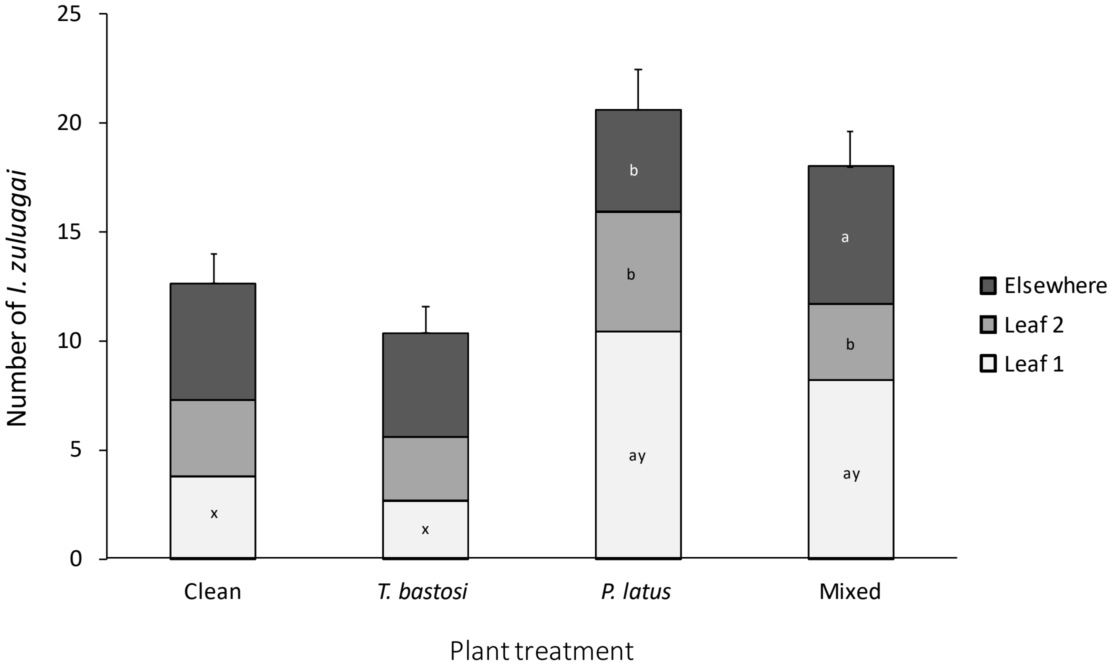

Fig 3. Average number of predators captured on leaf 1, leaf 2 and elsewhere on the plant (leaf 3, 4 and bud) (mean ± SE) after 24 hours. Letters x and y indicate significant differences in the number of mites encountered on leaves in different treatments ($P < 0.05$). Letters a, b and c indicate significant differences in the distribution of mites within each treatment ($P < 0.05$).

