RENATO DE ALMEIDA SARMENTO

TOMATO INDUCED DEFENSES MEDIATING ECOLOGICAL INTERACTIONS AMONG ARTHROPODS

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

VIÇOSA
MINAS GERAIS - BRASIL
2007

RENATO DE ALMEIDA SARMENTO

TOMATO INDUCED DEFENSES MEDIATING ECOLOGICAL INTERACTIONS AMONG ARTHROPODS

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

APROVADA: 12 de fevereiro de 2007.

Dra. Madelaine Venzon
(Co-Orientadora)

Prof. Eraldo Rodrigues de Lima
(Co-Orientador)

Prof. José Henrique Schoereder

Prof. Derly José Henriques da Silva

Prof. Angelo Pallini Filho
(Orientador)

AGRADECIMENTOS

A Deus, por estar presente em todos os momentos de minha vida, vitórias e derrotas, alegrias e tristezas, me iluminando e guiando em toda essa longa jornada.

Minha gratidão eterna aos meus pais, pela confiança, oportunidades oferecidas, apoio, estímulo e pelo amor incondicional.

Às minhas irmãs Renata e Roberta, que muito me incentivaram nos momentos que precisei e ao amigo e cunhado Raimundo Wagner de Souza Aguiar.

À Madelaine Venzon pelos valiosos comentários dados ao trabalho.

Ao professor e amigo Julcemar Didonet, que foi de fundamental importância para o alcance desse objetivo.

Aos professores da UFT e amigos: Eduardo, Gil, Joênes e Jair.

Em especial ao prof. Angelo Pallini, que foi mais do que um orientador, foi um amigo sincero e dedicado que muito contribuiu para a realização desse trabalho e também para minha formação profissional e pessoal.

Aos pesquisadores da University of Amsterdam: Arne Janssen, Maurice W. Sabelis, Martijn Egas e Jun Bruin.

Aos colegas da University of Amsterdam: Nayanie Aratchige, Roos van Maanen, Michiel van Wijk, Amir Grosman, Tom Groon, Nicola Tien, Merijn Kant, Mathias Spichtig, Isa Lesna e Paulien de Bruijn.

Aos colegas da Entomologia da UFV: Felipe de Lemos, Ana Paula, Berghem, Adrián, Claudinei, Eugênio (mossoró), Hatano (Japa), Cláudia, Romero, Anderson,

Hamilton, Adenir (morangueiro), Fadini, Luciano, Edmar e Marla pela boa convivência durante a realização do trabalho.

Aos colegas e ex-colegas de república Alexson Mello, Douglas Pina, Marcelo Corrêa e Jefferson Siqueira.

Aos amigos tocantinenses e companheiros em Viçosa: Manoel Mota, Rodrigo Fidelis, Hélio Barros, Raimundo Rocha, Ronaldo Coimbra e Daniel Fragoso.

À "Dona" Paula, que mais que uma secretária eficiente, é uma pessoa amável e bem humorada.

Aos funcionários do departamento "seu" Chico, Evaristo e Samir.

Todas as pessoas que de uma forma ou de outra contribuíram para que atingisse este meu objetivo.

À FAPEMIG pelo apoio financeiro ao projeto de pesquisa.

À University of Amsterdam (UvA) pelo treinamento concedido, e à WOTRO-NWO (Netherlands Foundation for the Advancement of Tropical Research) pelo apoio financeiro.

À Universidade Federal de Viçosa (UFV) pela oportunidade de realização do curso e ao CNPq pela concessão de bolsa de estudo.

Ofereço

Aos meus pais:

Raimundo Roberto Gonçalves Sarmento

&

Jacira Almeida Sarmento

BIOGRAFIA

RENATO DE ALMEIDA SARMENTO, filho de Raimundo Roberto Gonçalves Sarmento e Jacira Almeida Sarmento, nasceu em 10 de maio de 1979 na cidade de Gurupi, TO.

Em 1996 iniciou o curso de Agronomia na Universidade do Tocantins onde foi bolsista de Iniciação Científica sob a orientação do professor Julcemar Didonet. Concluiu o curso em janeiro de 2001.

Em abril de 2001, iniciou o Curso de Mestrado em Entomologia na Universidade Federal de Viçosa sob a orientação do Prof. Angelo Pallini. Concluiu o curso em 28 de fevereiro de 2003.

Em março de 2003, iniciou o Curso de Doutorado em Entomologia na Universidade Federal de Viçosa sob a orientação do Prof. Angelo Pallini, submetendo-se à defesa de tese em fevereiro de 2007.

SUMÁRIO

RESUMO

SARMENTO, Renato de Almeida, D.Sc., Universidade Federal de Viçosa, fevereiro de 2007. **Defesas induzidas em plantas de tomate mediando interações ecológicas entre artrópodes.** Orientador: Angelo Pallini Filho. Co-Orientadores: Madelaine Venzon e Eraldo Rodrigues de Lima.

Neste estudo foi investigado o efeito das defesas induzidas diretas e indiretas de plantas de tomate sobre características ecológicas do ácaro vermelho *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), pelo fato desse ácaro ser praga importante do tomateiro. Também foi avaliado o efeito de defesas indiretas de plantas de tomate, tais como a emissão de compostos voláteis induzidos por herbivoria, no comportamento de forrageamento do coccinelídeo predador *Cycloneda sanguinea* Linnaeus (Coleoptera: Coccinellidae), e se este predador usa pistas voláteis para detectar a presença de predadores heteroespecíficos em seus locais de alimentação e oviposição. Foi observado que fêmeas do ácaro *T. evansi* foram atraídas ao invés de repelidas por voláteis provenientes de plantas de tomate infestadas com ácaros da mesma espécie. Além disso, a fecundidade de fêmeas de *T. evansi* em folhas de tomate que previamente receberam dano desses ácaros foi aproximadamente duas vezes maior que em folhas de plantas de tomate limpas, enquanto a fecundidade em folhas de plantas induzidas foi similar àquela obtida em plantas limpas. Em seguida, foi observado que a atividade de inibidores de protease foi aproximadamente três vezes mais baixa em folhas danificadas pelo ácaro *T. evansi* do que em folhas limpas e duas vezes menor que em folhas limpas de plantas danificadas (folhas induzidas). Contudo, quando folhas de plantas de tomate foram danificadas pelo ácaro rajado *Tetranychus urticae* Koch (Acari: Tetranychidae), observou-se um aumento expressivo dos níveis desses inibidores de protease nessas folhas. Em seguida, observou-se que a menor taxa de oviposição de fêmeas de *T. evansi* em folhas de plantas de tomate que foram previamente infestadas com espécimens de *T. urticae* coincidiu com a preferência de fêmeas de *T. evansi* por plantas de tomate infestadas por ácaros da mesma espécie, quando testada contra plantas infestadas com o ácaro rajado *T. urticae*. Estes resultados indicam que o ácaro *T. evansi* é capaz de enfraquecer o sistema de defesa de suas plantas hospedeiras, o que explicaria a maior atratividade dessas plantas a *T. evansi*, bem como o melhor desempenho de fêmeas deste ácaro em plantas infestadas por ácaros da mesma espécie, quando comparada a plantas limpas. Já o desempenho inferior de fêmeas de *T. evansi* em folhas de plantas de tomate que receberam danos do ácaro rajado *T. urticae,* pode ter sido determinada pela baixa qualidade nutricional de tais plantas, causada pelos altos níveis de inibidores de protease nessas plantas. Adicionalmente, a densidade relativa de teia produzida por fêmeas de *T. evansi* foi aproximadamente três vezes menor em discos feitos de folhas de plantas que foram previamente infestadas com ácaros da mesma espécie do que em discos de folhas de plantas que foram previamente infestadas com *T. urticae*. Já a taxa de oviposição de fêmeas de *T. evansi* foi aproximadamente duas vezes maior em discos feitos de folhas de plantas de tomate que foram previamente infestadas com ácaros da mesma espécie do que em folhas de plantas que foram infestadas com o ácaro *T. urticae*. Isso mostra que fêmeas do ácaro *T. evansi* possuem flexibilidade para ajustar o investimento na produção de ovos *vs.* produção de teia, em função da qualidade de suas plantas-hospedeiras. Finalmente, foi observado que o coccinelídio predador *C. sanguinea,* usa pistas voláteis induzidas por herbivoria para selecionar suas presas e também usa odores para evitar locais ocupados pelo predador heteroespecífico *Eriopis connexa* Mulsant (Coleoptera: Coccinellidae), provavelmente para evitar competição por alimento e/ou predação intra-guilda.

ABSTRACT

SARMENTO, Renato de Almeida, D.Sc., Universidade Federal de Viçosa, February of 2007. **Tomato induced defenses mediating ecological interactions among arthropods**. Adviser: Angelo Pallini Filho, Co-Advisers: Madelaine Venzon and Eraldo Rodrigues de Lima.

In this study, we investigated the effect of tomato induced direct and indirect defenses on ecological traits of the red spider mite *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), given the fact that this spider mite is such a successful pest species. It was also investigated the effect of tomato induced indirect defenses, such as the emission of volatile compounds, on the foraging behavior of the ladybird predator *Cycloneda sanguinea* Linnaeus (Coleoptera: Coccinellidae), and whether this predator can use volatile cues to assess the presence of heterospecific predators on a given patch. We observed that *T. evansi* mites were attracted rather than repelled by the volatiles from tomato plants infested with conspecifics. Furthermore, the fecundity of *T. evansi* females on leaves of tomato plants damaged by conspecifics was a factor 2 higher than on leaves of clean tomato plants, whereas fecundity on induced plants was similar to that on clean plants. Lastly, we found that the proteinase inhibitor activity was approximately 3-fold lower in damaged leaves than in clean leaves, and 2-fold lower than in undamaged leaves from a damaged plant. However, when tomato plantas were damaged by the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), we found that the levels of proteinase inhibitors in the leaves of such plants increased instaed of decreiased. Additionally, the lower oviposition rate of *T. evansi* females on leaves of tomato plants that previously received damage by *T. urticae* was matched by the preference of *T. evansi* to plants infested with conspecifics mites, when tested *versus* plants infested with *T. urticae* mites. These results indicate that *T. evansi* is capable of weakening the defense system of its host plant, which would explain the attractiveness of infested plants to the mites and the higher performance on plants infested by conspecifics compared to clean plants. We favor the possibility that the preference of *T. evansi* females to plants infested by conspecifics, when tested versus plants infested by *T. uricae* was determined by the lower nutritional quality of the plants caused by the higher levels of proteinase inhibitors in such plants. Furthermore, the relative web density produced by *T. evansi* was a factor 3-fold lower on leaf-discs from leaves of plants that were previously damaged by conspecifics than in leaf-discs from leaves of plants that received damage by *T. urticae.* Nevertheless, the ovipositional rate of *T. evansi* females was approximately 2-fold higher on tomato leaves that were previously damaged by conspecifics mites compared to leaves of plants that were previously damaged by *T. urticae*. We conclude that *T. evansi* mites have the flexibility to adapt their investment in eggs *vs.* web production depending on the quality of their host plants. Finally, we observed that *C. sanguinea* can use volatile cues to assess patch profitability and avoid patches with the heterospecific predator *Eriopis connexa* Mulsant (Coleoptera: Coccinellidae), probably to avoid competition for food and/or intraguild predation.

Introduction and General Conclusions

Plant defense research has emerged from the fields of phytopathology and agronomy but is now a central topic in the more general fields of plant physiology and ecology as well. Plant defense against herbivores arthropods involve a combination of constitutive and inducible defenses that decrease the performance of such herbivores (Baldwin & Preston, 1999). These constitutive and inducible defenses are crucial for resistance against non-pathogen pests such as herbivorous insects and mites (Kant, 2006). Constitutive defenses involve those traits that are always on/in the plant and when removed, neutralized or knocked-out make the plant more susceptible to attackers. Examples are sticky trichomes (Eisner et al., 1998) and constitutively produced secondary metabolites (Wittstock & Gershenzon, 2002). The defense state of a plant can also be adjusted upon signals that are associated with attack (Agrawal, 2005). Such adjustments involve structural reinforcements (e.g. thickening of cell walls or other structural barriers) or increased of toxic compounds, such as nicotine in tobacco (Steppuhn et al., 2004), glucosinolates in Arabidopsis (Thatcher et al., 2005; Grubb & Abel, 2006) and proteinase inhibitors in several plant species such as tomato (Chen et al., 2005). Typical induced-defense compounds can be autotoxic (Gog et al., 2005), costly to produce (Walters & Boyle, 2005) and to function relatively late in the interaction as a defense (Morris et al., 2006). In addition to *direct defenses* (that are per definition the one-to-one type interactions between a plant and an attacker) plant can defend themselves *indirectly* via the third trophic level: predators and parasitoids of herbivores (Sabelis et al., 2001; 2006). Plant traits (constitutive or inducible) that facilitate the presence of such natural enemies by any means are the mediators of indirect defenses. For example, plants can provide shelter (Romero & Benson, 2005; Matos, 2006) or alternative food (Kost & Heil, 2005) to predators thereby arresting the herbivore's enemy. They can also produce specific signals – volatile organic compounds – upon attack that help natural enemies in finding their food and/or ovipositional sites (Janssen et al., 1997; Grostal & Dicke, 1999; Gnanvossou et al., 2003).

The above implies that plants should assess the nature of its attackers in order to mount an appropriate defense response. How specific plants can recognize particular attackers depends highly on the particular interaction under study. Some responses are merely wound-responses but in other cases the interaction seems to be specific (Wasternack et al., 2006). Plant defenses can be activated by the so-called elicitors (Hahn, 1996), which can be plant cell wall fragments that are typically generated during the pathogen/herbivore-plant interaction, pathogen or herbivore-derived products secreted during the interaction (e.g. saliva) (Hahn, 1996), or a conjugation of both. Only few herbivore elicitors of plant defenses have been identified. The first was ß-glucosidase, an enzyme from *Pieris brassicae* regurgitant, potent in inducing the emission of volatiles such that *Cotesia glomerata* was attracted (Mattiacci et al., 1995). The second was reported by Alborn et al. (1997), and was called *volicitin* since also this compound specifically induced sufficient emission of plant-volatiles to attract parasitic wasps. Volicitin (N[17-hidroxilinolenoil]-L-glutamina) is a conjugate of a plant-derived fatty acid to an insect gut amino acid. Later, several other fatty-acid amino-acid (FACS) conjugates from *Manduca sexta* oral secretions, potent in inducing various defense responses in *Nicotina attenuate* were isolated as well (Halitschke et al., 2001). How

FACS are perceived by plants is unclear although a membrane bound protein that interacts with volicitin has been reported (Truitt et al., 2004). Alternatively, glucose oxidase (GOX) from *Spodoptera exigua* saliva was found to repress the expression of plant genes involved in defenses such of those genes involved in the production of herbivore-induced volatiles (Bede et al., 2006). It has been proposed that this happens when direct defenses are inadequate (Kahl et al., 2000), since in many cases it appeared that these volatiles had the property of making the plant more attractive to predators and parasitoids of herbivores (Pare et al., 1998; Pare & Tumlinson, 1999). The attraction of natural enemies of a pest species is referred to as the indirect defense response (Sabelis et al., 2001). It is believed that the direct and the indirect defense strategies act in concert (Baldwin & Preston, 1999). However, hardly anything is known about the properties of their interactions and their interdependence (Kant et al., 2004).

In tomato, the indirect defense response is characterized by an increase in producing volatile compounds like monoterpenes, and a variety of sesquiterpenes, aromatics, aldehydes, ketones, alcohols, and esters (Andersson et al., 1980; Lundgren et al., 1985; Buttery et al., 1987; Smith et al., 1996; Ament et al., 2004). When infested with spider mites, leaves of tomato plants start to emit more phenolic methyl salicylate (MeSA) and homoterpene 4,8, 12-trimethyltrideca1,3,7,11-tetraeno (TMTT) (Dicke et al., 1998). However, different herbivore species can induce different volatiles (Vercammen et al., 2001; Thaler et al., 2002). Additionally, it has been well established that predatory mites, discriminate between prey-infested and uninfested plants on the basis of odours (for reviews, see Janssen et al., 1997; Dicke et al., 1990; Sabelis et al., 1999).

The direct defense response of tomato plants upon herbivory is characterized by an increase in signaling compounds like jasmonate (JA), ethylene (Et), salicylate (SA) and systemin, which results in local and systemic changes, such as the accumulation of polyphenol oxidase, peroxidase (POD), chitinase, callose synthase and proteinase inhibitors (Figure 1) (McCloud & Baldwin, 1997; Walling, 2000). Proteinase inhibitors are proteins that inhibit digestive enzymes in the gut of arthropod herbivores, which can reduce their growth, reproduction and consequently causing herbivore death (Green & Ryan, 1972; Broadway & Duffey, 1986; Ryan, 1990; Gatehouse, 2002; Lawrence & Koundal, 2002; Pompermayer et al., 2003).

However, direct defense responses of tomato plants against stylet-feeders (such as phloem-feeding whiteflies, aphids, and mesophyll-feeding mites and thrips) and leaf-chewing insects (such as lepidopteran larvae) appear to be different. Caterpillars stimulate polyphenol oxidase and lipoxigenase activity, leaf miners only POD activity (Stout et al., 1994). Also spider mites feeding on tomato plants induce the accumulation of proteinase inhibitors, while whiteflies do not (Walling, 2000). Li et al., (2002) showed that the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) induces a rapid jasmonate regulated in tomato. This spider mite has been recovered from over 900 plant species comprising 124 different plant families (Egas et al., 2003).

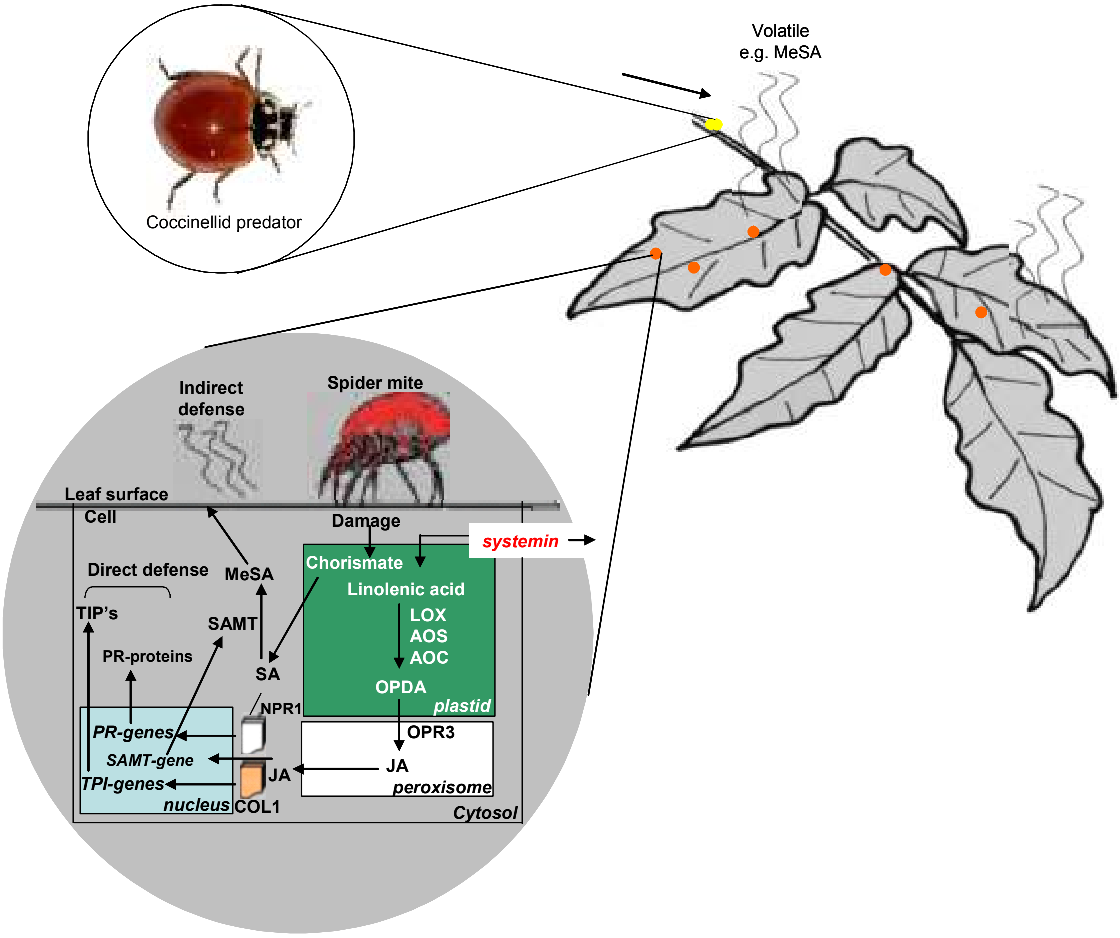

Figure 1. Overview of the induced defenses response. Herbivores (such as spider mites) induce formation of linolenic acid-derived JA (octadecanoid-signaling pathway) and chorismate-derived SA (phenylpropanoid-signaling pathway). The peptide systemin mediates transmission of the systemic wound signal. SA induces an NPR1-dependent signaling pathway that results in accumulation of Pathogenesis Related-proteins (PR-proteins) and JA induces a COL1-dependent signaling pathway that results in accumulation of (trypsin) proteinase inhibitors (TPIs). These are direct defense. Moreover, JA stimulates expression of *salicylic acid methyl transferase* (SAMT) and thereby induces conversion of SA into MeSA which is released as a volatile. Induced volatiles (TMTT and other terpenoids are not shown) are released into the air after which natural enemies of herbivores (such as coccinellid predators) can use these odours to locate their preys and ovipositional sites as well. This we call the indirect defense response (Kant, 2006).

The red spider mite, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) has been reported as pest of several solanaceous crops, but preferring to feed on tomato (Jeppson et al., 1975; DeMoraes & McMurtry, 1985). However, there are no previous studies that investigated the effect of *T. evansi* damage on the induction of defense-responses, such as the acumulation of proteinase inhibitors in tomato plants.