Discussion

Iphiseiodes zuluagai preferred plants infested with *P. latus* and mixed infested plants to clean plants (Fig. 1). On clean plants, there is no food, so this preference is apparently associated with the presence of prey as a positive stimulus. Numerous studies have shown that predators prefer infested plants to clean plants (Janssen 1999; Nomikou *et al.*, 2005; Oliveira *et al.*, 2008; Sarmento *et al.*, 2011). The role of the various cues produced by the plant and by the prey, used by *I. zuluagai* to find the plants, is unclear. Probably, the plants attacked by *P. latus*, *T. bastosi* or by both herbivores release herbivore-induced volatiles, which are differentially attractive to the predators. Additionally, the predatory mites may have been arrested by the prey present on the plants, where also cues such as web, eggs and faeces were released by the herbivores. When offered choice between clean plants and plants with *T. bastosi*, the predators preferred clean plants (Fig. 1). This result was not expected, considering that predatory mites were found earlier to prefer leaves with *T. bastosi* over clean leaves (Sarmento *et al.*, 2011) and also preferred plants with mixed diet over clean plants as shown above. Indeed, it is strange that predators prefer clean plants to plants with *T. bastosi*. However, a reason for the difference between results of the two studies maybe that the set-ups were different, here we used entire plants that were infested for 3 days, whereas Sarmento *et al*. (2011) used detached leaves of plants infested for 1 week. Moreover, we evaluated the experiment only 24 hours after the mites were released and Sarmento and colleagues evaluated every hour for 7 hours and removed the mites at each evaluation. As they removed the mites every hour, these mites had no time to commute between the leaves. So, they evaluated the first choice of each mite. However, in a natural system they are free to move within and between plants. Therefore, our evaluation gave them time to experience and change their choice. Another possible explanation is the presence of web produced by *T. bastosi*, which certainly differs from the previous experiments. Although some predatory mites can cope with the web produced by their prey (Takafuji & Chant 1976; Sabelis 1981; Lemos *et al*., 2010), other predators are hindered by the web and tend to avoid it (Takafuji & Chant 1976; Sabelis 1981; Moraes & Flechtmann 2008; Messelink *et al*., 2009; Venzon *et al*., 2009, Franco *et al*., 2010). *Iphiseiodes zuluagai* was reported to reduce predation when web produced by a tetranychid mite was present (Franco *et al*., 2010). We observed that *I. zuluagai* got trapped in the web on plants with a high infestation of *T. bastosi* (Silva, L.C.R, pers. obs.). So, if they seem to avoid plants with *T. bastosi*, how they can prefer plants with mixed diet over clean plants? Probably, the gain of this mite by feeding on a mixed diet may be larger than the risk of being trapped in a dense web produced by *T. bastosi,* When the predatory mite got trapped in the web it becomes more susceptible to other predator attacks, they also spend a lot of energy trying to go out from the web and may die. Hence, to choose a plant that offers this kind of risk the diet need to compensate. However, when *T. bastosi* is in association with *P. latus* the consumption of a mixed diet, can overcome those risks, because the toxin dilution or nutritional supplementation may result in a higher fitness, which does not happen when feeding on a diet consisting of *T. bastosi* alone

Iphiseiodes zuluagai showed a preference for plants infested with *P. latus* over plants infested with *T. bastosi* (Fig. 2). In the field, this predatory mite is found associated with these pest mites, and laboratory experiments have confirmed the suitability of these prey (Sarmento *et al*., 2011; Marques *et al*., 2015). Another phytoseiid mite that also occurs on *J. curcas*, *Euseius concordis*, commutes between leaves with *T. bastosi* and leaves with *P. latus* to mix its diet and enhance its fitness by consuming 34% of *T. bastosi* and 66% of *P. latus* (Marques 2018). Perhaps, *I. zuluagai* also commutes between plants to mix its diet and this explains why a small percentage (28%) of the predators recaptured was found on plants with *T. bastosi*. Moreover, these two species may differ in their defence against predators. For example, *P. latus* does not produce web, which may facilitate the foraging behaviour of the predator. In addition, maybe the volatiles released by plants infested by *P. latus* are more attractive.

Considering that *I. zuluagai* are positively affected by a mixed diet (Marques *et al*., 2015), we expected them to forage for a mixed-infested plant over plants infested by a single herbivore, but this is not what we found (Fig. 2). They did not show a significant preference for any of those combinations. Maybe the predators did not associate the presence of a mixed diet with the cues released by a plant attacked by both herbivores, so when they arrived on a plant and perceived the presence of prey, they were arrested there. However, we do not know how the mites behaved during the 24 hours of the experiment, hence, we do not know if they arrived and stayed on one plant or if they moved between plants. In addition, we do not know the role of volatiles produced by *J. curcas* plants. Probably, this lack of significant preference is because this mite lacks the experience with those volatiles. So, they were perhaps not capable of choosing the best food source based on the chemical cues. Thus, they use other cues, such as physical cues, to detect the presence of prey on the plant. However, both prey are part of the natural diet of this generalist mite, so maybe they need experience with a mixed diet plus the associated volatiles before being able to choose the patch with both prey. Even specialist predatory mites as *P. persimilis* need experience with the complex of prey-plant odours to be attracted to plants with its main prey (Drukker *et al*., 2000).

As the herbivores were placed on different leaves on the plants, according to their natural occurrence, we also assessed the position of the predatory mites on the plants. We found that in uninfested plants and plants infested by *T. bastosi*, the predatory mites were evenly distributed over the two leaves. On plants infested with *P. latus* and both herbivores together*,* the numbers of predators found on leaf 1, which harboured *P. latus*, were higher than on the other leaves. On plants infested with both herbivores, the numbers of mites that were found on leaf 1 and the rest of leaves were higher than number of mites encountered on leaf 2, which was infested with *T. bastosi.* Apparently, the preference of *I. zuluagai* for the newest leaf is associated with the presence of *P. latus*. However, they did not avoid leaves with *T. bastosi* on plants harbouring only this prey, whereas they apparently avoided leaves with *T. bastosi* on plants with both prey, showing that they sometimes avoid *T. bastosi*.

The results obtained in this study show that *I. zuluagai* has a preference for plants infested by both prey and plants infested by *P. latus* over clean plants. However, they did not show a preference for plants with both herbivores to singly infested plants, whereas the former would offer an optimal diet (Marques *et al*., 2015). Further studies should evaluate what causes the attraction and arrestment of *I. zuluagai* to plants with different combinations of prey. This information is crucial to determine if this predatory mite can be an efficient biological agent in the field.

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CHAPTER II

OLFACTORY RESPONSE OF A GENERALIST MITE TO VOLATILES PRODUCED BY SINGLE AND MULTIPLE INFESTATION

Laila Cristina Rezende Silva[1], Renato Almeida Sarmento[2] & Arne Janssen[3]

[1]Department of Entomology, Federal University of Viçosa (UFV), CEP 36570-000, Viçosa, MG, Brazil. Email: laila.rezende@ufv.br

[2]Federal University of Tocantins (UFT), PO Box 66, Gurupi, TO, Brazil. Email: rsarmento@mail.uft.edu.br

[3]IBED, Department of Evolutionary and Population Biology, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands. Email: arne.janssen@uva.nl

Abstract

Many natural enemies are known to respond to herbivore-induced plant volatiles, which are an indirect plant defence used to signal the presence of herbivores on plants to the natural enemies of these herbivores. The simultaneous occurrence of several herbivores on plants is known to change the volatiles produced by the plant in such a way that the volatile blend does not resemble any of the blends induced by each herbivore alone. Obviously, this may influence the response of the natural enemies to the volatiles of these plants. Here, I studied whether the attraction of the predatory mite *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae) to volatiles emanated by *Jatropha curcas* L. infested plants would change with the simultaneous presence of the two phytophagous mites *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) through Y-tube olfactometer assays. These two pests co-occur on *J. curcas* plants, are considered suitable prey for *I. zuluagai*, but together form a higher quality diet, increasing the fitness of the predator relative to single diets. Therefore, I investigated the response of the predatory mites to volatiles of plants attacked by either of the two prey species separately or together. Regardless of the infesting species, it was found that *I. zuluagai* did not discriminate between uninfested or infested plants, or between singly or multiply infested plants. Hence, *I. zulugai* does not show an innate response towards volatiles of infested *J. curcas* plants. I discuss the possible explanations for this lack of preference and suggest that studies on this interaction should be extended to experiments which test the effect of experience with those prey.