Furthermore, *T. evansi* appears to be an unfavorable prey for several species of phytoseiid mite predators (DeMoraes & McMurtry, 1985) including commercially available predatory mites, such as *Phytoseiulus persimilis* and *Neoseiulus californicus* that are currently widely used to control the related *T. urticae* in glasshouse crops (Drukker et al., 1997; Garthwaite, 2000; Escudero & Ferragut, 2005). This is partly due to the eggs of *T. evansi* containing a feeding depressant that means predators rarely consume a whole egg and if they do feed, they feed very slowly (DeMoraes & McMurtry, 1986). Additionally, *T. evansi* mites spin a chaotically and structured web over their colonies and these webs are hard to penetrate for most of predators (Sabelis & Bakker, 1992; Oliveira et al., 2005; Rosa et al., 2005).

Thesis overview

In this thesis, I have investigated the effect of tomato induced direct and indirect defenses on ecological traits of the red spider mite *T. evansi* (chapter 1, 2 and 3), given the fact that this spider mite is such successful pest species. I have also investigated the effect of tomato induced indirect defenses, such as the emission of volatile compounds on the foraging behavior of the ladybird predator *Cycloneda sanguinea*, which is among the natural enemies studied as biocontrol agents of mites and aphids in Brazil. I also investigated whether this predator can use volatile cues to assess patch profitability (chapter 4).

Chapter 1 - Herbivores weaken plant defense

In chapter 1, we first investigated the preference and performance of *T. evansi* mites to plants infested with conspecifics. To measure the induced direct defense of tomato plants, we subsequently measured the proteinase inhibitor activity in leaves of tomato plants damaged and induced by *T. evansi* mites, as well as, in clean leaves of such plants. This chapter formed the basis for chapters 2 and 3. In this chapter we show three aspects: first that *T. evansi* mites were attracted rather than repelled by the volatiles from tomato plants that were infested with conspecific mites. Second, we show that the fecundity of *T. evansi* females on leaves of tomato plants that were previous damaged by conspecifics mites was a factor two times higher than on leaves of clean tomato plants, whereas fecundity on induced plants was similar to that on clean plants. Lastly, we show that proteinase inhibitor activity was approximately 3-fold lower in damaged leaves than in clean leaves, and 2-fold lower than in undamaged leaves from a damaged plant. We conclude that *T. evansi* is capable of weakening the defense system of its host plant, which would explain the attractiveness of infested plants to the mites and the higher performance on plants infested by conspecifics compared to clean plants.

Chapter 2 - Herbivores use odours to assess host-plant quality

In chapter 2, we investigated tomato's direct defense response to the two-spotted spider mite *Tetranychus urticae* by measuring the proteinase inhibitor activity in damaged, induced and clean tomato leaves. We subsequently evaluated the effect of the damage caused by *T. urticae* on tomato plants on the fecundity of *T. evansi* females. We also investigated the preference of *T. evansi* to odours of tomato plants infested with conspecifics *vs.* tomato plants infested with *T. urticae*. We conclude that the higher performance and preference of *T. evansi* females to plants that received damage by conspecifics mites, when tested versus plants infested by *T. urticae*, both were determined by the higher nutritional quality of the plants. We also conclude that this higher nutritional quality was because of the lower levels of proteinase inhibitors in such plants.

Chapter 3 - Host-plant quality mediates trade-off between oviposition and web production of the spider mite Tetranychus evansi *Baker & Pritchard (Acari: Tetranychidae)*

The silk of the web produced by spider mites, such as *T. evansi*, over their colonies consists of proteins (amino acids) that would otherwise be available for growth and reproduction. Given this information and the observed in the previous chapters: *T. evansi* females had higher oviposition rate on leaves of plants that were previous damaged by conspecifics mites than on leaves of plants that were previous damaged by *T. urticae*, both compared to leaves of clean plants (chapter 1 and 2). In chapter 3 we investigated the effect of the damage caused by *T. evansi* and its closely relative *T. urticae* on the investment in eggs *vs.* web production of *T. evansi* females. We found that *T. evansi* mites have the flexibility to adapt their investment in eggs *vs.* web production depending on the quality of their host plant.

Chapter 4 - Use of odours by Cycloneda sanguinea Linnaeus (Coleoptera: Coccinellidae) to assess patch quality

In chapter 4, we investigated whether the ladybird predator *C. sanguinea* use volatile cues to asses patch profitability. We conclude that *C. sanguinea* can use volatile cues to assess patch profitability and avoid patches with heterospecific predators, probably to avoid competition for food and/or intraguild predation.

References

Agrawal, A. A. 2005. Future directions in the study of induced plant responses to herbivory. **Entomologia Experimentalis et Applicata**, 115, 97-105.

Alborn, T., Turlings, T. C. J., Jones, T. H., Stenhagen, G., Loughrin, J. H. & Tumlinson, J. H. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. **Science**, 276, 945-949.

Ament, K., Kant, M. R., Sabelis, M. W., Haring, M. A. & Schuurink, R. C. 2004. Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. **Plant Physiology**, 135, 2025-2037.

Andersson, B. A., Holman, R. T., Lundgren, L. & Stenhagen, G. 1980. Capillary gas chromatograms of leaf volatiles - A possible aid to breeders for pest and disease resistance. **Journal of Agricultural and Food Chemistry**, 28, 985-989.

Baldwin, I. T. & Preston, C. A. 1999. The eco-physiological complexity of plant responses to insect herbivores. **Planta**, 208, 137-145.

Bede, J. C., Musser, R. O., Felton, G. W. & Korth, K. L. 2006. Caterpillar herbivory and salivary enzymes decrease transcript levels of *Medicago truncatula* genes encoding early enzymes in terpenoid biosynthesis. **Plant Molecular Biology**, 60, 519-531.

Broadway, R. M. & Duffey, S. S. 1986. Plant proteinase-inhibitors - Mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. **Journal of Insect Physiology**, 32, 827-833.

Buttery, R. G., Ling, L. C. & Light, D. M. 1987. Tomato leaf volatile aroma components. **Journal of Agricultural and Food Chemistry**, 35, 1039-1042.

Chen, H., Wilkerson, C. G., Kuchar, J. A., Phinney, B. S. & Howe, G. A. 2005. Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. **Proceedings of the National Academy of Sciences of the United States of America**, 102, 19237-19242.

DeMoraes, G. J. & McMurtry, J. A. 1985. Comparison of *Tetranychus evansi* and *T. urticae* (Acari: Tetranychidae) as prey for eight species of Phytoseiid mites. **Entomophaga**, 30, 393-397.

DeMoraes, G. J. & McMurtry, J. A. 1986. Suitability of the spider mite *Tetranychus evansi* as prey for *Phytoseiulus persimilis*. **Entomologia Experimentalis et Applicata**, 40, 109-115.

Dicke, M., Takabayashi, J., Posthumus, M. A., Schutte, C. & Krips, O. E. 1998. Plant-phytoseiid interactions mediated by herbivore-induced plant volatiles: variation in production of cues and in responses of predatory mites. **Experimental & Applied Acarology**, 22, 311-333.

Dicke, M., Vanbeek, T. A., Posthumus, M. A., Bendom, N., Vanbokhoven, H. & Degroot, A. E. 1990. Isolation and identification of volatile kairomone that affects acarine predator-prey interactions - Involvement of host plant in its production. **Journal of Chemical Ecology**, 16, 381-396.

Drukker, B., Janssen, A., Ravensberg, W. & Sabelis, M. W. 1997. Improved control capacity of the mite predator *Phytoseiulus persimilis* (Acari: Phytoseiidae) on tomato. **Experimental & Applied Acarology**, 21, 507-518.

Egas, M., Norde, D. J. & Sabelis, M. W. 2003. Adaptive learning in arthropods: spider mites learn to distinguish food quality. **Experimental & Applied Acarology**, 30, 233-247.

Eisner, T., Eisner, M. & Hoebeke, E. R. 1998. When defense backfires: Detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. **Proceedings of the National Academy of Sciences of the United States of America**, 95, 4410-4414.

Escudero, L. A. & Ferragut, F. 2005. Life-history of predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis* (Acari: Phytoseiidae) on four spider mite species as prey, with special reference to *Tetranychus evansi* (Acari: Tetranychidae). **Biological Control**, 32, 378-384.

Garthwaite, D. 2000. Changes in biological control usage in Great Britain between 1968 and 1995 with particular reference to biological control on tomato crops. **Biocontrol Science and Technology**, 10, 451-457.

Gatehouse, J. A. 2002. Plant resistance towards insect herbivores: a dynamic interaction. **New Phytologist**, 156, 145-169.

Gnanvossou, D., Hanna, R. & Dicke, M. 2003. Infochemical-mediated niche use by the predatory mites *Typhlodromalus manihoti* and *T. aripo* (Acari: Phytoseiidae). **Journal of Insect Behavior**, 16, 523-535.

Gog, L., Berenbaum, M. R., DeLucia, E. H. & Zangerl, A. R. 2005. Autotoxic effects of essential oils on photosynthesis in parsley, parsnip, and rough lemon. **Chemoecology**, 15, 115-119.

Green, T. R. & Ryan, C. A. 1972. Wound-induced proteinase inhibitor in plant leaves - Possible defense mechanism against insects. **Science**, 175, 776-779.

Grostal, P. & Dicke, M. 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. **Behavioral Ecology**, 10, 422-427.

Grubb, C. D. & Abel, S. 2006. Glucosinolate metabolism and its control. **Trends in Plant Science**, 11, 89-100.

Hahn, M. G. 1996. Microbial elicitors and their receptors in plants. **Annual Review of Phytopathology**, 34, 387-412.

Halitschke, R., Schittko, U., Pohnert, G., Boland, W. & Baldwin, I. T. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera: Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. **Plant Physiology**, 125, 711-717.

Janssen, A., Bruin, J., Jacobs, G., Schraag, R. & Sabelis, M. W. 1997. Predators use volatiles to avoid prey patches with conspecifics. **Journal of Animal Ecology**, 66, 223-232.

Jeppson, L.R., Keifer, H. H. & Baker, E. W. 1975. **Mites injurious to economic plants**. Berkeley, University of California Press, 614p.

Kahl, J., Siemens, D. H., Aerts, R. J., Gabler, R., Kuhnemann, F., Preston, C. A. & Baldwin, I. T. 2000. Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. **Planta**, 210, 336-342.

Kant, M. R. 2006. **The consequences of herbivore variability for direct and indirect defenses of plants**. PhD thesis, University of Amsterdam, Amsterdam, 192p.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A. & Schuurink, R. C. 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. **Plant Physiology**, 135, 483-495.

Kost, C. & Heil, M. 2005. Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (*Phaseolus lunatus* - Fabaceae). **Basic & Applied Ecology**, 6, 237-248.

Lawrence, P. K. & Koundal, K. R. 2002. Plant protease inhibitors in control of phytophagous insects. **Electronic Journal of Biotechnology**, 5, 93-109.

Li, C. Y., Williams, M. M., Loh, Y. T., Lee, G. I. & Howe, G. A. 2002. Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. **Plant Physiology**, 130, 494-503.

Lundgren, L., Norelius, G. & Stenhagen, G. 1985. Leaf volatiles from some wild tomato species. **Nordic Journal of Botany**, 5, 315-320.

Matos, C. H. C. 2006. Mecanismos de defesa constitutiva em espécies de pimenta *Capsicum* e sua importância no manejo do ácaro branco *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae). Tese de Doutorado, Universidade Federal de Viçosa, Viçosa, 59p.

Mattiacci, L., Dicke, M. & Posthumus, M. A. 1995. Beta-Glucosidase - An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. **Proceedings of the National Academy of Sciences of the United States of America**, 92, 2036-2040.

McCloud, E. S. & Baldwin, I. T. 1997. Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*. **Planta**, 203, 430-435.

Morris, W. F., Traw, M. B. & Bergelson, J. 2006. On testing for a tradeoff between constitutive and induced resistance. **Oikos**, 112, 102-110.

Oliveira, E. E., Oliveira, C. L., Sarmento, R. A., Fadini, M. A. M. & Moreira, L. R. 2005. Biological aspects of the predator *Cycloneda sanguinea* (Linnaeus, 1763) (Coleoptera: Coccinellidae) fed with *Tetranychus evansi* (Baker & Pritchard, 1960) (Acari: Tetranychidae) and *Macrosiphum euphorbiae* (Thomas, 1878) (Hemiptera: Aphididae). **Bioscience Journal**, 21, 33-39.

Pare, P. W., Alborn, H. T. & Tumlinson, J. H. 1998. Concerted biosynthesis of an insect elicitor of plant volatiles. **Proceedings of the National Academy of Sciences of the United States of America**, 95, 13971-13975.

Pare, P. W. & Tumlinson, J. H. 1999. Plant volatiles as a defense against insect herbivores. **Plant Physiology**, 121, 325-331.

Pompermayer, P., Falco, M. C., Parra, J. R. P. & Silva, M. C. 2003. Coupling diet quality and Bowman-Birk and Kunitz-type soybean proteinase inhibitor effectiveness to *Diatraea saccharalis* development and mortality. **Entomologia Experimentalis et Applicata**, 109, 217-224.

Romero, G. Q. & Benson, W. W. 2005. Biotic interactions of mites, plants and leaf domatia. **Current Opinion in Plant Biology**, 8, 436-440.

Rosa, A. A., Gondim, M. G. C., Fiaboe, K. K. M., de Moraes, G. J. & Knapp, M. 2005. Predatory mites associated with *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on native solanaceous plants of coastal Pernambuco State, Brazil. **Neotropical Entomology**, 34, 689-692.

Ryan, C. A. 1990. Protease inhibitors in plants - genes for improving defenses against insects and pathogens. **Annual Review of Phytopathology**, 28, 425-449.

Sabelis, M. W. & Bakker, F. M. 1992. How predatory mites cope with the web of their Tetranychid prey - A functional view on dorsal chaetotaxy in the Phytoseiidae. **Experimental & Applied Acarology**, 16, 203-225.

Sabelis, M. W, Janssen, A., Pallini, A., Venzon, M., Bruin, J., Drukker, B. & Scutareanu, P. 1999. **Behavioural responses of predatory and herbivorous arthropods to induced plant volatiles: from evolutionary ecology to agricultural applications**, p.269-297. In Agrawal, A., Tuzun, S. & Bent, E. (eds.) Induced plant defenses against pathogens and herbivores: biochemistry, ecology, and agriculture, Minnesota, The American Phytopathological Society, 390p.

Sabelis, M. W., Janssen, A. & Kant, M. R. 2001. Ecology - The enemy of my enemy is my ally. **Science**, 291, 2104-2105.

Sabelis, M W., Takabayashi, J., Janssen, A., van Wijk, M., Sznajder, B., Aratchige, N., Lesna, I., Belliure, B., Kant, M. R. & R. C. Schuurink. 2006. **Chemically mediated, indirect effects on arthropod communities**. In: Ohgushi, T., Craig, T. P., Price, P. W. (eds) Indirect interaction webs: nontrophic through induced plant traits. Cambridge, Cambridge University Press, in press.

Smith, R. M., Marshall, J. A., Davey, M. R., Lowe, K. C. & Power, J. B. 1996. Comparison of volatiles and waxes in leaves of genetically engineered tomatoes. **Phytochemistry**, 43, 753-758.

Steppuhn, A., Gase, K., Krock, B., Halitschke, R. & Baldwin, I. T. 2004. Nicotine's defensive function in nature. **Plos Biology**, 2, 1684-1684.

Stout, M. J., Workman, J. & Duffey, S. S. 1994. Differential induction of tomato foliar proteins by arthropod herbivores. **Journal of Chemical Ecology**, 20, 2575-2594.

Thaler, J. S., Karban, R., Ullman, D. E., Boege, K. & Bostock, R. M. 2002. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. **Oecologia**, 131, 227-235.

Thatcher, L. F., Anderson, J. P. & Singh, K. B. 2005. Plant defence responses: what have we learnt from Arabidopsis? **Functional Plant Biology**, 32, 1-19.

Truitt, C. L., Wei, H. X. & Pare, P. W. 2004. A plasma membrane protein from *Zea mays* binds with the herbivore elicitor volicitin. **Plant Cell**, 16, 523-532.

Vercammen, J., Pham-Tuan, H. & Sandra, P. 2001. Automated dynamic sampling system for the on-line monitoring of biogenic emissions from living organisms. **Journal of Chromatography A**, 930, 39-51.

Walling, L. L. 2000. The myriad plant responses to herbivores. **Journal of Plant Growth Regulation**, 19, 195-216.

Walters, D. R. & Boyle, C. 2005. Induced resistance and allocation costs: What is the impact of pathogen challenge? **Physiological and Molecular Plant Pathology**, 66, 40-44.

Wasternack, C., Stenzel, I., Hause, B., Hause, G., Kutter, C., Maucher, H., Neumerkel, J., Feussner, I. & Miersch, O. 2006. The wound response in tomato - Role of jasmonic acid. **Journal of Plant Physiology**, 163, 297-306.

Wittstock, U. & Gershenzon, J. 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. **Current Opinion in Plant Biology**, 5, 300-307.

CHAPTER 1

Herbivores weaken plant defense

Renato Almeida Sarmento[1,2]*; Arne Janssen*[2]*; Angelo Pallini*[1]*; Felipe de Lemos*[1]

Madelaine Venzon[3] *and Maria Goreti Almeida Oliveira*[4]

[1]Department of Animal Biology/Entomology, Federal University of Viçosa, Brazil; [2]Institute for Biodiversity and Ecosystem Dynamics, Section Population Biology, University of Amsterdam, PO Box 94084, Amsterdam, The Netherlands; [3]EPAMIG (Agriculture and Livestock Research Enterprise of Minas Gerais, Brazil); [4]Department of Biochemistry and Molecular Biology, Federal University of Viçosa, Brazil

Abstract - Plants defend themselves against herbivores in several ways. They can produce specific blends of volatile chemicals in response to herbivore damage which are attractive to predators and parasitoids of the herbivores. Another type of defense is the synthesis of high levels of proteinase inhibitor enzymes in plant leaves after herbivore damage, which can reduce their growth and reproduction. The induced production of volatiles by plants often coincides with increased levels of defense against herbivores. It is therefore expected that herbivores would avoid plants that emit herbivore-induced volatiles. We studied such a response in the red spider mite *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), using a Y-tube olfactometer. We found that the mites were attracted rather than repelled by volatiles from tomato plants that were attacked by conspecifics. Further experiments showed that the fecundity of these mites on damaged tomato leaves was a factor 2 higher than on clean tomato plants, whereas fecundity on induced plants was similar to that on clean plants. This is in agreement with the observed attraction to damaged plants, but both higher oviposition and attraction are at odds with the hypothesis that herbivory induces a defense response in plants. To further investigate this, we assessed the levels of proteinase inhibitors in damaged, induced and clean tomato leaves. Contrary to what is commonly reported in the literature, proteinase inhibitor activity was approximately 3-fold lower in damaged leaves than in clean leaves, and 2-fold lower than in undamaged leaves from a damaged plant. We conclude that *T. evansi* is capable of weakening the defense system of its host plant, which would explain the attractiveness of infested plants to the mites and the higher performance on plants infested by conspecifics compared to clean plants.

Key words: Tomato plants, volatiles, *Tetranychus evansi*, direct defense, proteinase inhibitor

Resumo – Plantas se defendem contra o ataque de artrópodes herbívoros de várias maneiras. Elas podem produzir voláteis específicos em resposta ao dano do herbívoro, os quais são atrativos aos predadores e parasitóides que se alimentam desses herbívoros. Um outro tipo de defesa é a síntese de altos níveis de inibidores de protease nas folhas das plantas após o dano causado pelo herbívoro, o que pode levar à redução do crescimento e reprodução desses herbívoros. Adicionalmente, a produção de voláteis pelas plantas, induzidos por herbivoria, freqüentemente coincide com altos níveis de defesa contra herbívoros. Assim, espera-se que herbívoros evitem plantas que emitam voláteis induzidos por herbivoria. Neste estudo, tal resposta foi testada com o ácaro vermelho *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), utilizando um olfatômetro de tubo em Y. Observou-se que os ácaros foram atraídos ao invés de repelidos pelos voláteis provenientes de plantas de tomate infestadas com ácaros da mesma espécie. Observou-se em experimentos adicionais que a fecundidade desses ácaros em folhas de plantas de tomate previamente infestadas com ácaros co-específicos foi aproximadamente 2 vezes maior que em folhas de plantas limpas, enquanto que a fecundidade em folhas induzidas foi similar àquela em folhas limpas. Estes resultados coincidiram com a atração de fêmeas de *T. evansi* por plantas infestadas por co-especificos. Porém, a maior oviposição e atração contradizem a hipótese de que a herbivoria induz uma resposta de defesa em plantas. Para investigar isto, foram medidos os níveis de inibidores de protease em folhas de plantas de tomate limpas, danificadas e induzidas por *T. evansi.* Ao contrário do que é comumente documentado na literatura, a atividade de inibidores de protease em folhas de plantas de tomate danificadas pelo ácaro *T. evansi* foi aproximadamente 3 vezes menor que em folhas de plantas limpas, e 2 vezes menor do que em folhas limpas de plantas danificadas (folhas induzidas). Isso mostra que o ácaro *T. evansi* é capaz de enfraquecer o sistema de defesa de suas plantas-hospedeiro, o que explicaria a atratividade de plantas infestadas por fêmeas de *T. evansi* e o melhor desempenho em plantas infestadas por co-específicos comparada com plantas limpas.

Palavras-chave: plantas de tomate, voláteis, *Tetranychus evansi*, defesa direta, inibidor de protease.

Introduction

In response to herbivore damage, many plants produce a specific blend of volatile chemicals that attract predators and parasitoids, which then attack the herbivores and relieve the plant (Sabelis & van de Baan, 1983; Dicke et al., 1990; Turlings et al., 1990; Vet & Dicke, 1992; Sabelis et al., 2001). Herbivore damage is also known to induce the accumulation of high levels of chemicals within the plant tissue, which act as biochemical defense and interfere with the performance of the herbivores (Ryan, 1990). Examples of such chemicals are proteinase inhibitors, which are proteins that inhibit digestive enzymes in the gut of arthropod herbivores leading to reduction of their growth and reproduction (Ryan, 1990; Lawrence & Koundal, 2002; Pompermayer et al., 2003).