Keywords: volatiles, olfactometer, multiple infestation, predator

Introduction

Plants are known to protect themselves against attacks by herbivores through constitutive and induced defences. Both induced and constitutive defences can act directly or indirectly against herbivores (Dicke & Sabelis 1988; Karban *et al*., 1997; Karban & Baldwin 1997). Induced direct defences negatively affect herbivores through plant morphological and chemical traits (eg. trichomes, leaf toughness, secondary metabolites and anti-digestive proteins) (Karban & Baldwin 1997; Howe & Jander 2008; Hagenbucher *et al*., 2013). Induced indirect plant defences do not affect the herbivores directly, but indirectly through the attraction of natural enemies due to production of volatiles from plants under attack (Dicke & Sabelis 1988; Sabelis *et al*., 1999; Kessler & Baldwin 2002; Choh & Takabayashi 2010) or through the arrestment of natural enemies by providing them with food (Heil *et al*., 2001; Ness 2003; Choh *et al*., 2006; Choch & Takabayashi 2010; Yamawo & Suzuki 2017). The induced plant volatiles (HIPV) produced under herbivore attacks can be released by different parts of the plant into the atmosphere (War *et al*., 2011) and are important cues for predators and parasitoids to find prey on the attacked plant (Dicke & Sabelis 1988; Turlings *et al*., 1990). For example, experiments showed that *Phytoseiulus persimilis* and *Mataseiulus occidentalis* are attracted by volatiles emanating from bean leaves infested by their prey *Tetranychus urticae* and *Amblyseius potentillae* and *Amblyseius finandicus* are attracted by volatiles related to plant attacks by their prey, the phytophagous mite *Panonychus ulmi* (Sabelis & van de Baan 1983).

These blends of volatiles are complex in terms of composition, because they can differ depending on the species of herbivore, herbivore density, (Dicke *et al*., 1998; Horiuchi *et al.,* 2003; Shiojiri *et al*., 2010; Silva *et al*., 2017; Cai *et al*., 2013; Pinto-Zevallos *et al*., 2018), plant species and even plant cultivars (Van den Boom *et al*., 2004; Takabayashi *et al*., 1994). Obviously, many of the first studies of this phenomenon considered simple tritrophic systems, but in nature, plants are often attacked by several herbivores at the same time. When a host plant is attacked by more than one herbivore, the induced volatiles can be qualitatively and quantitatively different from volatiles induced by a single pest (Shiojiri *et al.,* 2001; Delphia *et al.,* 2007; Magalhães *et al*., 2018). Therefore, such multiple infestations can change the interaction between plants and natural enemies due to the modification of volatile compounds released by an infested plant. For example, Shiojiri et al. (2001) observed that the parasitoid *Cotesia plutellae* was attracted to cabbage plants infested by

Plutella xylostella, but not to plants infested by *P. xylostella* and *Pieris rapae* simultaneously, while *Cotesia glomerata* preferred plants infested by both prey than plants infested by *P. rapae.* Owing to these differences in volatile composition between singly and doubly-infested plants, it is difficult to predict the response of predators and parasitoids to the volatiles produced by plants simultaneously attacked by several herbivores (Shiojiri *et al*., 2001, 2002; Dicke *et al*., 2009). The capacity of predators and parasitoids to identify those volatiles can be innate or learned (Turlings *et al*., 1993; Dicke *et al*., 1990; Aratchige *et al*., 2004; Nomikou *et al*., 2005; Tatemoto & Shimoda 2008). Therefore, the rearing history can influence the attraction of predators (Drukker *et al*., 2000; van den Boom *et al*., 2002). Although many studies show the attractiveness of experienced predators to volatiles from plants infested by a single pest, studies with a naïve predatory mite responding to blends of volatiles of single versus multiple-infested plants need to further investigations.

The generalist predatory mite *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae) and its two prey the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) co-occur in plantations of the biodiesel plant *Jatropha curcas* L. in Brazil (Sarmento *et al*., 2011). This phytoseiid mite is blind, so it depends to a large extend to volatiles to locate prey. It is known that *I. zuluagai* feed on both prey and pollen and benefit from diets consisting of a mixture of eggs of both pest mites (Marques *et al*., 2015). However, it is not known if they are attracted by volatiles released from plants with single and multiple infestations. Therefore, we studied the innate olfactory response of predatory mites *I. zuluagai* to volatile blends induced by multiple and single infestations.

Material and Methods

Plant material and mites rearing

The experiments were conducted at the Federal University of Tocantins, Gurupi, Tocantins, Brazil. Plants were cultivated in plastic pots (2.8 L) and irrigated once per day. They were kept in a fine-meshed cage to prevent any pest infestation. The initial laboratory stock colonies of *I. zuluagai*, *T. bastosi* and *P. latus* originated from mites collected from *J. curcas* in an experimental field in Gurupi, state of Tocantins, Brazil (11°45′47″S, 49°02′57″W). Predators were reared on arenas consisting of flexible plastic discs floating on distilled water inside a plastic box. *Iphiseiodes zuluagai* were fed with castor bean pollen (*Ricinus communis* L.) and honey. *Tetranychus bastosi* were reared on uninfested plants of *J. curcas* that were renewed when necessary. *Polyphagotarsonemus latus* were reared on leaves of *J. curcas* placed on a foam layer surrounded by hydrophilic cotton wool to prevent mites to escape. All cultures were maintained in a climate-controlled room (25 ± 2 °C, 65 –70% relative humidity, and a 12 hr L/12 hr D photoperiod.)

Infestation of J. curcas plants

Jatropha curcas plants of 30 days old with four leaves were used in the following experiments. There were four treatments: Uninfested plants, *T. bastosi* infested plants, *P. latus* infested plants and mixed infested plants (*T. bastosi* + *P. latus*). The apical leaf (leaf 1) and the leaf below (leaf 2) received 100 adult females of herbivorous mites, according to the treatment. When a plant was infested by a single (*P. latus* or *T. bastosi*) prey, we released them on both leaves and when it was simultaneously infested with both species, the two species were placed on different leaves: *P. latus* on the apical leaf and *T. bastosi* on the leaf below, in agreement with their natural within-plant distribution. Three days later, the experiments were carried out.

Olfactometer experiments

The response of *I. zuluagai* to volatiles of plants infested with phytophagous mites were carried out in a Y-tube olfactometer (Sabelis & van de Baan, 1983; Janssen 1999). The olfactometer consisted of *Y*-shaped *glass tube* (27 cm long x 3.5 cm inner diameter) with a metal wire in the middle of its interior on which the mites can walk. Each arm was connected to a glass cage in which the plants were placed. The base of the Y-tube was connected to a vacuum pump that produced an airflow from the tube arms to the base. This airflow carried plant volatiles into the olfactometer tube. The air speed was measured with anemometers and was calibrated to 0.50 m/s in each arm.

Adult female predators were starved for 2 hours prior the experiment. One mite at a time was introduced at the base of the tube where it walked upwind along the metal wire. On the wire junction, it had to choose one volatile source. Its choice was recorded upon reaching the end of one of the arms. Each individual was observed for a maximum of 5 minutes. When an individual did not make a choice during this period, this was recorded as no choice. One replicate consisted of the response of twenty females (excluding those that made no choice) to two sets of plants, and new plants and predators were used for each replicate. To prevent unforeseen asymmetries in the set-up, the volatile sources were connected to the opposite arm after each five females that had made a choice. Each combination of treatments was replicated four times. To test whether there was a significant preference for one of the arms of the olfactometer in any of the replicates, we analysed the data with a generalized linear model (GLM) with a Poisson error distribution with side (left or right) and replicate and their interaction as factors. This was never significant. Subsequently, the preference for a certain volatile source was tested with a similar GLM with volatile source, replicate and their interaction as factors. A significant interaction between replicate and volatile would indicate that the preference for a volatile was not consistent over all replicates, but this was never the case. All statistical analyses were performed in R.