It has been suggested that host-plant preference and performance of herbivores are positively correlated (Agrawal, 2000). Thus, ovipositing females should lay eggs on the best possible host plant for larval survival and subsequent reproduction (Mayhew, 1997; West & Cunningham, 2002). The induced production of volatiles by plants often coincides with increased levels of defense against herbivores (Kant et al., 2004), thus it is expected that herbivores are attracted to clean plants instead of damaged and infested plants. For the red spider mite *Tetranychus evansi* (Acari: Tetranychidae), however, it was demonstrated that the mite prefers clean air to odours of clean tomato plants, and odours of plants infested with conspecifics to odours of clean plants (Sarmento, 2003). These results suggests that the mites use herbivore-induced volatiles from the damaged plants to localize their host plants. A potential reason for the preference of *T. evansi* for plants infested by conspecifics is that damaged plants may have a weakened instead of a stronger defense system, and the mites use herbivore-induced-volatiles as a cue indicating the decrease of defense. This would be contrary to the accepted notion that herbivore damage induces defense in plants. By colonizing plants with weakened direct defense, *T. evansi* would have better performance on those plants compared to clean plants.

Another reason for the preference for infested plants could be the presence of web produced by conspecifics. Spider mites such as *T. evansi* spin a chaotically structured web over their colonies and these webs are thought to function as defense against predators (Saito, 1977; Sabelis & Bakker, 1992). The silk of the web is made of proteins (Hazan et al., 1975) and its production may go at the expense of amino acids that would otherwise be available for growth and reproduction. Therefore, an additional reason for the preference of *T. evansi* for plants infested by conspecifics and not for clean plants could be that they can colonize webbed areas of infested plants and thus profit from web protection without having invested on its production.

These arguments led us to hypothesize that the presence and damage caused by *T. evansi* on tomato plants play a role in the preference and performance of conspecifics and that plants infested by conspecifics are more profitable to *T. evansi* than clean plants. To test this hypothesis, we performed three laboratory experiments. We first assessed the preference of *T. evansi* for odours of tomato plants infested with conspecifics *vs.* clean air. This experiment focused on determining if the preference of *T. evansi* for plants infested by conspecifics is because of the avoidance of clean tomato plants or because plants infested by conspecifics are more attractive to *T. evansi*. Subsequently, we assessed the fecundity of *T. evansi* on damaged, induced and clean tomato leaves. Lastly, we measured proteinase inhibitor activity on damaged, on induced and on clean tomato leaves to measure the induced direct defense of tomato plants.

Materials and Methods

Mite rearing and plant material

Spider mites (*T. evansi*) were obtained in 2002 from a natural infestation of tomato plants (*Lycopersicon esculentum* Mill variety Santa Clara I-5300) in a greenhouse at the Federal University of Viçosa. Mites were reared on tomato plants of the same variety.

Tomato seeds were sown in a commercial substrate, composed of vermiculite and organic fertilizer, in trays and kept inside mite-proof screen cages in a greenhouse. Plants (21 days old) were transplanted to plastic pots (2L) that contained a mixture of soil, bovine manure (3:1) and fertilizer (4-14-8 N-P-K). Tomato plants were further grown in mite-proof screen cages in a greenhouse until they were 45 days old and had at least four completely developed leaves. Plants were infested with *T. evansi* by putting four small infested tomato leaves on each plant kept inside a mite-proof screen cage in a greenhouse. The plants were incubated in this way for one week, resulting in 300-400 adult mites on each plant.

Olfactory choice experiment

The response of *T. evansi* to odours of clean and infested tomato plants was determined in two-choice tests in a Y-tube olfactometer (Sabelis & van de Baan, 1983; Janssen et al., 1999). The olfactometer consisted of a glass tube in the form of a Y, with a white Y-shaped metal wire in the middle to channel the mites. The base of the tube was connected to an air pump that produced an airflow from the arms of the tube to the base. Odour sources (consisting of a group of three plants) were placed in a tray (l x w x h = 30 x 20 x 8 cm) that was placed inside a second, water-containing tray (60 x 39 x 6). A Plexiglas container (50 x 36 x 43 cm) was put over the plants so that it rested in the water-containing outer tray. In this way, a water barrier was created to prevent escape of spider mites, and the water served as an air tight seal for the container at the same time. The containers had an air inlet and outlet (diameter 1 cm) at the opposing walls. The inlet and outlet were covered with mite-proof gauze. The odour sources were connected to the arms of the olfactometer with plastic tubes equipped with a valve to regulate wind speed. The airflow through both arms of the Y-tube was measured with hot-wire anemometers, and calibrated with the valves. When wind speeds in both arms are equal, the odours form two neatly separated fields in the base of the Y-tube with the interface coinciding with the metal wire (Sabelis & van de Baan, 1983). The wind speed was *c.* 0.45 m/s in each arm.

Adult female *T. evansi* were collected in a Petri dish (8 cm Ø) and kept inside a climate box without food for 24 h (28 ± 2°C relative humidity 70 ±10%) before the experiment. Mites were introduced into the olfactometer, one at a time, by disconnecting the pump and putting the female on the metal wire at the base of the Y-tube. After the pump was reconnected, the female started moving upwind to the junction of the wire, where she had to choose for one of the two arms. Each individual was observed until it had reached the end of one of the arms or for a maximum of 5 min and was then removed. After removing each mite, the metal wire in the middle of the tube was cleaned with a piece of cotton wool soaked in alcohol (70%) to remove any possible traces (silken threads, faeces) left by the mite (Pallini et al., 1997). Subsequently, the wire was left to dry before the next mite was tested. In this way, we tried to eliminate any effect of silk or residues deposited on the metal wire on the behavior of the subsequent mite (Gotoh et al., 1993). The containers with the odour sources were connected to the opposite arm of the olfactometer after each 5 mites tested to correct for any unforeseen asymmetry in the experimental set-up. To avoid pseudo-replication, the odour sources were changed and the set-up was cleaned after each 20 mites tested. Four replicates were done testing the attractiveness of mite-infested plants relative to air. Differences in fractions of mites choosing the two odour sources were tested using the replicated goodness-of-fit test (Sokal & Rohlf, 1995).

Spider mite fecundity

The oviposition rate of *T. evansi* was measured on damaged leaves, on induced leaves and on clean leaves. Each treatment was replicated on four different plants. Two leaves of each plant were infested with *T. evansi.* A non-drying glue (Bio-Controle®) was applied to the petiole of these leaves to prevent mites from moving to other leaves of the plants. These undamaged leaves were used as induced leaves. A total of four plants were used for this treatment. On two of these plants, leaves number 1 and 3 (counted from the oldest leaf) received mites, whereas leaves number 2 and 4 were kept clean. This treatment was reversed on the other two plants to ensure that each treatment (induced or damaged) was done on leaves of comparable age. For each treated plant, a control was prepared, consisting of a clean plant of the same age as the treated plant, and with glue applied to the same leaves, but without mites. The plants were kept inside mite-proof screen cages in a greenhouse. Seven days after infestation with mites, leaf-discs (20 mm Ø) were made from the leaflet of the plants; 10 discs were taken from the leaves with mites (damaged) and after mites, web and eggs were removed with a thin brush. A further 10 discs were prepared from clean leaves of the infested plants (induced). To correct for differences in age of leaves, 10 control discs were made of the leaves of the clean control plant that corresponded to the damaged leaves as well as 10 discs that corresponded to the induced leaves. The leaf-discs were kept individually in Petri dishes (8 cm Ø) containing wet cotton wool.

Because oviposition rate varies with age in spider mites (Sabelis, 1991), female mites of similar age were used to measure oviposition. To obtain such cohorts, several adult females were allowed to lay eggs on detached uninfested tomato leaves (on wet cotton wool) to produce an egg-wave. The adults were removed after 24 h and the eggs were reared to adulthood. All females used in the experiments were two days old since the last moulting stage. One female was maintained on each leaf disc, and were kept at a temperature of 28 ± 2ºC; relative humidity 70 ±10%, and 14 hours of light. The ovipositon rate was evaluated every 24 hours for a period of four days. After 48 hours, leaf-discs were replaced by new discs made from the other leaf of the same plant that received the same treatment. The results were analyzed using linear mixed models.

Proteinase inhibitors assays

The proteinase inhibitor activity in tomato leaves was measured in damaged leaves, induced leaves and controls as described above. To ensure that the infestation period of the plants and glue applied to their petiole did not affect the treatments, the following controls were done: leaves damaged for 4 h without glue, clean leaves with glue and clean leaves without glue. Each treatment was replicated on four different plants. The plants were incubated according to each treatment for one week, except for a treatment in which leaves were damaged for 4 h only.

Protein extraction

Crude protein was extracted from leaves of tomato plants that received the above-mentioned treatments. For each treatment, two samples of leaves of 300 mg were collected, pooled in 15-ml tubes, directly frozen in liquid nitrogen and stored at - 80°C. Subsequently, each sample was ground to powder in liquid nitrogen and homogenized in 1000 µl extraction buffer (0.1 M Tris-HCl buffer, pH 8.2 and 20 mM $CaCl_2$; 1:3 w/v). The liquid was centrifuged at 17200g for 30 min at 4°C, and the supernatant was collected.

Measurement of trypsin inhibitory activity

A standard spectrophotometric assay was used to measure trypsin inhibitory activity in the supernatant. A 50 µl aliquot of trypsin (4.7×10^{-5} M) was mixed with 50 µl of the plant extract and 500 µl extraction buffer (0.1 M Tris-HCl buffer, pH 8.2 and 20 mM $CaCl_2$). The mixture was incubated at room temperature for 5 min. The control for each volume of extract tested was obtained by the addition of 500 µl extraction buffer and 50 µl of trypsin (4.7×10^{-5} M). A 500 µl aliquot of the mixture (tests and controls) was added to 500 µl extraction buffer (0.1 M Tris-HCl buffer, pH 8.2 and 20 mM $CaCl_2$) and 500 µl D,L-BApNA (1.2 mM). Trypsin activity was monitored for 150 seconds at intervals of 30 seconds at 410 nm absorbance. The difference between the absorbance measured at 150 and 60 seconds was used to determine the trypsin activity. Measurements were done in triplicate per sample.

The results obtained were converted to milligrams trypsin inhibited per gram of protein according to the following equation: mg trypsin/g total protein = AB/1000CP with A= enzyme control – absorbance at 410 nm of the extract; B = the final incubation volume (600 μL) divided by the extract volume (50 μL) = 12; C = Trypsin factor (the result from the activity of 1μL of Trypsin on the substrate D,L-BApNA measured at 410 nm absorbance); for Trypsin D,L-BApNA = 0.019 (Kakade et al., 1974); $P_{(g/ml)}$ = protein concentration of the extracts, which was calculated by measuring the absorbance of each protein-concentration sample at 280 and 260 nm. The ratio of these two values was determined and used to select an appropriate correction factor from a table (Warburg & Christian, 1941). The absorbance at 280 nm was multiplied by this factor to yield the protein concentration in mg per ml. The protein concentration obtained was then converted to g/ml. Results were analyzed with an ANOVA followed by Tukey HSD.

Results

Olfactory choice

When given a choice between odours from tomato plants infested by conspecifics and clean air, *T. evansi* had a significant preference for tomato plants infested by conspecifics in two out of four replicates, a preference bordering significance in one replicate, and no preference in the fourth replicate. The replicates were not heterogeneous ($G_H = 3.26$, df = 3, $p > 0.05$), all replicates showed a similar trend, and the pooled results showed a highly significant preference for tomato plants infested by conspecifics ($G_p = 13.16$, d.f. = 1, $p < 0.001$) (Fig. 1).

There was no significant effect of the position of the odour sources on the preference of mites in the experiment, suggesting that asymmetries in the set-up due to directionality of light or other factors had no effect on choice of the mites.

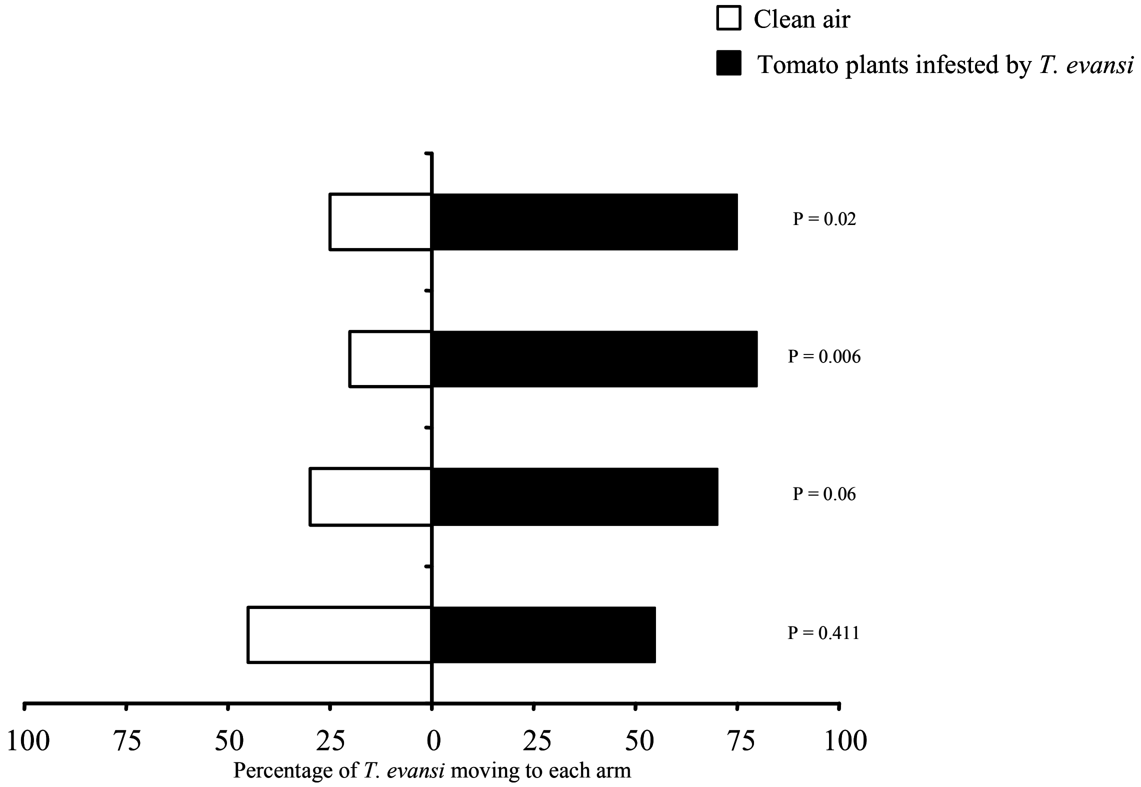

Figure 1. Choice of *T. evansi* females when offered volatiles from tomato plants infested by conspecifics (right) versus clean air (left). Each bar represents the results of a replicate, in which 20 mites were tested.

Spider mite fecundity

The rate of oviposition did not vary with time, but there was a significant effect of the treatments (Fig. 2, $F_{3,\,136} = 7.40$, $p = 0.0001$). The ovipositional rate was significantly higher on tomato leaves that had received damage by conspecifics than on clean tomato leaves or on tomato leaves that were induced by conspecifics (Tukey HSD, $p = 0.02$) (Fig. 2).

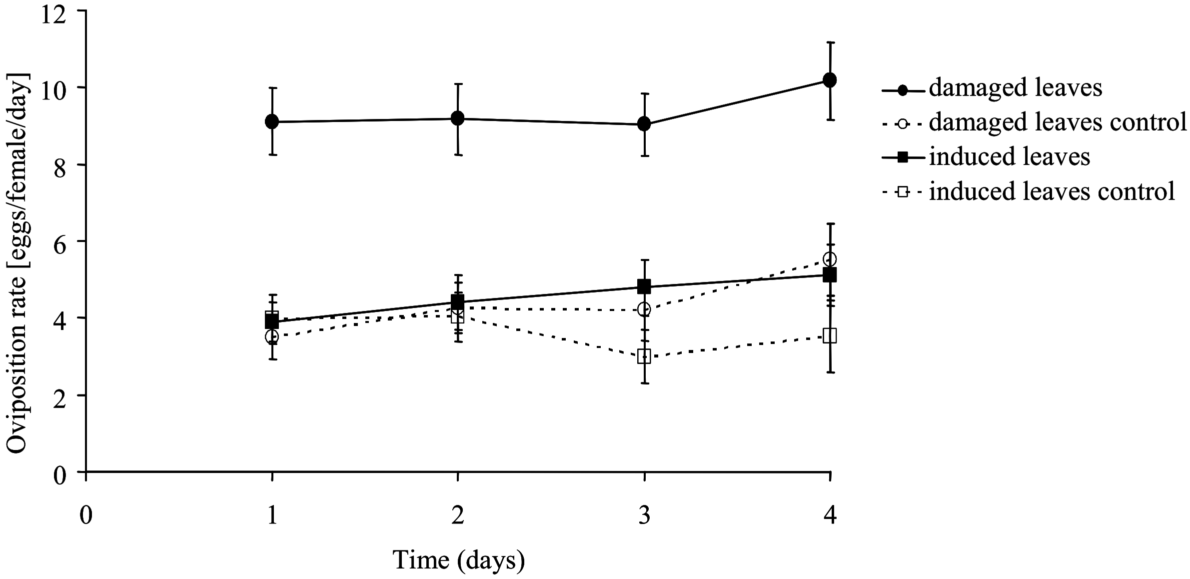

Figure 2. Average (± s.e.) ovipositional rate of *T. evansi* on tomato leaves that were induced by conspecific mites, induced and damaged by conspecifics, and on clean leaves.

Trypsin inhibitory activity

The trypsin inhibitory activity was determined in damaged, induced and clean tomato leaves. Statistical analysis revealed a significant effect of the treatments ($F_{3,12} = 118$, $p < 0.0001$). Post-hoc comparisons showed that damaged leaves had the lowest inhibitory activity, whereas clean leaves showed the highest inhibitory activity against trypsin (Fig. 3). In damaged leaves, the inhibitory activity was approximately 2-fold decreased compared to induced leaves and 3-fold decreased compared to clean leaves (Fig. 3).

There was no significant difference in proteinase inhibitory activity between leaves that were damaged for a period of four hours or of seven days (Fig. 4, T-test, $p = 0.31$). In addition, there was no significant difference between leaves from plants that received glue and leaves from plants that had not received glue (Fig, 4, T-test, $p = 0.12$), showing that the infestation period of the plants and the glue had no effect on induction of proteinase inhibitors.

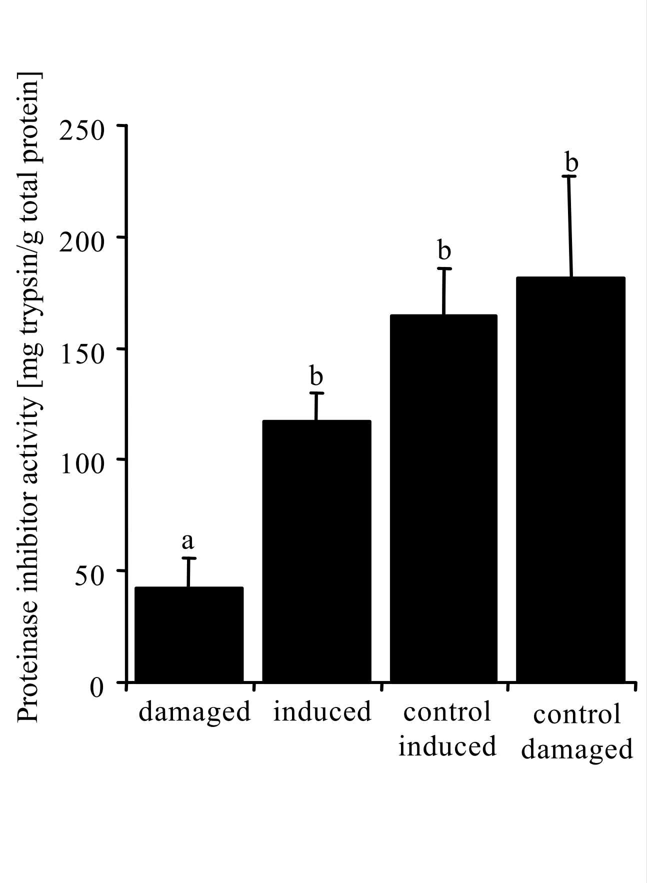

Figure 3. Proteinase inhibitor activity in extracts of damaged, induced and clean tomato leaves. The vertical bars indicate the means (+ s.e.) for each treatment. Different letters denote significant differences among treatments (planned comparisons).

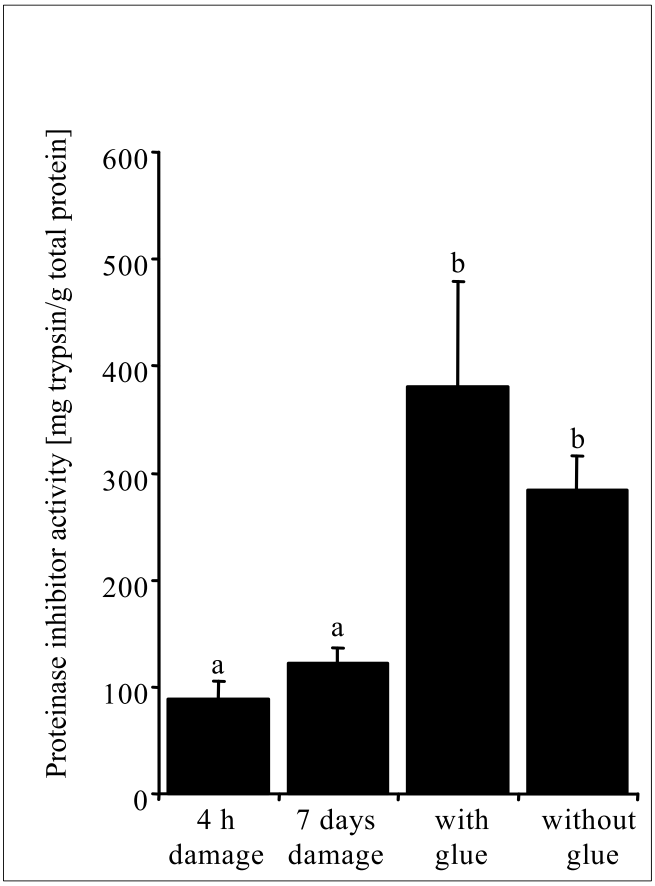

Figure 4. Average (± s.e.) proteinase inhibitor activity in extracts of leaves damaged for 4 hours without glue (4 h damage), leaves damaged for 7 days without glue (7 days damage), clean leaves with glue (with glue) and clean leaves without glue (without glue). Different letters denote significant differences among treatments (planned comparisons).