Results

The predators showed no preference when offered a choice between plants infested by *T. bastosi* vs clean plants (Fig. 1a, GLM: χ2 = 0.80, d.f. = 1, P = 0.37) or between plants infested by *P. latus* vs clean plants (Fig. 1a, GLM: χ2 = 0.20, d.f. = 1, P = 0.65). When given a choice between mixed infested vs clean plants, *I. zuluagai* showed a slight, non-significant preference for mixed infested plants (Fig. 1a, GLM: erχ2 = 3.22, d.f. = 1, P = 0.072). The predator showed a slight non-significant preference for *T. bastosi* when offered a choice between plants infested by *P. latus* vs plants infested by *T. bastosi* (Fig. 1b, GLM: χ2 = 2.46, d.f. = 1, P = 0.11). *Iphiseiodus zuluagai* showed no significant preference between mixed infested plants vs plants infested by *T. bastosi* (Fig. 1b, GLM: χ2 = 0.050, d.f. = 1, P = 0.82) and mixed infested plants vs plants infested by *P. latus (*Fig. 1b, GLM: χ2 = 0.601, d.f. = 1, P = 0.43).

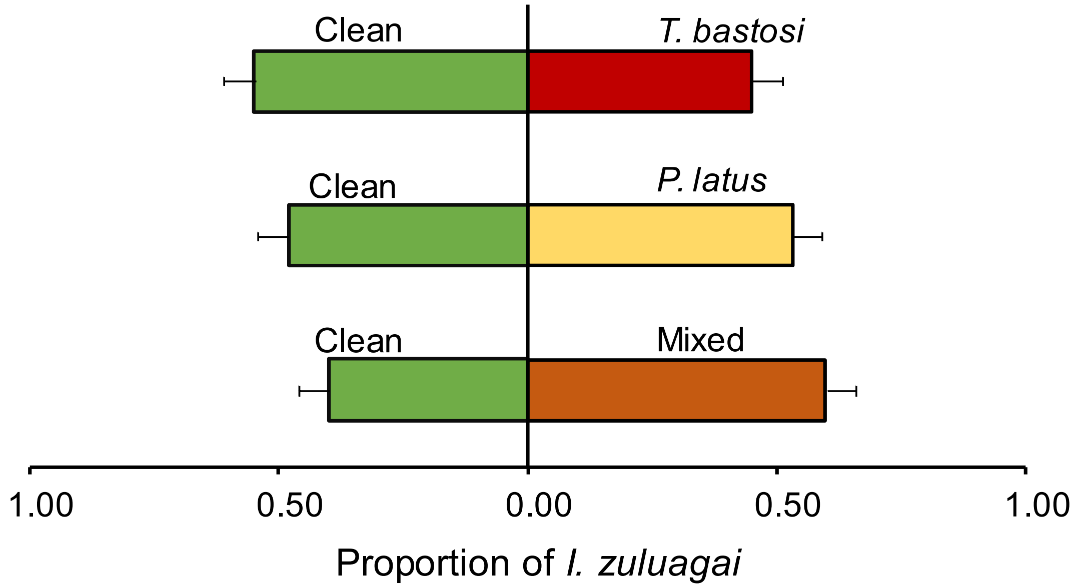

Figure 1. Response of the predator *I. zuluagai* in a Y-tube olfactometer to volatiles from clean *J. curcas* plants and *J. curcas* plants infested by *T. bastosi* and/or *P. latus*. Each bar represents the average (+ SE) of four replicates; one replicate is the choice of twenty predatory mites with one set of plants.