Discussion

Our results indicate that herbivores can use herbivore-induced volatiles to assess the level of plant defense. The olfactory choice experiment showed that females of *T. evansi* were attracted rather than repelled by volatiles from tomato plants that were attacked by conspecifics. Additional experiments showed that the fecundity of these mites on damaged tomato leaves was a factor 2 higher than on clean tomato leaves, whereas fecundity on induced leaves was similar to that on clean plants. However, both results are in disagreement with the hypothesis that herbivory induces a defense response in plants (McCall, et al., 1994; Stout et al., 1994; Karban & Baldwin, 1997; Stout et al., 1998; Tamayo, et al., 2000; Arimura et al., 2005). We therefore assessed the induction of proteinase inhibitors by *T. evansi* in damaged, induced and clean leaves.

Current literature supports the hypothesis that proteinase inhibitors are induced and accumulated in above-ground tissues of various plant species, including tomato, as a direct consequence of herbivore damage and wounding of leaves. Proteinase inhibitors make the plant tissue less suitable for invading pests (Green & Ryan, 1972; Strassner et al., 2002; Kant et al., 2004; Damle et al., 2005). Kant et al. (2004) demonstrated that the direct defense system of tomato plants was activated within one day after infestation with the two-spotted spider mite *T. urticae*. They suggested that the rapid increase in inhibitory compounds upon infestation is a specific response of the plant to the spider mite species used in their experiments. Moreover, the magnitude of the induction of the production of proteinase inhibitors may also depend on the amount of damage (Pearce et al., 1993; Alarcon & Malone, 1995), the type of herbivore that is feeding (Stout et al., 1998), or on the inducing agent that is applied (Botella et al., 1996). It is generally thought that this huge variation in type, distribution, and inducibility of proteinase inhibitors among plants is tightly linked with the ecological role of PI's in each species (Jongsma et al., 1994). Nevertheless, the general consensus is that herbivory induces production of PI's.

Contrary to this consensus, our results show that the levels of PI's in tomato plants decreased after infestation with the phytophagous mite *T. evansi*. The proteinase inhibitor activity was approximately 3-fold lower in damaged leaves than in clean leaves, and 2-fold lower than in induced leaves. This is the first study that shows that PI levels decreased after the plants were damaged by herbivores. These low levels are matched by the preference of *T. evansi* for infested plants and the higher performance on leaves that were infested with conspecifics compared to clean leaves. It suggests that the mites prefer plants infested by conspecifics because of the reduced levels of proteinase inhibitors.

An additional explanation for the preference and the higher performance of *T. evansi* on plants infested by conspecifics is that there is a trade-off between population growth capacity and web production by *T. evansi*. To defend themselves, spider mites spin a chaotically structured web over their colonies and these webs are hard to penetrate for most predators (Saito, 1977; Sabelis & Bakker, 1992). The silk of this web consists of proteins (Hazan et al., 1975) and its production may thus go at the expense of amino acids that would otherwise be available for growth and reproduction. By colonising plants already infested by conspecifics, the mites would not need to produce much web because those plants already contain web. However, we measured oviposition on damaged plant tissue after having removed the web. Unless the mites equate damage with the potential presence of web, we would expect the mites to produce similar amounts of web in all treatments, and thus would have similar oviposition rates in all treatments.

We therefore conclude that the higher oviposition rate of *T. evansi* on damaged leaves is caused by its capacity to weaken the defense system of their host plant, which would explain the attractiveness of infested plants to the mites and the higher performance on plants infested by conspecifics compared to clean plants. This is the first example of an herbivore that down-regulates the synthesis of plant defensive compounds. It is an established fact that PI's are induced in high amounts in tissues after herbivore damage, and are involved in protection of the plants against herbivores (Ryan, 1990; Boulter, 1993). Down-regulating the synthesis of proteinase inhibitors would allow herbivores to overcome this plant defense. The mechanisms involved in this are a subject of further studies.

Acknowledgments

We thank Merijn Kant (Max Planck Institute for Chemical Ecology, Jena, Germany) for his expert advice and discussion on PI assays. Maria C. Rosado is thanked for support and assistance with the experiments. This research was supported in part by CNPq-Brazil and WOTRO-NWO (Netherlands Foundation for the Advancement of Tropical Research). AJ received a scholarship from CAPES, Brazil (PVE). AP and MV received scholarship from CNPq, Brazil.

References

Agrawal, A. A. 2000. Host-range evolution: Adaptation and trade-offs in fitness of mites on alternative hosts. **Ecology**, 81, 500-508.

Alarcon, J. J. & Malone, M. 1995. The influence of plant age on wound induction of proteinase inhibitors in tomato. **Physiologia Plantarum**, 95, 423-427.

Botella, M. A., Xu, Y., Prabha, T. N., Zhao, Y., Narasimhan, M. L., Wilson, K. A., Nielsen, S. S., Bressan, R. A. & Hasegawa, P. M. 1996. Differential expression of soybean cysteine proteinase inhibitor genes during development and in response to wounding and methyl jasmonate. **Plant Physiology**, 112, 1201-1210.

Boulter, D. 1993. Insect pest-control by copying nature using genetically-engineered crops. **Phytochemistry**, 34, 1453-1466.

Damle, M. S., Giri, A. P., Sainani, M. N. & Gupta, V. S. 2005. Higher accumulation of proteinase inhibitors in flowers than leaves and fruits as a possible basis for differential feeding preference of *Helicoverpa armigera* on tomato (*Lycopersicon esculentum Mill*, Cv. Dhanashree). **Phytochemistry**, 66, 2659-2667.

Dicke, M., Sabelis, M. W., Takabayashi, J., Bruin, J. & Posthumus, M. A. 1990. Plant strategies of manipulating predator-prey interactions through allelochemicals - Prospects for application in pest-control. **Journal of Chemical Ecology**, 16, 3091-3118.

Gotoh, T., Bruin, J., Sabelis, M. W. & Menken, S. B. J. 1993. Host race formation in *Tetranychus urticae* - genetic differentiation, host-plant preference, and mate choice in a tomato and a cucumber strain. **Entomologia Experimentalis et Applicata**, 68, 171-178.

Green, T. R. & Ryan, C. A. 1972. Wound-induced proteinase inhibitor in plant leaves - possible defense mechanism against insects. **Science**, 175, 776-779.

Hazan, A., Gertler, A., Tahori, A. S. & Gerson, U. 1975. Spider mite webbing.3. Solubilization and amino-acid composition of silk protein. **Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology**, 51, 457-462.

Janssen, A., Pallini, A., Venzon, M. & Sabelis, M. W. 1999. Absence of odour-mediated avoidance of heterospecific competitors by the predatory mite *Phytoseiulus persimilis*. **Entomologia Experimentalis et Applicata**, 92, 73-82.

Jongsma, M. A., Bakker, P. L., Visser, B. & Stiekema, W. J. 1994. Tyrpsin inhibitor activity in mature tobacco and tomato plants is mainly induced locally in response to insect attack, wounding and virus infection. **Planta**, 195, 29–35.

Kakade, M. L., Rackis, J. J., McGhee, J. E. & Puski, G. 1974. Determination of trypsin-inhibitor activity of soy products - Collaborative analysis of an improved procedure. **Cereal Chemistry**, 51, 376-382.

Karban, R & Baldwin, I. T. 1997. **Induced Responses to Herbivory,** Chicago, University Of Chicago Press, 330p.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A. & Schuurink, R. C. 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. **Plant Physiology**, 135, 483-495.

Lawrence, P. K. & Koundal, K. R. 2002. Plant protease inhibitors in control of phytophagous insects. **Electronic Journal of Biotechnology**, 5, 93-109.

Mayhew, P. J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. **Oikos**, 79, 417-428.

McCall, P. J., Turlings, T. C. J., Loughrin, A. T. P. & Tumlinson, J. H. 1994. Herbivore-induced volatile emission from cotton (*Gossypium hirsutum* L.) seedlings. **Journal of Chemical Ecology**, 20, 3039-3050.

Pallini, A., Janssen, A. & Sabelis, M. W. 1997. Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. **Oecologia**, 110, 179-185.

Pearce, G., Johnson, S. & Ryan, C. A. 1993. Purification and characterization from tobacco (*Nicotiana tabacum*) leaves of 6 small, wound-inducible, proteinase isoinhibitors of the potato inhibitor-li family. **Plant Physiology**, 102, 639-644.

Pompermayer, P., Falco, M. C., Parra, J. R. P. & Silva-Filho, M. C. 2003. Coupling diet quality and bowman-birk and kunitz-type soybean proteinase inhibitor effectiveness to *Diatraea saccharalis* development and mortality. **Entomologia Experimentalis et Applicata**, 109, 217-224.

Ryan, C. A. 1978. Proteinase-inhibitors in plant leaves - biochemical model for pest-induced natural plant protection. **Trends in Biochemical Sciences**, 3, 148-150.

Ryan, C. A. 1990. Protease inhibitors in plants - Genes for improving defenses against insects and pathogens. **Annual Review of Phytopathology**, 28, 425-449.

Sabelis, M. W. & Van de Baan, H. E. 1983. Location of distant spider mite colonies by phytoseiid predators - Demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. **Entomologia Experimentalis et Applicata**, 33, 303-314.

Sabelis, M. W. 1991. **Life history evolution of spider mites**. p.23–49. In: Schuster R; Murphy P. W. (eds) The Acari: reproduction, development and life-history strategies. Chapman & Hall, London.

Sabelis, M. W. & Bakker, F. M. 1992. How predatory mites cope with the web of their tetranychid prey - A functional view on dorsal chaetotaxy in the phytoseiidae. **Experimental & Applied Acarology**, 16, 203-225.

Sabelis, M. W., Janssen, A. & Kant, M. R. 2001. Ecology - The enemy of my enemy is my ally. **Science**, 291, 2104-2105.

Saito, Y. 1977. Study of the spinning behaviour of the spider mite (Acarina: Tetranychidae). I. Method for the quantitative evaluation of the mite webbing and the relationship between webbing and walking. **Japanese Journal of Applied Entomology and Zoology**, 21, 27-34.

Sarmento, R. A. 2003. **Resposta comportamental de artrópodes em teia alimentar de tomateiro**. Dissertação de mestrado, Universidade Federal de Viçosa, Viçosa, 61p.

Sokal, R. R. & Rohlf, F. J. 1995. **Biometry: the principles and practice of statistics in biological research,** New York, Freeman, 887p.

Stout, M. J., Workman, K. V., Bostock, R. M. & Duffey, S. S. 1998. Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. **Oecologia**, 113, 74-81.

Stout, M. J., Workman, J. & Duffey, S. S. 1994. Differential induction of tomato foliar proteins by arthropod herbivores. **Journal of Chemical Ecology**, 20, 2575-2594.

Strassner, J., Schaller, F., Frick, U. B., Howe, G. A., Weiler, E. W., Amrhein, N., Macheroux, P. & Schaller, A. 2002. Characterization and cDNA-microarray expression analysis of 12-oxophytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. **Plant Journal**, 32, 585-601.

Tamayo, M. C., Rufat, M., Bravo, J. M. & Segundo, B. S. 2000. Accumulation of a maize proteinase inhibitor in response to wounding and insect feeding, and characterization of its activity toward digestive proteinases of *Spodoptera littoralis* larvae. **Planta**, 211, 62-71.

Turlings, T. C. J., Tumlinson, J. H. & Lewis, W. J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. **Science**, 250, 1251-1253.

Vet, L. E. M. & Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. **Annual Review of Entomology**, 37, 141-172.

Warburg, O. & Christian, W. 1941. Isolation and crystallization of the glycolytic enzyme enolase. **Biochemische Zeitschrift**, 310, 385–421.

West, S. A. & Cunningham, J. P. 2002. A general model for host plant selection in phytophagous insects. **Journal of Theoretical Biology**, 214, 499-513.

CHAPTER 2

Herbivores use odours to assess host-plant quality

Renato Almeida Sarmento[1,2], *Arne Janssen*[2], *Angelo Pallini*[1] *and Madelaine Venzon*[3]

[1]Department of Animal Biology/Entomology, Federal University of Viçosa, Brazil; [2]Institute for Biodiversity and Ecosystem Dynamics, Section Population Biology, University of Amsterdam, PO Box 94084, Amsterdam, The Netherlands; [3]EPAMIG (Agriculture and Livestock Research Enterprise of Minas Gerais, Brazil)

Abstract - The direct defense response of plants to herbivory is characterized by the activation of signaling cascades, which leads to the formation of specific products and physiological changes that lower the plant quality as food for herbivorous arthrophods. We investigated the direct defense response of tomato when attacked by the two-spotted spider mite *Tetranychus urticae* by measuring the proteinase inhibitor activity in damaged, induced and clean tomato leaves. We subsequently evaluated the effect of the damage caused by *T. uticae,* on tomato plants, on the fecundity of females of the closely related spider mite species *T. evansi*. Lastly, we investigated the preference of *T. evansi* to odours of tomato plants infested with conspecific mites *vs.* tomato plants infested with *T. urticae*. We found that the levels of proteinase inhibitors in the leaves of tomato plants increased after infestation of the plants with the two-spotted spider mite *T. urticae* and that the lower oviposition rate of *T. evansi* females on leaves of tomato plants that previously received damage by *T. urticae* was matched by the preference of *T. evansi* to plants infested with conspecifics mites when tested *versus* plants infested with *T. urticae* mites. We conclude that the lower performance of *T. evansi* females on leaves of plants that received damage by *T. urticae* and their preference for plants infested by conspecifics rather than plants infested by *T. urticae*, are in agreement with the lower nutritional quality of the plants caused by the higher levels of proteinase inhibitors in plants damaged by *T. urticae*.

Key words: *T. evansi*, proteinase inhibitors, tomato plants, plant defense, nutritional quality.

Resumo – As defesas diretas de plantas em resposta à herbivoria são caracterizadas pela ativação de vias metabólicas sinalizadoras, que levam à formação de produtos específicos e mudanças fisiológicas que reduzem a qualidade nutricional da planta para herbívoros. Neste trabalho, foi investigada a resposta de defesas diretas de plantas de tomate ao ácaro rajado *Tetranychus urticae*, pela avaliação da atividade de inibidores de protease em folhas de plantas de tomate limpas, danificadas e induzidas por esse ácaro. Em seguida foi avaliado o efeito do dano causado pelo ácaro *T. urticae*, em plantas de tomate, na fecundidade de fêmeas de *Tetranychus evansi*. Por último foi acessada a preferência de *T. evansi* aos odores provenientes de plantas de tomate infestadas por co-específicos *vs.* plantas infestadas pelo ácaro rajado *T. urticae.* Os resultados mostraram que os níveis de inibidores de protease em folhas de tomate aumentaram após a infestação das plantas com o ácaro *T. urticae* e que a menor taxa de oviposição de fêmeas de *T. evansi* em folhas de plantas de tomate que foram previamente infestadas pelo ácaro rajado *T. urticae*, coincidiram com a preferência de *T. evansi* por plantas infestadas por ácaros co-específicos, quando testados *vs.* plantas infestadas pelo ácaro *T. urticae*. Esses resultados indicam que a performance de fêmeas de *T. evansi* foi inferior em folhas de plantas que previamente receberam dano do ácaro *T. urticae*, bem como a preferência por plantas infestadas por co-específicos, foram determinadas pela qualidade nutricional inferior das plantas, decorrente dos níveis mais altos de inibidores de protease em plantas danificadas pelo ácaro *T. urticae*.

Palavras-chave: *Tetranychus evansi*, *Tetranychus urticae*, inibidor de protease, plantas de tomate, defesa direta, qualidade nutricional

Introduction

Plant resistance to arthropod herbivores is often mediated by a combination of constitutive and inducible defenses that negatively affect the feeding, growth, or reproduction of the attacking pest (Karban et al., 1997; Karban & Baldwin, 1997; Walling, 2000). Induced defenses are characterized by changes in morphology and/or increases in secondary metabolites or defense-associate proteins (Ament et al., 2004). If such changes lower the food quality for herbivores, they are referred to as direct defenses. These defenses can be activated by products in herbivore saliva or regurgitant (McCloud & Baldwin, 1997) but also by plant products that result from herbivore damage (Pare et al., 1998). In general, the direct defense response of plants to herbivory is characterized by the activation of signaling pathways, which leads to the formation of specific products and physiological changes that lower the food quality and therefore interfering with the performance of the herbivore (Walling, 2000; Lawrence & Koundal, 2002).

In tomato, the herbivore-induced defense response is characterized by an increase in signaling compounds like jasmonate (JA), ethylene (Et), salicilate (SA) and systemin, which results in local and systemic changes, such as the accumulation of polyphenol oxidase, peroxidase (POD), chitinase, callose synthase, and wound-inducible proteinase inhibitors (WIPIs) (Walling, 2000). Proteinase inhibitors are proteins that inhibit digestive enzymes in the gut of arthropod herbivores, which can reduce their growth and reproduction (Green & Ryan, 1972; Broadway & Duffey, 1986; Ryan, 1990; Lawrence & Koundal, 2002; Pompermayer et al., 2003). However, the direct defense response of tomato plants against stylet-feeders (such as phloem-feeding whiteflies, aphids and mesophyll-feeding mites and thrips) and leaf-chewing insects (such as lepidopteran larvae) appears to be different. Caterpillars stimulate polyphenol oxidase and lipoxigenase activity, leaf miners only POD activity (Stout et al., 1994). Also spider mites feeding on tomato plants induce the accumulation of proteinase inhibitors, while whiteflies feeding do not (Walling, 2000; Ament et al., 2004; Kant et al., 2004). However, the magnitude of the accumulation of proteinase inhibitors may also depend on the type of herbivore that is feeding (Stout et al., 1998). Furthermore, it was previous demonstrated that the levels of PI's in tomato leaves decreased after infestation with the red spider mite *Tetranychus evansi* (chapter 1).

These results led us to hypothesize that the induction of such defenses may also vary with spider mite species. To investigate this, we assessed the direct defense response of tomato plants to the two-spotted spider mite *T. urticae* by measuring the proteinase inhibitor activity in damaged, induced and in clean tomato leaves. Because it was observed that the levels of proteinase inhibitors in the leaves of tomato plants increased after infestation of the plants with *T. urticae* and that the amount of proteinase inhibitors in plant tissues is indicative of the nutritional quality of such plants (Karban et al., 1997; Agrawal, 1999; Thaler et al., 2002), we subsequently measured the oviposition rate of *T. evansi* females on tomato leaves that were damaged and induced by *T. urticae*, as well as, on clean tomato leaves. This experiment focused on determining whether higher levels of proteinase inhibitors in the leaves of tomato plants negatively influence the performance of *T. evansi* females.

Earlier experiments showed that the lower levels of proteinase inhibitors were matched by the higher performance and preference of *T. evansi* to plants infested with conspecifics compared to clean leaves or clean air (Sarmento, 2003; chapter 1). These results lead us to hypothesize that *T. evansi* can use herbivore-induced volatile cues to assess host-plant quality. To investigate this, we assessed the preference of *T. evansi* to odours of tomato plants infested with conspecifics (plants where the leaves contained lower levels of PI's, on which *T. evansi* showed higher performance compared to clean leaves) *vs.* tomato plants infested by *T. urticae* (plants where the leaves contained higher levels of PI's, on which *T. evansi* showed lower performance compared to clean leaves).

Materials and Methods

Mite rearing and plant material

Tetranychus evansi was obtained in 2002 from a natural infestation of tomato plants (*Lycopersicon esculentum* Mill variety Santa Clara I-5300) in a greenhouse at the Federal University of Viçosa. The population of *T. urticae* was obtained from strawberry plants, in a greenhouse at the Federal University of Viçosa and were subsequently transferred to tomato plants of the same variety.

Tomato seeds were sown in a commercial substrate, composed of vermiculite and organic fertilizer, in trays and kept in mite-proof screen cages in a greenhouse. Plants (21 days old) were transplanted to plastic pots (2L) that contained a mixture of soil, bovine manure (3:1) and fertilizer (4-14-8 N-P-K). Tomato plants were further grown in mite-proof screen cages in a greenhouse until they were 45 days old and had at least four completely developed leaves. Plants were infested either with *T. evansi* or *T. urticae* by putting four small, infested tomato leaves on each plant, inside a mite-proof screen cage in a greenhouse. The plants were incubated in this way for one week, resulting in 300 – 400 adult mites on each plant.

Proteinase inhibitors assays

The proteinase inhibitor activity was measured in leaves of tomato plants that were damaged and induced by *T. urticae*, and in clean leaves. Each treatment was replicated on four different plants. Two leaves of each plant were infested with *T. urticae.* A non-drying glue (Bio-Controle®) was applied to the petiole of these leaves to prevent mites from moving to other leaves of the plants. These undamaged leaves were used as induced leaves. On two of these plants, leaves number 1 and 3 (counted from the oldest leaf) received mites, whereas leaves number 2 and 4 were kept clean. This treatment was reversed on the other two plants to ensure that each treatment (induced or damaged) was done on leaves of comparable age. For each treated plant, a control was prepared, consisting of a clean plant of the same age as the treated plant, and with glue applied to the same leaves, but without mites. The plants were kept inside mite-proof screen cages in a greenhouse. The plants were incubated according to each treatment for one week.

Protein extraction

Crude protein was extracted from leaves of tomato plants that received the above-mentioned treatments. For each treatment, two samples of 300 mg were collected, pooled in 15-ml tubes, directly frozen in liquid nitrogen and stored at - 80°C. Subsequently, each sample was ground to powder in liquid nitrogen and homogenized in 1000 µl extraction buffer (0.1 M Tris-HCl buffer, pH 8.2 and 20 mM $CaCl_2$; 1:3 w/v). The liquid was centrifuged at 17200g for 30 min at 4°C, and the supernatant was collected.