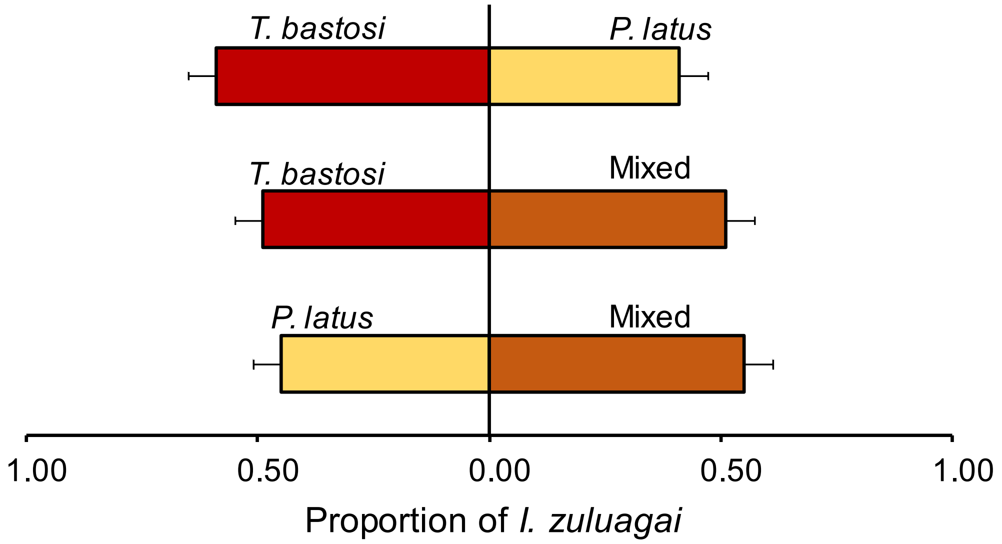

Figure 2. Response of the predator *I. zuluagai* in a Y-tube olfactometer to volatiles from *J. curcas* plants infested with different combinations of the prey mites. Each bar represents the average (+ SE) of four replicates; one replicate is the choice of twenty predatory mites with one set of plants.

Discussion

We observed that the predatory mite *I. zuluagai* showed no significant preference for clean plants vs infested plants with *P. latus*, *T. bastosi* or plants infested by both simultaneously. Many studies showed that plants under attack are more attractive to predators than clean plants, (Janssen 1999; Venzon *et al*., 1999; van den Boom *et al*., 2002; Fadini *et al*., 2010) but we did not observe this pattern of attraction. We also showed that *I. zuluagai* did not have significant preference for volatiles from singly-infested and multiply-infested plants.

The attack of plants by several herbivores simultaneously can lead to the production of different volatile blends than attacks by each species separately (Shiojiri *et al*., 2001; Delphia *et al*., 2007; Moyaeri *et al*., 2007; Magalhães *et al*., 2018). Consequently, these changes in volatile composition may affect attraction of natural enemies. Previous studies showed that multiple infestations can either increase or reduce natural enemy attraction (Shiojiri *et al*., 2001; Moyaeri *et al*., 2007; Zhang *et al*., 2009; Lima *et al*., 2016). It was somewhat curious that *I. zuluagai* did not show significant attraction to any treatment, even when a clean plant was the alternative option, because we previously observed that *I. zuluagai* prefer single and multiple infested plants over clean plants in release and recapture experiments (Chapter 1).