Measurement of trypsin inhibitory activity

A standard spectrophotometric assay was used to measure trypsin inhibitory activity in the supernatant. A 50 µl aliquot of trypsin (4.7 x 10^{-5} M) was mixed with 50 µl of the plant extract and 500 µl extraction buffer (0.1 M Tris-HCl buffer, pH 8.2 and 20 mM $CaCl_2$). The mixture was incubated at room temperature for 5 min. The control for each volume of extract tested was obtained by the addition of 500 µl extraction buffer and 50 µl of trypsin (4.7 x 10^{-5} M). A 500 µl aliquot of the mixture (tests and controls) was added to 500 µl extraction buffer (0.1 M Tris-HCl buffer, pH 8.2 and 20 mM $CaCl_2$) and 500 µl D,L-BApNA (1.2 mM). Trypsin activity was monitored for 150 seconds at intervals of 30 seconds at 410 nm absorbance. The difference between the absorbance measured at 150 and 60 seconds was used to determine the trypsin activity. Measurements were done in triplicate per sample.

The results obtained were converted to milligrams trypsin inhibited per gram of protein according to the following equation: mg trypsin/g total protein = AB/1000CP with A= enzyme control – absorbance at 410 nm of the extract; B = the final incubation volume (600 µL) divided by the extract volume (50 µL) = 12; C = Trypsin factor (the result from the activity of 1µL of Trypsin on the substrate D,L-BApNA measured at 410 nm absorbance); for Trypsin D,L-BApNA = 0.019 (Kakade et al., 1974); $P_{(g/ml)}$ = protein concentration of the extracts, which was calculated by measuring the absorbance of each protein-concentration sample at 280 and 260 nm. The ratio of these two values was determined and used to select an appropriate correction factor from a table (Warburg & Christian, 1941). The absorbance at 280 nm was multiplied by this factor to yield the protein concentration in mg per ml. The protein concentration obtained was then converted to g/ml. Results were analyzed with an ANOVA followed by Tukey HSD.

Spider mite fecundity

The oviposition rate of *T. evansi* was measured on leaves damaged and induced by *T. urticae*, as well as, on clean leaves as described above. Each treatment was replicated on four different plants. Seven days after the infestation of the plants with *T. urticae*, leaf-discs (20 mm Ø) were made from the leaflets of the plants; 10 discs were taken from the leaves with mites (damaged) and mites, web and eggs were removed by using a thin brush. A further 10 discs were prepared from the clean leaves of the infested plants (induced). To correct for differences in age of leaves, 10 control discs were made of the leaves of the clean control plant that corresponded to the damaged leaves as well as 10 discs that corresponded to the induced leaves. The leaf-discs were kept individually in Petri dishes (8 cm Ø) containing wet cotton wool.

Because oviposition rate varies with age in spider mites (Sabelis, 1991), female mites of similar age were used to measure oviposition. To obtain such cohorts, several adult females of *T. evansi* were allowed to lay eggs on detached uninfested tomato leaves (on wet cotton wool) to produce an egg-wave. The adults were removed after 24 h and the eggs were reared to adulthood. All females used in the experiments were two days old since the last moulting stage. One female was maintained on each leaf disc, and were kept at a temperature of 28 ± 2ºC; relative humidity 70 ±10%, and 14 hours of light. The ovipositon rate was evaluated every 24 hours for a period of four days. After 48 hours, leaf-discs were replaced by new discs made from the other leaf of the same plant that received the same treatment. The results were analyzed using Linear Mixed Models.

Olfactory choice experiment

The response of *T. evansi* to odours of tomato plants infested by *T. urticae vs.* tomato plants infested by conspecifics was determined in two-choice tests in a Y-tube olfactometer (Sabelis & van de Baan, 1983; Janssen et al., 1999). The olfactometer consisted of a glass tube in the form of a Y, with a white Y-shaped metal wire in the middle to channel the mites. The base of the tube was connected to an air pump that produced an airflow from the arms of the tube to the base. Odour sources (each consisting of a group of three plants) were placed in a tray (l x w x h = 30 x 20 x 8 cm) that was also placed inside a second water-containing tray (60 x 39 x 6). A Plexiglas container (50 x 36 x 43 cm) was put over the plants so that it rested in the water-containing outer tray. In this way, a water barrier was created to prevent escape of spider mites, and the water served as an air tight seal for the container at the same time. The containers had an air inlet and outlet (diameter 1 cm) at opposing walls. The inlet and outlet were covered with mite-proof gauze. The odour sources were connected to the arms of the olfactometer with plastic tubes equipped with a valve to regulate wind speed. The airflow through both arms of the Y-tube was measured with hot-wire anemometers, and calibrated with the valves. When wind speeds in both arms are equal, the odours form two neatly separated fields in the base of the Y-tube with the interface coinciding with the metal wire (Sabelis & van de Baan, 1983). The wind speed was *c.* 0.45 m/s in each arm.

Adult female *T. evansi* were collected in a Petri dish (8 cm Ø) and kept inside a climate box without food for 24 h (28 ± 2°C relative humidity 70 ±10%) before the experiment. Mites were introduced into the olfactometer, one at a time, by disconnecting the pump and putting the female on the metal wire at the base of the Y-tube. After the pump was reconnected, the female started moving upwind to the junction of the wire, where she had to choose for one of the two arms. Each individual was observed until she had reached the end of one of the arms or for a maximum of 5 min and was then removed. After removing each mite, the metal wire in the middle of the tube was cleaned with a piece of cotton wool soaked in alcohol (70%) to remove any possible traces (silken threads, faeces) left by the mite (Pallini et al., 1997). Subsequently, the wire was left to dry before the next mite was tested. In this way, we tried to eliminate any effect of silk or residues deposited on the metal wire on the behavior of the subsequent mite (Gotoh et al., 1993). The containers with the odour sources were connected to the opposite arm of the olfactometer after each 5 mites tested to correct for any unforeseen asymmetry in the experimental set-up. To avoid pseudo-replication, the odour sources were changed and the set-up was cleaned after each 20 mites tested. Four replicates were done testing the attractiveness of plants infested by *T. urticae* relative to plants infested by conspecifics. Differences in fractions of mites choosing the two odour sources were tested using the replicated goodness-of-fit test (Sokal & Rohlf, 1995).

Results

Trypsin inhibitory activity

The trypsin inhibitory activity was determined in leaves that were damaged and induced by *T. urticae* and in clean tomato leaves (controls). Statistical analysis revealed a significant effect of the treatments ($F_{3,16} = 52.15$, $p < 0.05$). Post-hoc comparisons showed that damaged leaves had the highest inhibitory activity, whereas clean leaves showed the lowest inhibitory activity against trypsin (Fig. 1). In damaged leaves, the inhibitory activity was approximately 2-fold increased compared to induced clean leaves (controls, Fig. 1).

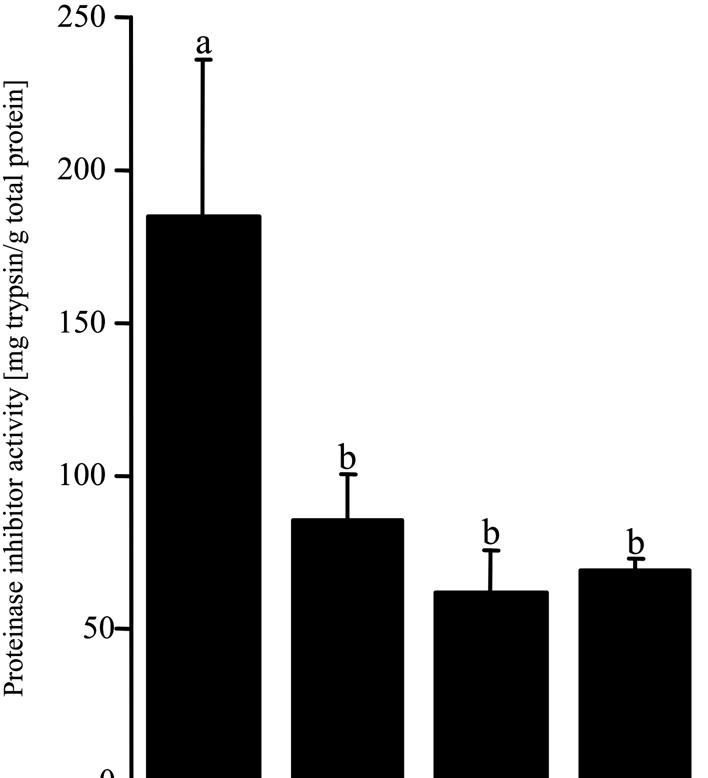

Figure 1. Proteinase inhibitor activity in extracts of leaves that were damaged and induced by *T. urticae* and in clean tomato leaves. The vertical bars indicate the means (+ s.e.) for each treatment. Different letters denote significant differences among treatments (planned comparisons).

Spider mite fecundity

The rate of oviposition did not vary with time, but there was a significant effect of treatment (Fig. 2, $F_{3,\ 156} = 42.07$, $p < 0.001$). The oviposition rate of *T. evansi* was significantly lower on leaves that previously received damage by *T. urticae* than on tomato leaves that were induced by these mites or on clean leaves (induced control and damaged control) (Tukey HSD, $p < 0.001$). Furthermore, the oviposition rate of *T. evansi* was significantly lower on leaves that were induced by *T. urticae* than on clean leaves (control for damage and induced leaves) (Tukey HSD, $p < 0.001$).

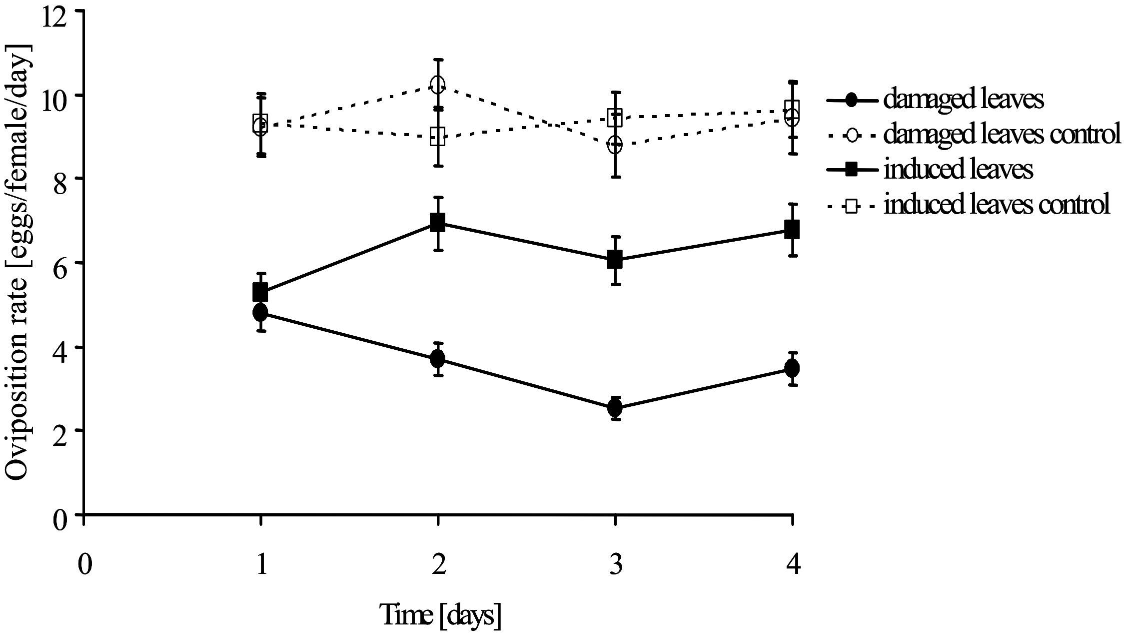

Figure 2. Average (± s.e.) ovipositional rate of *T. evansi* on leaves that were damaged and induced by *T. urticae* and on clean tomato leaves.

Olfactory choice

When given a choice between odours from tomato plants infested by conspecifics and tomato plants infested by *T. urticae*, *T. evansi* had a clear and significant preference for tomato plants infested by conspecifics in two out of four replicates and a preference bordering significance in the other two replicates. The replicates were not heterogeneous ($G_H = 7.66$, df = 3, $p > 0.05$), all replicates showed a similar trend, and the pooled results showed a highly significant preference of *T. evansi* females for tomato plants infested by conspecifics ($G_p = 16.79$, d.f. = 1, $p < 0.0001$).

There was no significant effect of the position of the odour sources on the preference of mites in the experiment, suggesting that asymmetries in the set-up due to directionality of light or other factors had no effect on choice of the mites.

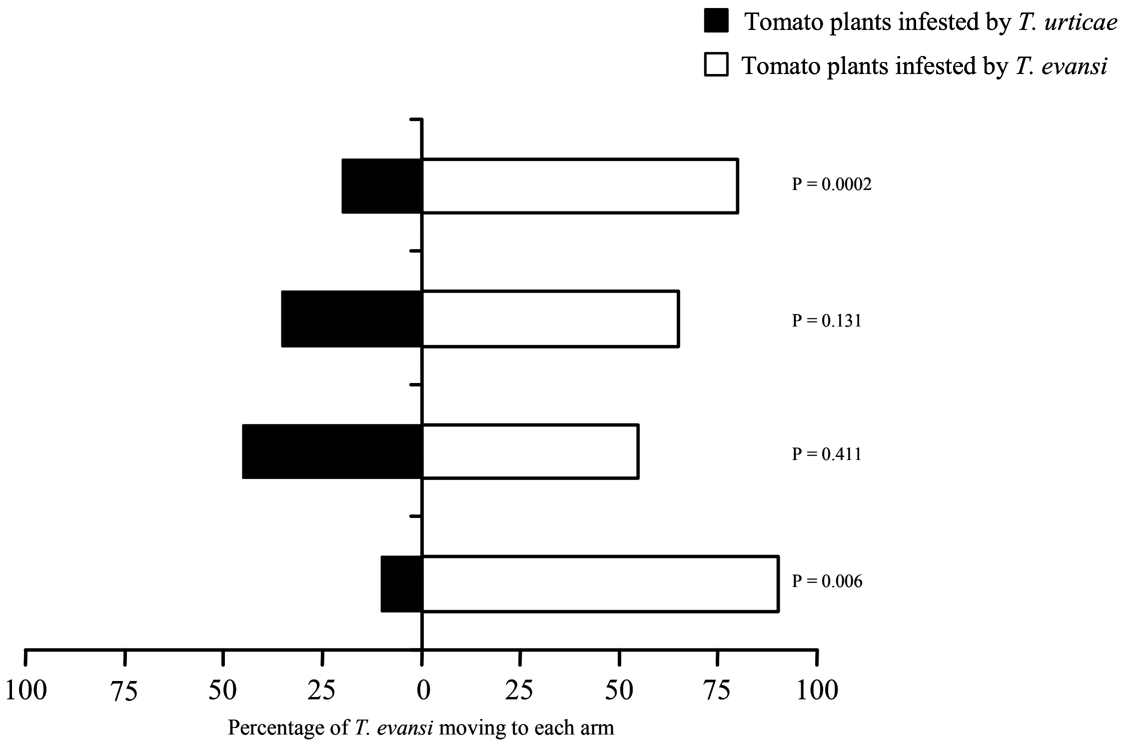

Figure 3. Choice of *T. evansi* females when offered volatiles from tomato plants infested by conspecifics (right) versus tomato plants infested by *T. urticae* (left). Each bar represents the results of a replicate, in which 20 mites were tested.

Discussion

Our results show that herbivores can use volatile cues to assess the quality of their host plants. It was observed that the levels of proteinase inhibitors in the leaves of tomato plants increased after infestation of the plants with the two-spotted spider mite *T. urticae*. Furthermore, the lower oviposition rate of *T. evansi* females on leaves of tomato plants that previously received damage by *T. urticae* was matched by the preference of *T. evansi* to plants infested with conspecifics mites, when tested versus plants infested with *T. urticae* mites. We previously found that the levels of proteinase inhibitors in the leaves of tomato plants decreased after infestation of the plants with the red spider mite *T. evansi*. Additionally, these low levels of PI's were matched by the higher performance of *T. evansi* females to plants that were infested with conspecifics compared to clean plants (chapter 1). These results show that the induction of such defenses may vary with spider mite species. The two spotted spider mite, *T. urticae,* has been recovered from over 900 plant species comprising 124 different plant families, while the red spider mite *T. evansi* has been reported as solanaceous crop pest, but preferring to feed on tomato (Jeppson et al., 1975; DeMoraes & McMurtry, 1985; Egas et al., 2003). The non-specificity of *T. urticae* mites to tomato plants could therefore have triggered the higher levels of proteinase inhibitors in leaves of plants infested by conspecific mites.

In general, the defense response of plants to herbivory is characterized by the activation of signaling compounds, which leads to the accumulation of specifics products such as proteinase inhibitors that interfere with the performance of the herbivore (Green & Ryan, 1972; Broadway & Duffey, 1986; Ryan, 1990; Lawrence & Koundal, 2002; Pompermayer et al., 2003). Additionally, JA-regulated proteins such as proteinase inhibitors and polyphenol oxidases implicate as anti-feedants against herbivores arthropods (Broadway & Duffey, 1986; Constabel et al., 1995). Therefore, the amount of proteinase inhibitors in plant tissues is indicative of the nutritional quality of such plants (Thaler et al., 2002). Consequently, plants containing higher levels of such proteinase inhibitors would be less profitable to herbivores arthropods compared to plants with lower levels of such PI's. This would explain the preference of *T. evansi* females to plants infested with conspecifics (lower levels of PI's) when tested *versus* plants damaged by *T. urticae* (higher levels of PI's).

However, it is relatively known the role of jasmonates in regulating the synthesis of phytochemicals, such as proteinase inhibitors, that have a direct effect on spider mites. Several lines of evidence indicate that induced defense of tomato plants against spider mites is regulated by this octadecanoid pathway (Karban et al., 1997; Enkerli et al., 1993; Felton et al., 1994; Duffey & Stout, 1996; Li et al., 2002; Ament et al., 2004). Additionally, the lower performance of *T. evansi* females on leaves of plants that were previously damaged by *T. urticae* mites may be attributed to secondary metabolites whose biosynthesis is regulated by JA (Keinanen et al., 2001; Memelink et al., 2001). Resistance of wild tomato species to spider mites is associated with defensive phytochemicals (e.g. methyl ketones, sesquiterpenes, and acyl sugars) that kill, repel, or entrap the herbivores (Williams et al., 1980). However, these compounds are typically found in secretions of glandular trichomes and generally do not accumulate to high levels in cultivated tomato plants (Farrar & Kannedy, 1992).

Moreover, as noted by Thaler et al., (2002), reduced egg production by spider mites grown on jasmonic acid-treated tomato plants (plants containing high level of PI's), suggests a nutritional rather than toxic mechanism of resistance. Therefore, we favor the possibility that the lower performance of *T. evansi* females on leaves of plants that received damage by *T. urticae* and their preference to plants infested by conspecifics, when tested versus plants infested by *T. urticae*, both were determined by the lower nutritional quality of the plants caused by the higher levels of proteinase inhibitors in such plants.

Acknowledgments

We thank Merijn Kant (Max Planck Institute for Chemical Ecology, Jena, Germany) for his expert advice and discussion on PI assays. Ana Paula Sérvulo de Andrade and Felipe de Lemos are thanked for support and assistance with the experiments. This research was supported in part by CNPq-Brazil and WOTRO-NWO (Netherlands Foundation for the Advancement of Tropical Research). AJ received a scholarship from CAPES, Brazil (PVE). AP and MV received a scholarship from CNPq, Brazil.

References

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. **Ecology**, 80, 1713-1723.

Ament, K., Kant, M. R., Sabelis, M. W., Haring, M. A. & Schuurink, R. C. 2004. Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. **Plant Physiology**, 135, 2025-2037.

Broadway, R. M. & Duffey, S. S. 1986. Plant proteinase-inhibitors - Mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exiqua*. **Journal of Insect Physiology**, 32, 827-833.

Constabel, C. P., Bergey, D. R. & Ryan, C. A. 1995. Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. **Proceedings of the National Academy of Sciences of the United States of America**, 92, 407-411.

DeMoraes, G. J. & McMurtry, J. A. 1985. Comparison of *Tetranychus evansi* and *T. urticae* (Acari: Tetranychidae) as prey for eight species of Phytoseiid mites. **Entomophaga**, 30, 393-397.

Duffey, S. S. & Stout, M. J. 1996. Antinutritive and toxic components of plant defense against insects. **Archives of Insect Biochemistry & Physiology**, 32, 3-37.

Egas, M., Norde, D. J. & Sabelis, M. W. 2003. Adaptive learning in arthropods: spider mites learn to distinguish food quality. **Experimental & Applied Acarology**, 30, 233-247.

Enkerli, J., Gisi, U. & Mosinger, E. 1993. Systemic acquired-resistance to phytophthora-infestans in tomato and the role of pathogenesis-related proteins. **Physiological & Molecular Plant Pathology**, 43, 161-171.

Farrar, R. R & Kannedy, G. G. 1992. **Insect and mite resistance in tomato**, p.122-142. In: Kalloo, G., Monographs on Theoretical & Applied Genetics. New York, Springer-Verlag, 358p.

Felton, G. W., Bi, J. L., Summers, C. B., Mueller, A. J. & Duffey, S. S. 1994. Potential role of lipoxygenases in defense against insect herbivory. **Journal of Chemical Ecology**, 20, 651-666.

Gotoh, T., Bruin, J., Sabelis, M. W. & Menken, S. B. J. 1993. Host race formation in *Tetranychus urticae* - genetic differentiation, host-plant preference, and mate choice in a tomato and a cucumber strain. **Entomologia Experimentalis et Applicata**, 68, 171-178.

Green, T. R. & Ryan, C. A. 1972a. Wound-induced proteinase inhibitor in plant leaves - possible defense mechanism against insects. **Science**, 175, 776-778.

Green, T. R. & Ryan, C. A. 1972b. Wound-induced proteinase inhibitor in tomato leaves - Evidence for a proteinase inhibitor inducing factor. **Federation Proceedings**, 31, A862-&.

Janssen, A., Pallini, A., Venzon, M. & Sabelis, M. W. 1999. Absence of odour-mediated avoidance of heterospecific competitors by the predatory mite *Phytoseiulus persimilis*. **Entomologia Experimentalis et Applicata**, 92, 73-82.

Jeppson, L. R., Keifer, H. H. & Baker, E. W. 1975. **Mites injurious to economic plants**. Berkeley, University of California Press, 614p.

Kakade, M. L., Rackis, J. J., McGhee, J. E. & Puski, G. 1974. Determination of trypsin-inhibitor activity of soy products - Collaborative analysis of an improved procedure. **Cereal Chemistry**, 51, 376-382.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A. & Schuurink, R. C. 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. **Plant Physiology**, 135, 483-495.

Karban, R & Baldwin, I. T. 1997. **Induced resistance to herbivory**. Chicago University Press, Chicago.

Karban, R., Agrawal, A. A. & Mangel, M. 1997. The benefits of induced defenses against herbivores. **Ecology**, 78, 1351-1355.

Keinanen, M., Oldham, N. J. & Baldwin, I. T. 2001. Rapid HPLC screening of jasmonate-induced increases in tobacco alkaloids, phenolics, and diterpene glycosides in *Nicotiana attenuata*. **Journal of Agricultural & Food Chemistry**, 49, 3553-3558.

Lawrence, P. K. & Koundal, K. R. 2002. Plant protease inhibitors in control of phytophagous insects. **Electronic Journal of Biotechnology**, 5, 93-109.

Leite, G. L. D., Picanco, M., Zanuncio, J. C. & Marquini, F. 2003. Factors affecting mite herbivory on eggplants in Brazil. **Experimental & Applied Acarology**, 31, 243-252.

Li, C. Y., Williams, M. M., Loh, Y. T., Lee, G. I. & Howe, G. A. 2002. Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. **Plant Physiology**, 130, 494-503.

McCloud, E. S. & Baldwin, I. T. 1997. Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*. **Planta**, 203, 430-435.

Memelink, J., Verpoorte, R. & Kijne, J. W. 2001. ORCAnization of jasmonate - responsive gene expression in alkaloid metabolism. **Trends in Plant Science**, 6, 212-219.

Pallini, A., Janssen, A. & Sabelis, M. W. 1997. Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. **Oecologia**, 110, 179-185.

Pare, P. W., Alborn, H. T. & Tumlinson, J. H. 1998. Concerted biosynthesis of an insect elicitor of plant volatiles. **Proceedings of the National Academy of Sciences of the United States of America**, 95, 13971-13975.

Pompermayer, P., Falco, M. C., Parra, J. R. P. & Silva, M. C. 2003. Coupling diet quality and Bowman-Birk and Kunitz-type soybean proteinase inhibitor effectiveness to *Diatraea saccharalis* development and mortality. **Entomologia Experimentalis et Applicata**, 109, 217-224.

Ryan, C. A. 1990. Protease inhibitors in plants - Genes for improving defenses against insects and pathogens. **Annual Review of Phytopathology**, 28, 425-449.

Sabelis, M. W. 1991. Life-history evolution of spider mites, p.23-50. In: Schuster, R. & Murphy, P.W. (eds.) **The Acari: reproduction, development and life-history strategies,** London, Chapman & Hall, 554p.

Sabelis, M. W. & Van de Baan, H. E. 1983. Location of distant spider-mite colonies by phytoseiid predators - Demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. **Entomologia Experimentalis et Applicata**, 33, 303-314.

Sarmento, R. A. 2003. **Resposta comportamental de artrópodes em teia alimentar de tomateiro**. Dissertação de mestrado, Universidade Federal de Viçosa, Viçosa, 61p.

Sokal, R. R. & Rohlf, F. J. 1995. *Biometry:* **The principles and practice of statistics in biological research**. New York: Freeman.

Stout, M. J., Workman, J. & Duffey, S. S. 1994. Differential induction of tomato foliar proteins by arthropod herbivores. **Journal of Chemical Ecology**, 20, 2575-2594.

Stout, M. J., Workman, K. V., Bostock, R. M. & Duffey, S. S. 1998. Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. **Oecologia**, 113, 74-81.

Thaler, J. S., Karban, R., Ullman, D. E., Boege, K. & Bostock, R. M. 2002. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. **Oecologia**, 131, 227-235.

Walling, L. L. 2000. The myriad plant responses to herbivores. **Journal of Plant Growth Regulation**, 19, 195-216.

Warburg, O. & Christian, W. 1941. Isolation and crystallization of the glycolytic enzyme enolase. **Biochemische Zeitschrift**, 310, 385–421.

Williams, W. G., Kennedy, G. G., Yamamoto, R. T., Thacker, J. D. & Bordner, J. 1980. 2-Tridecanone – Naturally-occurring insecticide from the wild tomato *Lycopersicon hirsutum F. glabratum*. **Science**, 207, 888-889.

CHAPTER 3

Host-plant quality mediates trade-off between oviposition and web production of the spider mite *Tetranychus evansi* Baker & Printchard (Acari: Tetranychidae)

Renato Almeida Sarmento[1,2]*; Arne Janssen*[2]*; Angelo Pallini*[1] *and Madelaine Venzon*[3]

[1]Department of Animal Biology/Entomology, Federal University of Viçosa, Brazil; [2]Institute for Biodiversity and Ecosystem Dynamics, Section Population Biology, University of Amsterdam, PO Box 94084, Amsterdam, The Netherlands; [3]EPAMIG (Agriculture and Livestock Research Enterprise of Minas Gerais, Brazil)

Abstract - To defend itself against its predators, the red spider mite *T. evansi* spins a chaotically structured web over its colonies. The silk of this web consists of proteins, and its production may thus go at the expense of amino acids that would otherwise be available for growth and reproduction. Given the observation that *T. evansi* females had higher oviposition rate on leaves of plants that were previously damaged by conspecific mites than on leaves of plants that were previously damaged by the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), both compared to leaves of clean plants, we investigated the effect of the damage caused by *T. evansi* and its close relative *T. urticae* on the investment in eggs *vs.* web production by *T. evansi* females. We found that the ovipositional rate of *T. evansi* females was approximately 2-fold higher on tomato leaves that were previously damaged by conspecific mites compared to leaves of plants that were previously damaged by *T. urticae*. Furthermore, the relative web density produced by *T. evansi* was a factor 3-fold lower on leaf-discs from leaves of plants that were previously damaged by conspecifics than in leaf-discs from leaves of plants that received damage by *T. urticae.* We conclude that *T. evansi* mites have the flexibility to adapt their investment in eggs *vs.* web production depending on the quality of their host plants.

Key words: Proteinase inhibitors, plant defense, tomato, nutritional status.

Resumo – Para se defender do ataque de predadores, o ácaro *T. evansi* produz uma grande quantidade de teia sobre suas colônias. Os fios dessa teia são compostos de proteinas, o que requer um grande gasto de aminoácidos que poderiam ser usados pelos ácaros para o crescimento e reprodução. Adicionalmente, foi observado anteriormente que fêmeas de *T. evansi* possuem maior taxa de oviposição em folhas de plantas de tomate que foram previamente danificadas por ácaros co-específicos do que em folhas de plantas que receberam dano do ácaro rajado *Tetranychus urticae* Koch (Acari: Tetranychidae), ambas comparadas com folhas de plantas limpas. Neste trabalho, no entanto, foi investigado o efeito do dano causado por *T. evansi* e seu parente próximo, *T. urticae*, no investimento em produção de ovos *vs.* produção de teia por fêmeas do ácaro *T. evansi*. Os resultados mostraram que a densidade relativa de teia produzida por fêmeas de *T. evansi* foi aproximadamente três vezes menor em discos feitos de folhas de plantas de tomate que receberam o dano de ácaros co-específicos do que em discos provenientes de plantas danificadas pelo ácaro *T. urticae.* Além disso, a taxa de oviposição de fêmeas de *T. evansi* foi aproximadamente duas vezes maior em discos provenientes de plantas danificadas por co-específicos, quando comparada à obtida em discos provenientes de plantas danificadas pelo ácaro rajado *T. urticae*. Isso mostra que o ácaro *T. evansi* é capaz de adaptar seu investimento na produção de ovos *vs.* produção de teia em função da qualidade das suas plantas-hospedeiro.

Palavras-chave: Inibidor de protease, defesa de planta, plantas de tomate, status nutricional

Introduction

Spider mites spend much time feeding on the leaf parenchyma, they are sluggish and therefore easy victims for much more agile predatory mites. To defend themselves against these enemies, they hide themselves in a web of self-produced silken threads (Sabelis & Bakker, 1992). Predatory mites can overcome this barrier by specialized behaviour and by morphological adaptations of their dorsal setae that help them to minimize body contact with silken threads in a web, thereby lowering the chance to get stuck in the web (Sabelis & Bakker, 1992; Sabelis et al., 1994). Species of spider mites differ in the amount of silk produced and in the structure of the web (Saito, 1977; Gerson, 1985). Additionally, there is a strong positive relationship between the capacity for population increase, the degree of local population aggregation and the investment in silk production across species of spider mites (Sabelis, 1991; Sabelis & Janssen, 1994). On one hand of the continuum, slowly reproducing spider mite species spin little silk. On the other hand, there are prolific spider mite species that spin a profuse, chaotically structured web over their colonies. For example, the red spider mite *Tetranychus evansi* (Acari: Tetranychidae) is known for its extreme investment in silk production (Baker & Pritchard, 1960; DeMoraes et al., 1987).

Tetranychus evansi was originally described from specimens collected on Mauritius in 1960 (Baker & Pritchard, 1960; DeMoraes & McMurtry, 1986). It has since been reported in the USA and Brazil (Bolland et al., 1998), and more recently in eastern and southern Africa (Knapp et al., 2003), Spain and Portugal (Ferragut & Escudero, 1999; Bolland & Vala, 2000). It has been reported as pest of several solanaceous crops, such as tomato (Jeppson et al., 1975; Moraes & McMurtry, 1985). The chaotic web with which *T. evansi* cover their colonies is hard to penetrate for most predators (Oliveira et al., 2005; Rosa et al., 2005), and *T. evansi* has therefore become a major pest (Maluf et al., 2001; Rosa et al., 2005; Oliveira et al., 2005).

The positive correlation across-species between population growth capacity and silk production in the spider mites is counterintuitive, because the two traits are expected to be subject to a trade-off. This is because the silk of the web produced by spider mites consists of proteins (Hazan et al., 1975) and its production may thus go at the expense of amino acids that would otherwise be available for growth and reproduction. Preliminary experiments with the closely related of *T. evansi*, the two-spotted spider mite *T. urticae*, have shown that selected lines with increased investment in silk also incur a cost in terms of lower egg production (Sabelis, unpublished data). Additionally, previous experiments showed that *T. evansi* had higher oviposition rate on leaves of plants that were previous damaged by conspecific mites than on leaves of plants that were previous damaged by the two-spotted spider mite *T. urticae*, both compared to leaves of clean plants (chapter 1 and 2). Therefore, if *T. evansi* is subject to a trade-off between population growth and silk production, we would expect females of these mites to produce less web on plants where they had higher oviposition rate (plants damaged by conspecifics) than on plants where they had lower oviposition (plants damaged by *T. urticae*). To investigate such a trade-off, we evaluated the effect of the damage caused by *T. evansi* and the closely related *T. urticae* on the production of web and eggs by *T. evansi*. Specifically, we measured the oviposition rate and the relative density of the web produced by *T. evansi* females on leaf-discs from leaves of tomato plants that were previously damaged by conspecifics, on leaves of plants previously damaged by *T. urticae* and on leaves of clean plants (controls).

Materials and Methods

Mite rearing and plant material

Tetranychus evansi was obtained in 2002 from a natural infestation of tomato plants (*Lycopersicon esculentum* Mill variety Santa Clara I-5300) in a greenhouse at the Federal University of Viçosa. The population of *T. urticae* was obtained from strawberry plants in a greenhouse at the Federal University of Viçosa and were subsequently transferred to tomato plants of the same variety.

Tomato seeds were sown in a commercial substrate, composed of vermiculite and organic fertilizer, in trays and kept in mite-proof screen cages in a greenhouse. Plants (21 days old) were transplanted to plastic pots (2L) that contained a mixture of soil, bovine manure (3:1) and fertilizer (4-14-8 N-P-K). Tomato plants were further grown in mite-proof screen cages in a greenhouse until they were 45 days old and had at least four completely developed leaves. Plants were infested with either *T. evansi* or *T. urticae* by putting four small, infested tomato leaves on each plant, inside a mite-proof screen cage in a greenhouse. The plants were incubated in this way for one week, resulting in 300 – 400 adult mites on each plant. Subsequently, the plants were used in the experiments.

Experimental set up

Egg production and the relative web density of *T. evansi* females were measured on leaves of plants that were previously damaged by *T. urticae*, on leaves of plants damaged by *T. evansi* and on leaves of clean plants. Each treatment was replicated on four different plants. The plants were kept in mite-proof screen cages in a greenhouse.

Seven days after infestation with mites, leaf-discs (Ø = 1 cm) were made from the leaflets of the plants; five discs were taken from the leaves of each plant. For the treatments where the plants were previously infested with *T. evansi* or *T. urticae*, the discs were made after the mites, the web and eggs produced by the mites were removed using a fine brush. The leaf-discs were kept in group of 15 in trays (30 x 20 cm) containing foam covered with wet cotton wool and filled with water to prevent the mites from escaping from the discs.

Egg production and relative web density

Tetranychus evansi females of similar age were used to measure oviposition and web production. To obtain such cohorts, several adult females were allowed to lay eggs on detached uninfested tomato leaves on wet cotton wool, inside a tray. The adults were removed after 24 h and the eggs were reared until adulthood. Subsequently, 2-day-old mated females of this cohort were maintained individually on a tomato leaf disc (Ø = 1 cm), inside a climate box (28 ± 2ºC; relative humidity 70 ±10%), for a period of four days. Subsequently the eggs produced were counted and the relative web density was estimated. The results were analyzed using Generalyzed Linear Models (g.l.m.).

Web quantification

The relative web density was measured by using red soil particles (Ø = 0.177 mm). By using a thin brush, the particles were sprinkled over the whole leaf-disc, covering the whole webbed area. Subsequently, the number of particles on the leaf and the number of grains in the web was quantified and the relative web density was calculated as the fraction of soil particles that was caught in the web. The results were analyzed using generalized linear models (g.l.m.) with a binomial error distribution (R Development Core Team, 2006).

Results

Oviposition rate

There was a significant effect of the treatment on the rate of oviposition of *T. evansi* (Fig. 1, $F_{2,\ 10} = 18.76$, $p < 0.001$). The oviposition rate was significantly higher on tomato leaves that previously received damage by conspecifics than on leaves that were previously damaged by *T. urticae* or on clean tomato leaves (Tukey HSD, $p < 0.05$) (Fig. 1). Furthermore, the oviposition rate of *T. evansi* was significantly lower on leaves that were previously damaged by *T. urticae* than on clean leaves (control) (Tukey HSD, $p < 0.01$).

Relative web production

There was high variation in web production among replicates, which might have been caused by heterogeneity in the plant material (Tukey HSD, $p < 0.01$). Nevertheless, there was a significant effect of treatment on the web production of *T. evansi* (Fig. 2, $F_{2,\ 48} = 14.22$ $p < 0.001$). The relative web density was a factor 3-fold higher on leaf discs from leaves of plants that were previously damaged by *T. urticae* than on leaf-discs from leaves of plants that had received damage by *T. evansi* (Tukey HSD, $p < 0.05$). The web density on clean leaf discs did not differ to that on leaves of plants that received damage by *T. evansi* or *T. urticae*.

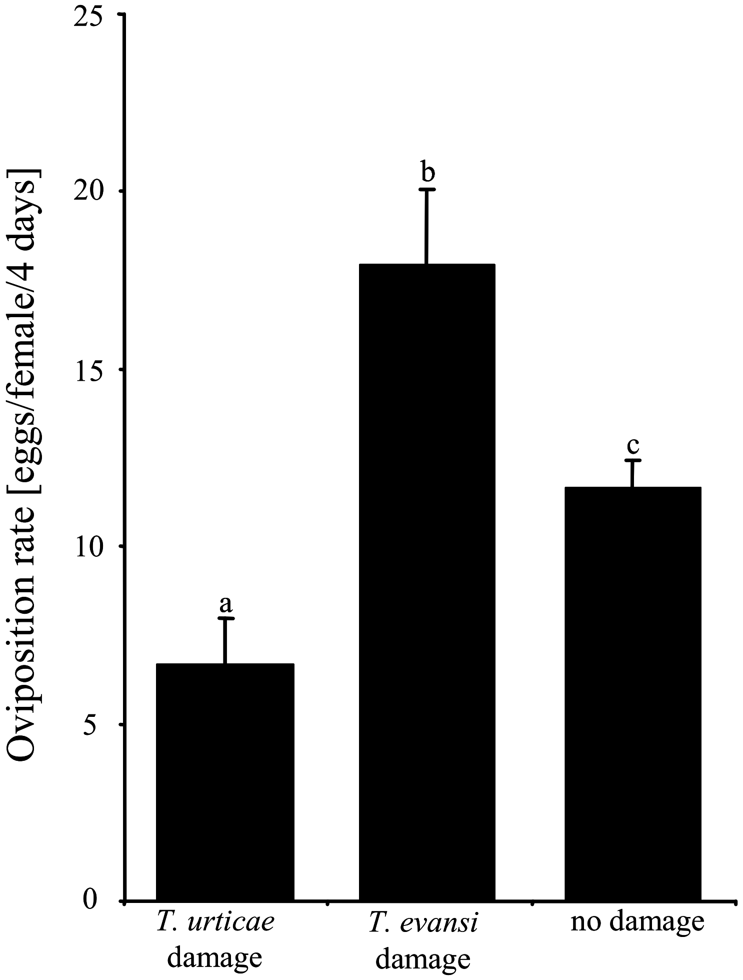

Figure 1. Average (± s.e.) oviposition rate of *T. evansi* on tomato leaf discs that were previously infested with conspecific mites, with the heterospecific two-spotted spider mite *T. urticae*, and on clean leaves.

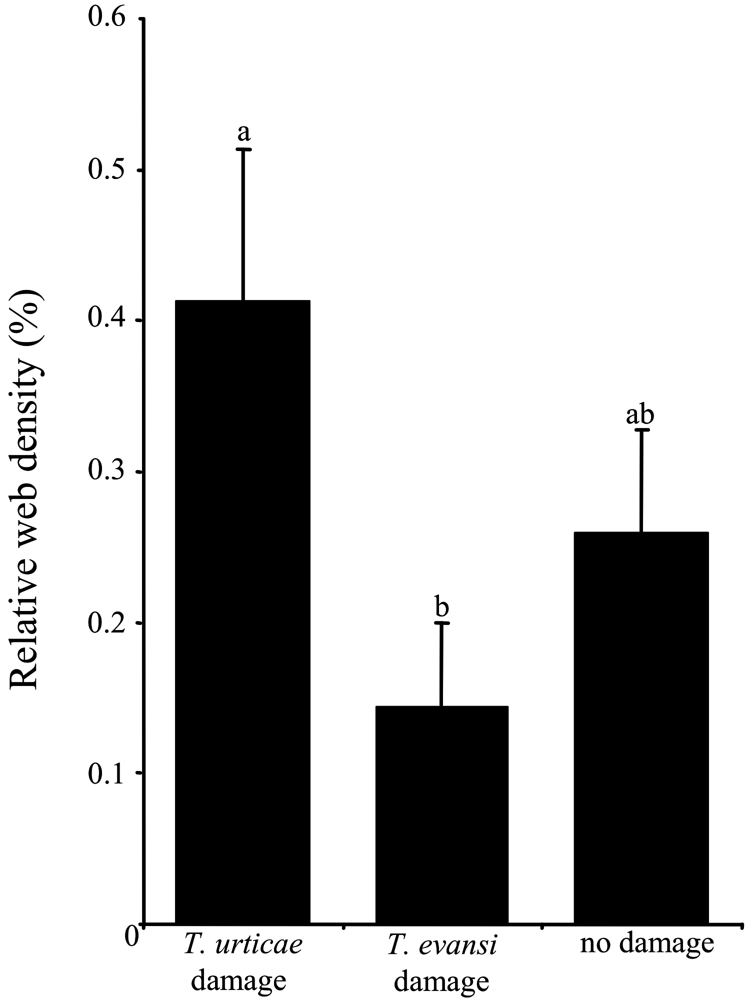

Figure 2. Average (± s.e.) relative web density of *T. evansi* on tomato leaf-discs that were previously infested with conspecific mites, with the heterospecific two-spotted spider mite *T. urticae*, and on clean leaves.

Discussion

Our results show that *T. evansi* mites have the flexibility to adapt their investment in eggs *vs.* web production depending on the quality of the host plant. The oviposition rate of *T. evansi* was approximately 2-fold higher on tomato leaves that were previously damaged by conspecific mites compared to leaves that were previously damaged by *T. urticae*. Furthermore, the relative web density produced by *T. evansi* was a factor three times lower on leaf-discs from leaves of plants that were previously damaged by conspecifics than on leaf-discs from leaves of plants that received damage by *T. urticae.* This suggests that spider mites that invest resources in indirect defence such as the production of web, allocate fewer resources to population growth, such as egg production.

It was previously observed that the higher performance of *T. evansi* on leaves that were damaged by conspecifics was matched by lower levels of proteinase inhibitors in the leaves, compared to clean leaves (chapter 1). In contrast, the lower performance of *T. evansi* females on leaves that previously received damage by *T. urticae* was accompanied by higher levels of proteinase inhibitors in the plants compared to the controls (chapter 2). Proteinase inhibitors are proteins associated with plant defence that are induced by herbivore damage and accumulate in above-ground plant tissues. They render the plant tissue less suitable for invading pests (Green & Ryan, 1972; Strassner et al., 2002; Kant et al., 2004; Damle et al., 2005). The amount of proteinase inhibitors in plant tissues is indicative of the nutritional quality of such plants (Thaler et al., 2002). Thus, one interpretation of these results is that *T. evansi* is capable of adjusting silk investment levels to host plant quality and as a consequence to the costs of investment in defense, for instance the production of web, the mites would allocate fewer resources to population growth, such as the production of eggs. Moreover, we favour the possibility that this flexibility in web and egg production by *T. evansi,* varying with host plant quality might be linked with the trade-off between population growth capacity and web production.