In the experiments reported in chapter 1, many other cues besides volatiles could have affected the behaviour of the predators, which can explain the difference in responses here and in chapter 1. In the previous experiments they had access to the prey, could touch them and their products such as exuviae and web, they could feed and feel vibrations besides perceiving chemical cues. Moreover, the time given to the predatory mites to make a choice was longer in the experiments with the plants than in the tests with the olfactometer. So, there were many more interactions in the release and recapture experiments which may have influenced the preference of those mites. Teodoro *et al.* (2009) showed that *I. zuluagai* did respond to volatiles of coffee plants attacked by *Oligonychus ilicis* in similar olfactometer essays as performed here, hence, the olfactometer is in principle suitable for this species. However, further experiments should verify whether the strain used here also responds well in this olfactometer. However, the predators did not show a significant preference for coffee plants attacked by *Brevipalpus phoenicis* over uninfested plants, even with *B. phoenicis* being part of the diet of *I. zuluagai* (Teodoro *et al.,* 2009). Another explanation for the lack of preference is that *I. zuluagai* shows no innate preference for volatiles of *J. curcas* plants with either of the two prey species, as has been found in other studies (Teodoro *et al*., 2009; Drukker *et al*., 2000; Pinto-Zevallos *et al*., 2018). The lack of preference of *I. zuluagai* in our essays may then have been caused by the fact that predatory mite females had no experience with these host plants and prey, as is known for other species. For example, Drukker *et al*., (2000) showed that naïve predatory mites *P. persimilis*, which are considered extreme specialist predators of tetranychid mites, did not show a significant preference for volatiles of *T. urticae*-infested bean leaves over clean air, indicating a weak innate response. However, when they gave the predators an experience with infested bean leaves on which they could feed on spider mite eggs while being exposed to the herbivore-induced plant volatile, *P. persimilis* showed a strong preference for infested leaves over clean air. So, the attraction to herbivore-induced plant volatiles can be modified by experience in various predators (Drukker *et al*., 2000; Malekinia *et al*., 2014; Pinto-Zevallos *et al*., 2018). Thus, experiments with experienced predatory mites need to be carried out to verify whether the same phenomenon occurs in *I. zuluagai*. Moreover, there are no studies on the volatile compounds from *J. curcas* infested by any pest. Thus, although *T. bastosi* and *P. latus* are suitable prey for *I. zuluagai* (Sarmento *et al*., 2011; Marques *et al*., 2015), the blend of volatiles emanating from plants infested by either of them alone or in combination are not attractive to naïve predatory mites. Maybe the lack of response could be an indicative of a low efficiency of this mite for biological control of those mites on *J. curcas*. However, the specialist predatory mite *P. persimilis* also does not show an innate preference for plants with its prey, the two-spotted spider mite, which it efficiently controls in greenhouses and outdoors. Hence, an innate preference for volatiles of plants attacked by a pest is not necessary for efficient control, but perhaps being able to learn the association between volatiles and the presence of the pest on a plant is efficient. Therefore, it needs to be established if *I. zuluagai* is capable of learning the association between plant volatiles and the presence of prey, as was found for *P. persimilis* (Drukker et al. 2000).

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GENERAL CONCLUSION

In this dissertation, I show that different prey attacking a plant can affect the foraging behaviour of a predatory mite. In chapter 1, I show that the predatory mite *I. zuluagai,* despite benefitting from a mixed diet consisting of two prey species, does not show a preference for plants infested with these two prey over plants infested with a single prey. Moreover, I show that this mite has a significant preference either for clean or infested plants, depending on the prey that is attacking, leading us to believe that several cues, such as physical, chemical and vibrational cues, are involved in this interaction.

The significant preference of *I. zuluagai* for infested plants indicates that this mite uses several cues for the location of its prey. Thus, in the second chapter, I accessed the foraging behaviour of this mite based only on volatile chemical cues. I investigated if volatiles emanating from plants with prey were in fact the main cues used by these predators in locating their prey. I found that predatory mites without experience with plants infested with those prey had no significant preference for the volatiles of any of the tested combinations. This indicates that this mite shows no innate preference for the volatiles of plants infested with their prey, as has been found for several other predators and leaves open the question whether experience with these volatiles associated with food may change their response.

In conclusion, *I. zuluagai* has significant preference for attacked plants when it could choose between plants and access various cues released by the attacked plant and the prey. Although, this mite did not demonstrate an innate response based on olfactory cues, they had an innate preference when other cues were involved, as shown in chapter 1, where they showed preferences even without previous. experience. Therefore, it is important to verify if its behaviour is plastic, and whether the olfactory response can change as a consequence of experience.