To defend their colonies against predators, spider mites, such as *T. evansi*, spin a chaotically structured web over them and these webs are hard to penetrate for many species of predator (Saito, 1977; Sabelis & Bakker, 1992; Rosa et al., 2005). The silk of this web consists of proteins (Hazan et al., 1975) and its production may thus go at the expense of amino acids that would otherwise be available for growth and reproduction. Thus, spider mites that strongly invest in silk production should pay off the costs caused by the higher production of web in terms of lower egg production. Furthermore, across-species patterns represent different optima for different environments, which indicates that *T. evansi* has to coordinate different life history strategies of reproductive allocation (egg production) with defence strategies (web production), and that this strategies may depend on either the nutritional status of their host plants or the presence of heterospecific competitors on such plants, or even a combination of both.

We have observed that the two-spotted spider mite *T. urticae* is not capable to overcome the web produced by *T. evansi* mites (Sarmento, pers. obs.). Hence, an additional explanation for the higher investment in web production in plants that were damaged by *T. urticae* could be that *T. evansi* equates cues related to the damage caused by heterospecifics competitors (*T. urticae*) with the potential presence of such competitors, and therefore produces more web on those plants in order to reduce competition with *T. urticae*. Hence, *T. evansi* mites may not only invest more in production of web to defend their colonies against predators, but also to protect them against heterospecifics competitors. Our results indicate that there is a trade-off between reproduction (egg production) and silk production by *T. evansi* mites, and that this trade-off is orchestrated by the quality of their host plants. Further experiments are underway to investigate this.

Acknowledgments

We thank Ana Paula Sérvulo de Andrade and Felipe de Lemos for support and assistance with the experiments. We thank CNPq-Brazil and CAPES-Brazil for financial support. AJ received a scholarship from CAPES, Brazil (PVE), and AP and MV from CNPq, Brazil.

References

Baker, E. W. & Pritchard, A. E. 1960. The tetranychid mites of Africa. **Hilgardia**, 29, 455-574.

Bolland, H. R., Gutierrez, J. & C. H. W. Flechtmann. 1998. **World catalogue of the spider mite family** (Acari: Tetranychidae). (Leiden Brill Academic Publishers), 392p. Leiden.

Bolland, H. R. & Vala, F. 2000. First record of the spider mite *Tetranychus evansi* (Acari: Tetranychidae) from Portugal. **Entomological Berichten**, 60, 180.

Damle, M. S., Giri, A. P., Sainani, M. N. & Gupta, V. S. 2005. Higher accumulation of proteinase inhibitors in flowers than leaves and fruits as a possible basis for differential feeding preference of Helicoverpa armigera on tomato (*Lycopersicon esculentum* Mill, Cv. Dhanashree). **Phytochemistry**, 66, 2659-2667.

DeMoraes, G. J. & McMurtry, J. A. 1985. Comparison of *Tetranychus evansi* and *T. urticae* (Acari: Tetranychidae) as prey for eight species of phytoseiid mites. **Entomophaga**, 30, 393-397.

DeMoraes, G. J. & McMurtry, J. A. 1986. Suitability of the spider-mite *Tetranychus evansi* as prey for *Phytoseiulus persimilis*. **Entomologia Experimentalis et Applicata**, 40, 109-115.

DeMoraes, G. J., McMurtry, J. A. & Baker, E. W. 1987. Redescription and distribution of the spider mites *Tetranychus evansi* and *T. marianae*. **Acarologia**, 28, 333-343.

Ferragut, F. & Escudero, A. 1999. *Tetranychus evansi* Baker & Pritchard (Acari, Tetranychidae), una nueva araña roja en los cultivos hortícolas españoles, **Boletín de Sanidad Vegetal Plagas** 25: 157-164.

Gerson, U. 1985. **Webbing**. p.223-232. In Helle, W. & Sabelis, M. W. (Eds.) Spider mites: their biology, natural enemies and control. New York, Elsevier, (World Crop Pests, 1A) 404p.

Green, T. R. & Ryan, C. A. 1972. Wound-induced proteinase inhibitor in plant leaves - Possible defense mechanism against insects. **Science**, 175, 776-778.

Hazan, A., Gertler, A., Tahori, A. S. & Gerson, U. 1975. Spider mite Webbing. 3. Solubilization and amino acid composition of silk protein. **Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology**, 51, 457-462.

Jeppson, L. R., Keifer, H. H. & Baker, E. W. 1975. **Mites injurious to economic plants**. Berkeley, University of California Press, 614p.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A. & Schuurink, R. C. 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. **Plant Physiology**, 135, 483-495.

Knapp, M., Wagener, B. & Navajas, M. 2003. Molecular discrimination between the spider mite *Tetranychus evansi* Baker & Pritchard, an important pest of tomatoes in southern Africa, and the closely related species *T. urticae* Koch (Acarina: Tetranychidae). **African Entomology**, 11, 300-304.

Maluf, W. R., Campos, G. A. & Cardoso, M. D. 2001. Relationships between trichome types and spider mite (*Tetranychus evansi*) repellence in tomatoes with respect to foliar zingiberene contents. **Euphytica**, 121, 73-80.

Oliveira, E. E., Oliveira, C. L., Sarmento, R. A., Fadini, M. A. M. & Moreira, L. R. 2005. Biological aspects of the predator *Cycloneda sanguinea* Linnaeus, 1763 (Coleoptera: Coccinellidae) fed with *Tetranychus evansi* Baker & Pritchard, 1960 (Acari: Tetranychidae) e *Macrosiphum euphorbiae* Thomas, 1878 (Hemiptera: Aphididae). **Bioscience Journal**, 21, 33-39.

R Development Core Team (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rosa, A. A., Gondim, M. G. C., Fiaboe, K. K. M., de Moraes, G. J. & Knapp, M. 2005. Predatory mites associated with *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on native solanaceous plants of coastal Pernambuco State, Brazil. **Neotropical Entomology**, 34, 689-692.

Sabelis, M. W. 1991. **Life-history evolution of spider mites**, p.2-49. In R. Schuster & Murphy, P. W. (eds), The Acari: Reproduction, Development, and Life-History Strategies. Amsterdam, Chapman & Hall, 554p.

Sabelis, M. W. & Bakker, F. M. 1992. How predatory mites cope with the web of their tetranychid prey - A Functional view on dorsal chaetotaxy In The Phytoseiidae. **Experimental & Applied Acarology**, 16, 203-225.

Sabelis, M. W., Bakker, F. M. & Bolland, H. R. 1994. Hypotrichy In The Phytoseiidae - Adaptation or phylogenetic constraint. **Experimental & Applied Acarology**, 18, 241-245.

Sabelis, M. W. & Janssen, A. 1994. **Evolution of life-history patterns in the phytoseiidae**, p.70-78. In: Houck, M. A. (eds) Mites: ecological and evolutionary analyses of life-history patterns, New York, Chapman & Hall, 384p.

Saito, Y. 1977. Study of the spinning behaviour of the spider mite (Acarina: Tetranychidae). I. Method for the quantitative evaluation of the mite webbing and the relationship between webbing and walking. **Japanese Journal of Applied Entomology and Zoology**, 21, 27-34.

Strassner, J., Schaller, F., Frick, U. B., Howe, G. A., Weiler, E. W., Amrhein, N., Macheroux, P. & Schaller, A. 2002. Characterization and cDNA-microarray expression analysis of 12-oxophytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. **Plant Journal**, 32, 585-601.

Thaler, J. S., Karban, R., Ullman, D. E., Boege, K. & Bostock, R. M. 2002. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. **Oecologia**, 131, 227-235.

CHAPTER 4

Use of odours by *Cycloneda sanguinea* Linnaeus (Coleoptera: Coccinellidae) to assess patch quality

Renato Almeida Sarmento^{1,2*}*; Madelaine Venzon*^{3}*; Angelo Pallini*^{1}*; Eugenio Eduardo de Oliveira*^{4} *and Arne Janssen*^{2}

Accepted by Entomologia Experimentalis et Applicata 04/12/06

[1]Department of Animal Biology/Entomology, Federal University of Viçosa, CEP: 36570-000, Viçosa-MG, Brazil; [2]Institute for Biodiversity and Ecosystem Dynamics, Section Population Biology, University of Amsterdam, PO Box 94084, Amsterdam, The Netherlands; [3]EPAMIG (Agriculture and Livestock Research Enterprise of Minas Gerais, Brazil); [4]Zoological Institute/Department of Animal Physiology, University of Cologne, Weyertal 119, 50923 Cologne, Germany

Abstract - Adult ladybirds are likely to encounter different species of prey when foraging for oviposition sites. Optimal oviposition theory predicts that females should lay eggs in those sites that are the most suitable for offspring development. Therefore, factors which directly affect offspring mortality, such as the presence of predators and food, are expected to play an important role in the assessment of patch profitability by ladybird predators. By using a Y-tube olfactometer, we tested whether the ladybird predator *Cycloneda sanguinea* Linnaeus (Coleoptera: Coccinellidae) can use volatile cues to asses patch profitability and avoid predator-rich patches. We assessed the foraging behaviour of *C. sanguinea* in response to odours associated with tomato plants infested with a superior prey, *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae) and with a inferior prey, *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae), in the presence or not of the heteroespecific predator, *Eriopis connexa* Mulsant (Coleoptera: Coccinellidae). Females of *C. sanguinea* significantly preferred plants infested by *M. euphorbiae* to plants infested by *T. evansi* and avoided odours emanating from plants on which *E. connexa* females were present. Our results show that *C. sanguinea* use volatile cues to asses patch profitability and to avoid patches with heterospecific competitor or intraguild predator.

Key words: Infochemicals, *Eriopis connexa*, aphids, mites, food competition, intraguild predation.

Resumo - Predadores coccinelídeos encontram freqüentemente diferentes tipos de presas quando estão forrageando por locais de oviposição. A teoria da oviposição ótima prediz que fêmeas de coccinelídeos deveriam depositar seus ovos naqueles locais mais apropriados para o desenvolvimento de sua prole. Desta forma, os fatores que afetam diretamente a sobrevivência da prole, como a presença de alimento e predadores, devem desempenhar um importante papel na avaliação da qualidade de locais para oviposição por esses predadores. Este trabalho testou se o predador *Cycloneda sanguinea* Linnaeus (Coleoptera: Coccinellidae) usa pistas voláteis para acessar a qualidade de locais para oviposição, bem como a presença de predadores heteroespecíficos nesses locais. Em um olfatômetro de tubo em Y, foi avaliado o comportamento de forrageamento de *C. sanguinea* em resposta aos odores associados com plantas onde outro predador generalista estava presente, *Eriopis connexa* Mulsant (Coleoptera: Coccinellidae) bem como as presas *Tetranychus evansi* Baker & Printchard (Acari: Tetranychidae) ou *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae). Os resultados mostraram que fêmeas de *C. sanguinea* foram atraídas por voláteis de plantas de tomate que infestadas com *T. evansi*. Além disso, *C. sanguinea* preferiu significativamente odores de plantas infestadas com *M. euphorbiae* a odores provenientes de plantas infestadas com *T. evansi* e evitaram odores emanados de plantas em que fêmeas de *E. connexa* estavam presentes. Os resultados obtidos neste trabalho mostram que *C. sanguinea* usa pistas voláteis para evitar locais onde predadores heteroespecíficos estão presentes, provavelmente para evitar competição por alimento ou predação intra-guilda.

Palavras-chave: Voláteis, coccinelídeos, afídeos, ácaros, competição por alimento, predação intra-guilda.

Introduction

The assessment of patch quality by female insects is assumed to be an important step in their foraging process (Dixon, 1959; Gutierrez et al., 1984; Dixon, 2000). Factors which directly affect offspring mortality (e.g., presence of predators and food) are expected to play an important role in the assessment of patch suitability by insect females, and it is likely to drive the evolution of the oviposition behaviour (Hemptinne et al., 1993; Fréchette et al., 2003; Rieger et al., 2004). Furthermore, foraging theory developed for invertebrate predators, and ladybirds in particular, indicates that if the females are to maximize their fitness, they should be reluctant to lay eggs close to those places that are less suitable for the development of their offspring (Mangel, 1987; Hemptinne et al., 1992; Hodek, 1993; Kindlmann & Dixon, 1993; Frechetté et al., 2006). This should be so since neonate have a limited dispersion capacity and need to quickly find a meal (Kindlmann & Dixon, 1993; Tschanz et al., 2005).

However, enemy-free patches can be also as important as the presence and the quality of food in the patches (Mangel, 1989; Ruzicka, 2001ab; Griffin & Yeargan, 2002ab; Frechetté et al., 2003, 2006). The patch dynamics and enemy-free space hypotheses are not mutually exclusive, and may provide complementary explanations (Frechetté et al., 2006). Therefore, females are expected to assess the general quality of prey for choosing an optimal ovipositional site, and consequently maximize their fitness (Stephens & Krebs, 1986; Hodek, 1993; Doumbia et al., 1998; Tschanz et al., 2005). However, when searching for a more suitable ovipositing patch, some predators are capable to assess not only the presence and quality of prey, but also the presence of competitors and intraguild predators (Obata, 1997; Ruzicka, 1994, 1996, 2001b, 2003, 2006; Li et al., 1997; Yasuda et al., 2000). This assessment occurs not only when they face a competitor or predator, but also when they detect cues associated with the presence of such competitors or predators (Obata, 1986; Sengonca & Liu, 1994; Janssen et al., 1997; Ruzicka & Havelka, 1998; Ruzicka, 2001a; Grostal & Dicke, 1999; Hemptinne & Dixon, 2000; Al Abassi et al., 2001; Ninkovic et al., 2001; Hemptinne et al., 2001; Ninkovic & Pettersson, 2003; Pettersson et al., 2005).

The ladybird predator *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) has generalist feeding habits and it is among the potential natural enemies studied as biocontrol agents of *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae) and *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), which are important pests of tomato plants in Brazil (Flechtmann, 1983; Maluf et al., 2001; Eichler & Reis, 1976; Cardoso & Lazzari, 2003). Although *C. sanguinea* can consume different prey types, there are indications that the predator has higher fitness when fed with aphids (Vandenberg & Gordon, 1988; Isikber & Copland, 2002). It is able to complete its biological cycle when fed only on *M. euphorbiae*, but not when fed only on *T. evansi* mites (Oliveira et al., 2005).

The co-occurrence of *C. sanguinea* and other coccinellid predators such as *Eriopis connexa* Mulsant (Coleoptera: Coccinellidae) in different crops, tomato included, has been described (Barbosa & França, 1981; Gassen, 1986; Hohmann, 1989; Grutzmacher & Link, 2000; Resende et al., 2006). Moreover, *E. connexa* was shown to efficiently prey on *T. evansi* and on *M. euphorbiae* (Sarmento et al., 2007). The simultaneous occurrence of predators, such as *C. sanguinea* and *E. connexa*, on a patch may impose oviposition of one or both species in a non optimal patch (Yasuda et al., 1999; Griffin & Yeargan, 2002ab). Therefore, besides competition for patch rich prey, predators may also face intraguild predation (Magalhães et al., 2005; Ruzicka, 2006).

In this study we investigated whether *C. sanguinea* can use volatile cues to asses patch suitability and to avoid patches housing heterospecific predators. Previous experiments showed that *C. sanguinea* prefer odours of tomato plants infested either by *M. euphorbiae* or *T. evansi*, when clean plants were offered as alternative. The predator avoided odours from clean plants, when tested versus clean air (Sarmento, 2003). For this reason, we first assessed the preference of *C. sanguinea* to odours of tomato plants infested with *T. evansi* vs. clean air. This experiment focused on determining if the preference of *C. sanguinea* to plants infested by these herbivores is because of the avoidance of clean tomato plants or because plants infested by herbivores are more attractive to *C. sanguinea*. Subsequently, we investigated the preference of *C. sanguinea* for odours from tomato plants infested with *T. evansi* or with *M. euphorbiae*. Lastly, we assess the capacity of *C. sanguinea* to use odours to detect the presence of the heterospecific predator *E. connexa*. We also tested whether the prey preference of *C. sanguinea* would change with *E. connexa* presence.

2. Material and Methods

Arthropod rearing and plant material

Spider mites (*T. evansi*) were obtained in 2002 from a natural infestation of tomato plants in experimental greenhouse conditions. Predators (*C. sanguinea* and *E. connexa*) and aphids (*M. euphorbiae*) were collected from tomato plants cultivated in experimental fields. Each species of predator (*C. sanguinea* and *E. connexa*) was reared separately in the laboratory in transparent plastic tubes (2.0 cm Ø x 10 cm high) containing a piece of wet cotton wool as water source, and they were fed with both diets (*M. euphorbiae* and *T. evansi*) on tomato leaves. The eggs deposited on the walls of the tubes were collected daily and transferred to Petri dishes (5.0 cm Ø x 1.5 cm high), where they were kept until eclosion of larvae. Subsequently, larvae were kept individually in Petri dishes and fed with *M. euphorbiae* and *T. evansi* until adulthood. Cultures were kept inside a climate box at 25 ± 1ºC; relative humidity 70 ±10%, and 12 hours light.

Tomato seeds (*Lycopersicon esculentum* Mill variety Santa Clara I-5300) were sown in trays in a commercial substrate, composed of vermiculite and organic fertilizer and kept in mite-proof screen cages in a greenhouse. Plants of 21 days old were transplanted to plastic pots (2L) that contained a mixture of soil, bovine manure (3:1) and fertilizer (4-14-8 N-P-K). Tomato plants were further grown in mite-proof screen cages in a greenhouse until they were 45 days old and had at least four completely developed leaves. Herbivores were reared in the greenhouse on tomato plants placed in mite-proof screen cages (1.0 x 1.0 x 0.5 m) with a wooden frame. Tomato plants infested by *T. evansi* were obtained by placing four small infested leaves on each plant, which were placed inside a mite-proof screen cage in a greenhouse. To obtain aphid infested plants, four small tomato leaves infested with *M. euphorbiae* were put on each plant. The plants were incubated in this way for one week, resulting in 300 – 400 adult mites per plant and 150-180 aphids per plant.

Olfactometer experiments

The response of *C. sanguinea* to odour sources was determined in two-choice tests by using a Y-tube olfactometer (Sabelis & van de Baan, 1983; Janssen et al., 1999). The olfactometer consisted of a glass tube in the form of a Y. The base of the tube was connected to an air pump that produced an airflow from the arms of the tube to the base. The airflow through both arms of the Y-tube was measured with hot-wire anemometers, and calibrated with valves between the air outlet of the containers of the odour sources and the arms of the olfactometer. When wind speeds in both arms are equal, the odours form two neatly separated fields in the base of the Y-tube (Sabelis & van de Baan, 1983). Pilot experiments showed that the best wind speed to assess the foraging behaviour of *C. sanguinea* in a Y-tube olfactometer is 0.45 m/s. Therefore, the wind speed in each arm of the Y-tube was calibrated to *c.* 0.45 m/s. Each odour source consisted of three tomato plants either occupied or not by herbivores or heterospecific predators. The odour sources were placed in a tray (l x w x h = 30 x 20 x 8 cm) that was placed inside a second water-containing tray (60 x 39 x 6). A plexiglas container (50 x 36 x 43 cm) was put over the plants so that it rested in the water-containing outer tray. In this way, a water barrier was created to prevent escape of arthropods, and the water served as an air tight seal for the container at the same time. The containers had an air inlet and outlet (diameter 1 cm) at opposing walls. The inlet and outlet were covered with mite-proof gauze.

Adult females of *C. sanguinea* were used in the experiments because this stage is responsible for finding suitable food and ovipositional sites for the development of their offspring (Hemptinne et al., 1992; Kindlmann & Dixon, 1993). Two days-old mated females of *C. sanguinea* were starved for 24 h before the experiments. They were tested individually in the olfactometer by introducing them one at a time on the base of the tube after disconnecting the pump. After the pump was reconnected, the female started moving upwind to the junction of the tube, where she had to choose for one of the two arms. It was observed that each female spent some time walking in circles inside each arm of the tube, perceiving each of the odour sources and making a choice subsquently. Each female was observed from the time that she left the tube until she either reached the end of one arm or for a maximum of 5 min. When the female spent more than 5 min without reaching the end of the arms, it was scored as having made no choice. The percentage of predators that did not make a choice in each replicate was very low (1-2 %), and these predators were not included in the analyses. Each replicate experiment was continued until 20 females had responded to any of the odour sources. After five responses, the Y-tube was cleaned with alcohol (70%) and left to dry for 5 min. Subsequently, the odour sources were switched to the opposite arm of the olfactometer to correct for any unforeseen asymmetry in the experimental set-up.

Three replicate experiments were done using different sets of plants and insects for each experiment. The following comparisons were tested: (i) Clean air vs. tomato plants infested by *T. evansi*; (ii) tomato plants infested by *M. euphorbiae* vs. tomato plants infested by *T. evansi*; (iii) tomato plants infested by *M. euphorbiae* vs. tomato plants infested by *M. euphorbiae* + *E. connexa* (5 adult females per plant); and (iv) tomato plants infested by *T. evansi* vs. tomato plants infested by *M. euphorbiae* + *E. connexa* (5 adult females per plant). Differences in numbers of *C. sanguinea* females choosing the odour sources were tested using a g-test with expected fractions of 0.5 for each odour source. Pooled results were tested with a replicated goodness-of-fit test (Sokal & Rohlf, 1995).

Results

Identification of host plants

In one replicate, *C. sanguinea* females showed a significant preference to odours from plants infested by *T. evansi* when clean air was given as alternative (P = 0.02) (Figure 1A). The other two replicates, showed the same trend, but the preference was not significant (Figure 1A). However, the replicates were not heterogeneous ($G_H = 0.47$, d.f. = 2, P = 0.787) and the pooled results showed a significant preference of *C. sanguinea* towards odours from plants infested with *T. evansi* ($G_P = 9.87$, d.f. = 1, $P < 0.001$, Figure 1A). The same phenomenon was observed in previous experiments, where *C. sanguinea* females were more attracted to odours of tomato plants infested by *M. euphorbiae* when offered clean air as alternative (Sarmento, 2003).

Suitability of patches

Cycloneda sanguinea significantly preferred odours from plants infested with *M. euphorbiae* to plants infested by *T. evansi* (Figure 1B). There were no significant differences between replicates ($G_H = 1.63$, d.f. = 2, P = 0.436). There was significant preference for plants infested by *M. euphorbiae* in all replicates, resulting in a highly significant overall preference of *C. sanguinea* towards odours from those plants ($G_P = 26$, d.f. = 1, $P < 0.0001$, Figure 1B).

When given a choice between odours from plants infested with *M. euphorbiae vs.* plants infested with *M. euphorbiae* plus the heterospecific predator *E. connexa*, *C. sanguinea* preferred odours of plants without *E. connexa* (Figure 1C). The overall results showed a significant preference of *C. sanguinea* toward odours from plants infested with *M. euphorbiae* (G_P = 29.11, d.f. = 1, P < 0. 0001, Figure 1C), and all replicates were similar (G_H = 0.23, d.f. = 2, P = 0.889).

When *C. sanguinea* was offered a choice between odours from plants infested with *T. evansi vs.* plants infested with *M. euphorbiae* plus *E. connexa*, *C. sanguinea* had a clear and significant preference for odours of plants infested with *T. evansi* in all replicates (Figure 1D). Overall, a highly significant preference was found (G_P = 26, d.f. = 1, P < 0.0001, Figure 1D), and a trend that was homogeneous in all three replicates (G_H = 0.85, d.f. = 2, P = 0.651).

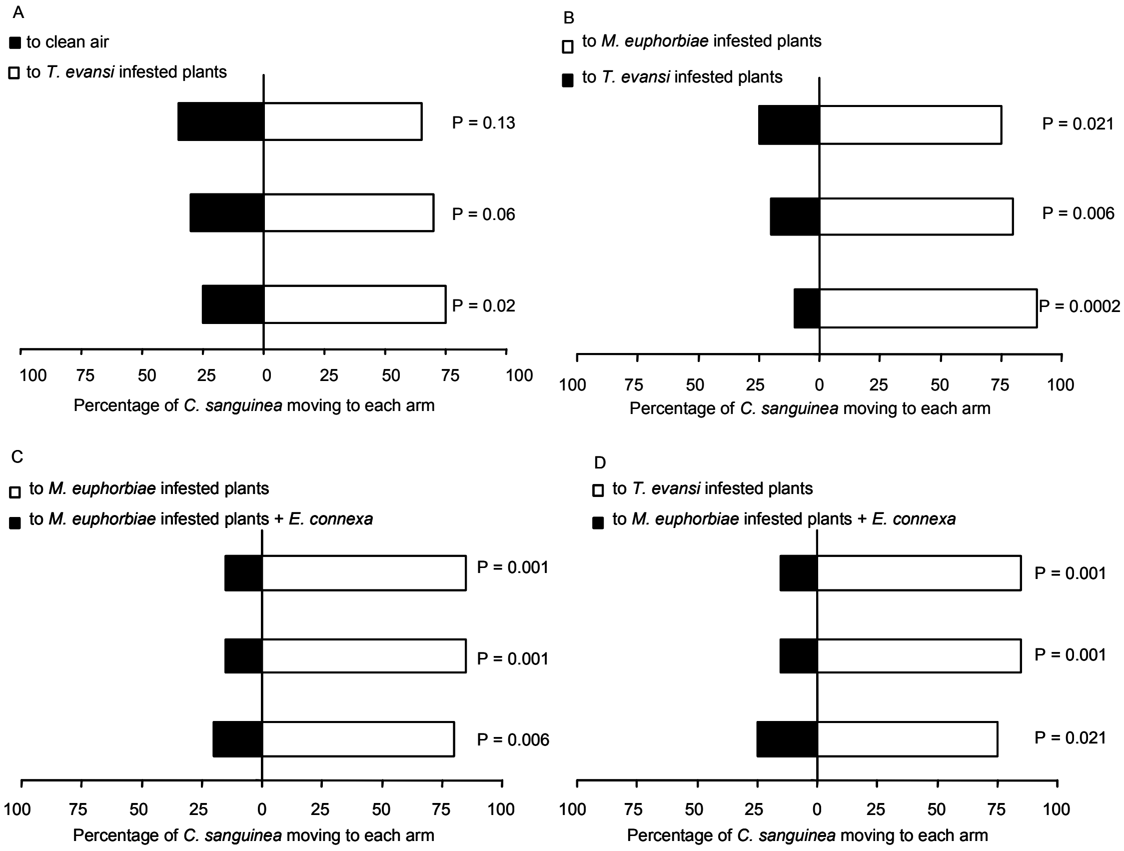

Figure 1. Choice of *Cycloneda sanguinea* females when offered odours of tomato plants infested by *Tetranychus evansi* versus clean air (A); tomato plants infested by *Macrosiphum euphorbiae* versus plants infested by *Tetranychus evansi* (B); tomato plants infested by *Macrosiphum euphorbiae* versus volatiles of tomato plants infested by *Macrosiphum euphorbiae* + *Eriopis connexa* (C) and volatiles of tomato plants infested by *Tetranychus evansi* versus volatiles of tomato plants infested by *Macrosiphum euphorbiae* + *Eriopis connexa* (D). Each bar represents the result of one replicate, in which 20 insects were tested.

Discussion

Our results indicate that *C. sanguinea* can use volatile cues to asses patch quality. Olfactory choice experiments showed that females of *C. sanguinea* were attracted to volatiles of tomato plants that were attacked by *T. evansi*. This is in accordance to some previous results with other predator species that are likely to use volatile cues to localize prey habitats (Tumlinson et al., 1992; Zhu et al., 1999; Al Abassi, 2000). We also show that *C. sanguinea* was capable of discriminating between odours of plants with different prey types and significantly preferred plants infested by its superior prey *M. euphorbiae* to plants infested by its inferior prey *T. evansi*. According to Oliveira et al. (2005), *C. sanguinea* females were not able to complete their biological cycle when fed only on *T. evansi* diet, but successfully completed its biological cycle when fed only on *M. euphorbiae* diet. Experiments carried out with *E. connexa* females showed that the predator had much more reserve metabolites in their fat body cells when fed with *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) than when fed with *T. evansi* mites (Sarmento et al., 2004). Besides the inferior nutritional quality of *T. evansi*, the presence of the web produced by *T. evansi* mites on the plants may diminish predation rate of ladybirds in patches constituted only by these mites (Oliveira et al., 2005, Sarmento et al., 2007).

Cycloneda sanguinea avoided odours emanating from plants infested by *M. euphorbiae* housing the heterospecific predator *E. connexa* and preferred plants with the aphid only. Furthermore, *C. sanguinea* significantly preferred plants infested by the inferior prey *T. evansi* to plants with the superior prey *M. euphorbiae* plus *E. connexa*. Thus, not only food quality orchestrates the assessment of patches by *C. sanguinea*, but also the presence of heterospecific predators. Intraguild predation is a widespread feeding behaviour frequently observed in aphidophagous guilds and may play a role on patch choice of ladybirds (Polis et al., 1989; Yasuda & Ohnuma, 1999; Yasuda et al., 2000; Burgio et al., 2002; De Clercq et al., 2003; Arim & Marquet, 2004).

It is not clear from our experiments the nature of the volatile used by *C. sanguinea* to perceive the presence of *E. connexa* on infested plants. Janssen et al. (1997) showed that the predatory mite *Phytoseiulus persimilis* avoided odours emanating from bean plants infested by its prey, the spider mite *Tetranychus urticae*, plus conspecifics predators. These authors suggested that *P. persimilis* can use volatiles (alarm pheromones) produced by adult preys in response to the presence of conspecific predators, to avoid such places. Results from Zhu et al. (1999) and Al Abassi et al. (2000) suggest that some ladybirds are attracted to (*E*)-β-farnesene, an aphid´s alarm pheromone. So, it is possibly that the presence of *E. connexa* on the plants and its feeding on aphids could have elicited the production of alarm pheromones by the aphids, and *C. sanguinea* may have used such volatiles to avoid plants housing *E. connexa.*

The avoidance of patch occupied by conspecific/heterospecific competitors/predators has been widely observed in many aphidophagous species (Ruzicka, 1994, 1996, 1997, 1998, 2001a). Furthermore, the enemy-free space hypothesis suggests that the presence of natural enemies may be as important (or more important) as food quality in shaping oviposition behaviour (Hodek, 1993; Frechetté et al., 2006). Natural selection could thus have favored the preference for oviposition sites that are free of enemies over those of high food quality. This hypothesis was originally developed to account for oviposition preferences of phytophagous insects, but as Sadeghi & Gilbert (1999) pointed out, selection pressures affecting oviposition in predators and phytophagous insects are likely to be similar. We therefore, favor the possibility that *C. sanguinea* can use volatile cues to assess patch profitability and avoid patches with *E. connexa*, avoiding thus competition for food and/or intraguild predation. However, further experiments at different time scales are underway to investigate this.

Acknowledgments

We thank Dr. André Luiz Matioli (Instituto Biológico de Campinas) for identification of the mites. Dr. Regina Celia Zonta de Carvalho is thanked for identification of the aphid species. CNPq-Brazil and CAPES-Brazil are thanked for financial support. AJ received a scholarship from CAPES-Brazil (PVE), and AP and MV from CNPq-Brazil.

References

Al Abasssi S, Birkett MA, Pettersson J, Pickett, JA, Wadhams, LJ & Woodcock CM (2000) Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. Journal of Chemical Ecology 26: 1765-1771.

Al Abasssi S, Birkett MA, Pettersson J, Pickett, JA, Wadhams, LJ & Woodcock CM (2001) Response of the ladybird parasitoid *Dinocampus coccinelae* to toxic alkaloids from the seven-spot ladybird, *Coccinella septempunctata*. Journal of Chemical Ecology 27: 33-43.

Arim M & Marquet PA (2004) Intraguild predation: a widespread interaction related to species biology. Ecology Letters 7: 557-564.

Barbosa S & França FH (1981) Pragas da batata e seu controle. Informe Agropecuário 76 (7): 55-61.

Burgio G, Santi F & Maini S (2002) On intra-guild predation and cannibalism in *Harmonia axyridis* (Pallas) and *Adalia bipunctata* L. (Coleoptera: Coccinellidae). Biological Control 24: 110-116.

Cardoso JT & Lazzari SMN (2003) Comparative biology of *Cycloneda sanguinea* (Linnaeus, 1763) and *Hippodamia convergens* (Guerin-Meneville, 1842) (Coleoptera, Coccinellidae) focusing on the control of *Cinara* spp. (Hemiptera, Aphididae). Revista Brasileira de Entomologia 47: 443-446.

De Clercq P, Peeters I, Vergauwe G & Thas O (2003) Interaction between *Podisus maculiventris* and *Harmonia axyridis*, two predators used in augmentative biological control in greenhouse crops. Biocontrol 48: 39-55.

Dixon AFG (1959) An experimental study of the searching behaviour of the predatory coccinellid beetle *Adalia decempunctata* (L.). Journal of Animal Ecology 28: 259-281.

Dixon AFG (2000) Insect predator-prey dynamics. Ladybird beetle and biological control. (Ed. by Press, C. U.). Cambridge, UK: Cambridge University.

Doumbia M, Hemptinne JL & Dixon AFG (1998) Assessment of patch quality by ladybirds: role of larval tracks. Oecologia 113: 197-202.

Eichler MR & Reis EM (1976) Seletividade fisiológica de inseticidas aos predadores de afídeos *Cycloneda sanguinea* (L., 1763) e *Eriopis connexa* (Germ., 1824) (Coleoptera: Coccinellidae). 3^{a} ed., Passo Fundo, EMBRAPA/CNPT, 20p.

Flechtmann CHW (1983) Rediscovery and redescription of *Tetranychus armenychus* Graminae (Acari, Prostigmata, Tetranychidae), a spider-mite from Paraguay. Journal of the Georgia Entomological Society 18: 6-8.

Fréchette B, Alauzet C & Hemptinne JL (2003) Oviposition behaviour of the two-spot ladybird beetle *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) on plants with conspecific larval tracks. Arquipélago - Life and Marine Science, Supplement 5: 73-77.

Fréchette B, Dixon AFG, Alauzet C, Boughenou N & Hemptinne JL (2006) Should aphidophagous ladybirds be reluctant to lay eggs in the presence of unsuitable prey? Entomologia Experimentalis et Applicata 118: 121-127.

Gassen DN (1986) Parasitos, patógenos e predadores de insetos associados à cultura de trigo. 1^a ed. EMBRAPA/CNPT, Passo Fundo, BR.

Griffin ML & Yeargan KV (2002a) Oviposition site selection by the spotted lady beetle *Coleomegilla maculata* (Coleoptera: Coccinellidae): choices among plant species. Environmental Entomology 31: 107-111.

Griffin ML & Yeargan KV (2002b) Factors potentially affecting oviposition site selection by the lady beetle *Coleomegilla maculate* (Coleoptera: Coccinellidae). Environmental Entomology 31: 112-119.

Grostal P & Dicke M (1999) Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. Behavioral Ecology 10: 422-427.

Grutzmacher AD & Link D (2000) Survey of the entomofauna associated with potato varieties during two crop seasons. Pesquisa Agropecuária Brasileira 35: 653-659.

Gutierrez J, Baumgaertner JU & Summers, CG (1984) Multitrophic models of predator-prey energetics. Canadian Entomologist 116: 923–963.

Hemptinne JL, Dixon AFG & J. Coffin. (1992) Attack strategy of ladybird beetles (Coccinellidae): factors shaping their numerical response. Oecologia 90: 238-245.

Hemptinne JL, Dixon AFG, Doucet JL & Petersen JE (1993) Optimal foraging by hoverflies (Diptera: Syrphidae) and ladybirds (Coleoptera: Coccinellidae): mechanisms. European Journal of Entomology 90: 451-455.

Hemptinne JL & Dixon AFG (2000) Defence, oviposition and sex: semiochemicals parsimony in two species of ladybird beetles (Coleoptera, Coccinellidae)? A short review. European Journal of Entomology 97: 443-447.

Hemptinne JL , Lognay G, Doumbia M & Dixon AFG (2001) Chemical nature and persistence of the oviposition deterring pheromone in the tracks of the larvae of the two spot ladybird, *Adalia bipunctata* (Coleoptera : Coccinellidae) Chemoecology 11: 43-47.

Hodek I. (1993) Habitat and food specificity in aphidophagous predators. Biocontrol Science and Technology 3: 91-100.

Hohmann CL (1989) Levantamento dos artrópodos associados à cultura da batata no município de Irati, Paraná. Anais da Sociedade Entomológica do Brasil 18: 53-60.

Isikber AA & Copland MJW (2002) Effects of various aphid foods on *Cycloneda sanguinea*. Entomologia Experimentalis et Applicata 102: 93-97.

Janssen A, Bruin J, Jacobs G, Schraag R & Sabelis MW (1997) Predators use volatiles to avoid prey patches with conspecifics. Journal of Animal Ecology 66: 223-232.

Janssen A, Pallini A, Venzon M. & Sabelis MW (1999) Absence of odour-mediated avoidance of heterospecific competitors by the predatory mite *Phytoseiulus persimilis*. Entomologia Experimentalis et Applicata 92: 73-82.

Kindlmann P & Dixon AFG (1993) Optimal foraging in ladybird beetles (Coleoptera, Coccinellidae) and its consequences for their use in biological-control. European Journal of Entomology 90: 443-450.

Li C, Roitberg, BD & Mackauer, M (1997) Effect of contact kairomone and experience on initial giving-up time. Entomologia Experimentalis et Applicata 84: 101-104.

Magalhães S, Tudorache C, Montserrat M, van Maanen R, Sabelis MW & Janssen A (2005) Diet of intraguild predators affects antipredator behavior in intraguild prey. Behavioral Ecology 16: 364-370.

Maluf WR, Campos GA & Cardoso MD (2001) Relationships between trichome types and spider mite (*Tetranychus evansi*) repellence in tomatoes with respect to foliar zingiberene contents. Euphytica 121: 73-80.

Mangel M (1987) Oviposition site selection and clutch size in insects. Journal of Mathematical Biology 25: 1-22.

Mangel M (1989) Evolution of host selection in parasitoids: does the state of the parasitoid matter? American Naturalist 133: 688-705.

Ninkovic V, Al Abassi S & Pettersson J (2001) The influence of aphid-induced plant volatiles on ladybird beetle searching behavior. Biological Control 21: 191-195.

Ninkovic V & Pettersson J (2003) Searching behaviour of the sevenspotted ladybird, *Coccinella septempunctata* – effects of plant-plant odour interaction. Oikos 100: 65-70.

Obata S (1986) Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). Entomophaga 31: 303-311.

Obata S (1997) The influence of aphids on the behaviour of adults of the ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). Entomophaga 42: 103-106.

Oliveira EE, Oliveira CL, Sarmento RA., Rezende LM & Fadini MAM (2005) Biological aspects of the predator *Cycloneda sanguinea* Linnaeus, 1763 (Coleoptera: Coccinellidae) fed with *Tetranychus evansi* Baker & Pritchard, 1960 (Acari: Tetranychidae) and *Macrosiphum euphorbiae* Thomas, 1878 (Homoptera: Aphididae). Bioscience Journal 21: 33-39.

Pettersson J, Ninkovic V, Glinwood R, Birkett MA & Pickett JA (2005) Foraging in a complex environment - semiochemicals support searching behaviour of the seven spot ladybird. European Journal of Entomology 102: 365-370.

Polis GA, Myers CA & Holt RD (1989) The ecology and evolution of intraguild predation - potential competitors that eat each other. Annual Review of Ecology and Systematics 20: 297-330.

Resende ALS, Silva EE, Silva VB, Ribeiro RLD, Guerra JGM & Aguiar-Menezes EL (2006) Primeiro registro de *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphididae) e sua associação com insetos predadores, parasitóides e formigas em couve (Cruciferae) no Brasil. Neotropical Entomology 35 (4): 551-555.

Rieger JF, Binckley CA & Resetarits WJ (2004) Larval performance and oviposition site preference along a predation gradient. Ecology 85: 2094-2099.

Ruzicka Z (1994) Oviposition-deterring pheromone in *Chrysopa oculata* (Neuroptera: Chrysopidae). European Journal of Entomology 91: 361-370.

Ruzicka Z (1996) Oviposition-deterring pheromone in Chrysopidae (Neuroptera): Intra- and interspecific effects. European Journal of Entomology 93: 161-166.

Ruzicka Z (1998) Further evidence of oviposition-deterring allomone in chrysopids (Neuroptera: Chrysopidae). European Journal of Entomology 95: 35-39.

Ruzicka Z & Havelka J (1998) Effects of oviposition-deterring pheromone and allomones on *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). European Journal of Entomology 95: 211-216.

Ruzicka Z (2001a) Oviposition responses of a aphidophagous coccinellids to tracks of a ladybird (Coleoptera: Coccinellidae) and lacewing (Neuroptera: Chrysopidae) larvae. European Journal of Entomology 98: 183-188.

Ruzicka Z (2001b) Responses of chrysopids (Neuroptera) to larval tracks of aphidophagous coccinellids (Coleoptera). European Journal of Entomology 98: 283-285.

Ruzicka Z (2003) Perception of oviposition-deterring larval tracks in aphidophagous coccinellids *Cycloneda limbifer* and *Ceratomegilla undecimnotata* (Coleoptera: Coccinellidae). European Journal of Entomology 100: 345-350.

Ruzicka Z (2006) Oviposition-deterring effects and heterospecifics larval tracks on *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). European journal of Entomology 103: 757-763.

Sabelis MW & Van de Baan HE (1983) Location of distant spider-mite colonies by phytoseiid predators - Demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. Entomologia Experimentalis et Applicata 33: 303-314.

Sadeghi H & Gilbert F (1999) Individual variation in oviposition preference, and its interaction with larval performance in an insect predator. Oecologia 118: 405-411.

Sarmento RA (2003) Resposta comportamental de artrópodes em teia alimentar de tomateiro. Dissertação de mestrado, Universidade Federal de Viçosa, Viçosa, 61p.

Sarmento RA, Oliveira HG, Holtz AM, Silva, SM, Serrão, JE & Pallini A (2004) Fat body morphology of *Eriopis connexa* (Coleoptera, Coccinelidae) in function of two alimentary sources. Brazilian Archives of Biology and Technology 47: 407-411.

Sarmento RA, Pallini A, Venzon M, Souza OFF, Molina-Rugama AJ & Oliveira CL (2007) Functional response of the predator *Eriopis connexa* (Coleoptera: Coccinellidae) to different prey types. Brazilian Archives of Biology and Technology 50: 121-126.

Sengonca C & Liu B (1994) Responses of the different instar predator, *Coccinella septempunctata* L (Coleoptera, Coccinellidae), to the kairomones produced by the prey and non-prey insects as well as the predator itself. Journal of Plant Diseases and Protection 101: 173-177.

Sokal RR & Rohlf FJ (1995) Biometry: The principles and practice of statistics in biological research. New York: Freeman, USA.

Stephens DW & Krebs JR (1986) Foraging theory. Princeton University Press, Princeton, USA.

Tschanz B, Schmid E & Bacher S (2005) Host plant exposure determines larval vulnerability - do prey females know? Functional Ecology 19: 391-395.

Tumlinson JH, Turlings TCJ & Lewis WJ (1992) The semiochemical complexes that mediate insect parasitoid foraging. Agricultural Zoology Reviews 5: 221-252.

Vandenberg N & Gordon RD (1988) The coccinellidae (Coleoptera) of south america, part I. A revision of the genus *Erythroneda Timberlake*, 1943. Revista Brasileira de Entomologia 32: 31-43.

Yasuda H & Ohnuma N (1999) Effect of cannibalism and predation on the larval performance of two ladybird beetles. Entomologia Experimentalis at Applicata 93: 63-67.

Yasuda H, Takagi T & Kogi K (2000) Effect of conspecific and heterospecific larval tracks on the oviposition behaviour of the predatory ladybird, *Harmonia axyridis* (Coleoptera: Coccinellidae). European Journal of Entomology 97: 551-553.

Zhu J, Cossé AA, Obrycki JJ, Boo KS & Baker TC (1999) Olfactory reactions of the twelve-spotted lady beetle, *Coleomegilla maculata* and the green lacewing, *Chrysoperla carnea* to semiochemicals released from their prey and host plant: electroantennogram and behavioural responses. Journal of Chemical Ecology 25: 1165-1177.