HAMILTON GOMES DE OLIVEIRA

HOW TO REALIZE THE POTENTIAL OF THE PREDATORY MITE PHYTOSEIULUS MACROPILIS TO CONTROL TWO-SPOTTED SPIDER-MITES IN STRAWBERRY?

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".

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Prof. Eraldo Rodrigues de Lima (Co-orientador)	Prof. Raul N.C. Guedes (Co-orientador)
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À minha esposa Cristina

À minha mãe Sebastiana

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BIOGRAFIA

Hamilton Oliveira, formado em Agronomia pela Universidade Federal de Viçosa no ano de 2000. Nesse mesmo ano iniciou o curso de Especialização no controle de pragas florestais sob a orientação do professor José Cola Zanuncio. Em 2001 iniciou o mestrado nessa instituição sob a orientação do Professor Angelo Pallini. Sob a orientação desse mesmo professor foi realizada o curso de Doutorado, cuja tese é aqui apresentada.

SUMÁRIO

RESUMO

OLIVEIRA, Hamilton Gomes de, D.Sc., Universidade Federal de Viçosa, novembro de 2006. **Como desenvolver o potencial do acaro predador *Phytoseiulus macropilis* para controlar o ácaro-rajado em plantas de morango?** Orientador: Angelo Pallini Filho. Co-Orientadores: Madelaine Venzon, Raul Narciso .C. Guedes, Eraldo Rodrigues de Lima e Jose Cola Zanuncio.

Controle biológico através de predadores que ocorrem naturalmente em suas regiões de origem é visto como uma promissora alternativa ao controle químico de pragas. No Brasil, estudos de campo demonstraram que o ácaro predador nativo, *Phytoseiulus macropilis* Banks é constantemente encontrado associado à populações do ácaro-rajado *Tetranychus urticae* Koch em plantações de morango no estado de Minas Gerais. A ocorrência desse predador é maior sobre plantas abandonadas do que sobre plantas ainda exploradas comercialmente e, consequentemente pulverizadas com acaricidas. Nessa tese foram avaliados os seguintes pontos: i) a capacidade de *P. macropilis* em controlar populações locais do ácaro-rajado *Tetranychus urticae,* ii) os efeitos letais e subletais dos acaricidas abamectin e dimetoato sobre o fitófago *T. urticae* e *P. macropilis* e iv) através de experimento de olfatometria os efeitos dos acaricidas sobre a capacidade de *P. macropilis* em detectar voláteis de plantas de morango infestadas com *T. urticae.* Os resultados mostraram que o ácaro predador *P. macropilis* é capaz de controlar populações locais de *T. urticae* e que se alimentando desse herbívoro o predador apresenta altas taxas de crescimento populacional. Mas, foi observado que a aplicação dos acaricidas abamectin e dimetoato é mais prejudicial ao predador que ao ácaro praga e, predadores que sobrevivem à aplicação de acaricidas apresentam baixas taxas de crescimento populacional em relação ao ácaro-rajado

quando expostos às mesmas doses de acaricidas. Além disso, esses predadores se tornam incapazes de detectar voláteis de plantas infestadas com *T. urticae*.

ABSTRACT

OLIVEIRA, Hamilton Gomes de, D.Sc., Universidade Federal de Viçosa, november, 2006. **How to realize the potential of the predatory mite *Phytoseiulus macropilis* to control two-spotted spider-mites in strawberry?** Adviser: Angelo Pallini Filho. Co-Advisers: Madelaine Venzon, Raul Narciso .C. Guedes, Eraldo Rodrigues de Lima e Jose Cola Zanuncio.

Biological control using naturally occurring predators has been attempted. In Brazil, field studies showed that the indigenous predatory mite *Phytoseiulus macropilis* (Banks) is constantly found associated with spider mites in strawberry crops in the Minas Gerais state. This predatory mite occurrence is high on abandoned strawberry fields, but on plants that are yet commercially explored and are sprayed with acaricides the occurrence of *P. macropilis* is less frequent. So, spider mite control is done through acaricides application in the area studied. The most applied acaricides in the area are abamectin and dimethoate. In this thesis it was studied the capacity of *P. macropilis* to be used as biological control agent against *T. urticae* in strawberry fields. It was evaluated i) the capacity of *P. macropilis* to control local population of *T. urticae*, ii) the lethal and sublethal effects of the acaricides abamectin and dimethoate on *T. urticae* and iii) studied by use of olfactory experiment, acaricides effects on the capacity of the *P. macropilis* to detect volatiles from strawberry infested plants with *T. urticae*. The results showed that *P. macropilis* is able to control *T. urticae* population and its reproduction and growth rates are high. However, Acaricides application is very harmful to *P. macropilis*. Predatory mite that survived the acaricide application have lower population growth rate than *T. urticae* when exposed to the same acaricide dose. Besides, surviving predators became unable to detect volatiles from infested plants with *T. urticae*.

CHAPTER 1: INTRODUCTION AND GENERAL DISCUSSION

Spider mites are common pest on many plants. Injury is caused as they feed, bruising the cells with their small whiplike mouthparts and ingesting the sap (Helle and Sabelis, 1985). Damaged areas typically appear marked with many small, light flecks, giving the plant a somewhat speckled appearance (Greco, 2005).

The dispersal ability of *T. urticae* in field is an important factor to be considered on pest control. Hussey and Parr (1963) indicated that the two spotted spider mites dispersed in the following ways: migration of newly emerged (presumably mated) females to oviposition sites; dispersal from infested plants, simply by dropping off; and movements over soil surface in accordance with the plane of polarized light.

There is direct evidence that the mites are able to suspend themselves on silken threads and thus be carried along by air currents (Kennedy and Smitley, 1985). Mites can also be dispersed on the clothing of greenhouse personnel or through the movement of infested plant material. Despite the dispersal ability of the mites, it is not uncommon to find infestations in one portion of the field throughout the season, and perhaps even from season to season. Patchy infestations in the field are characteristic of the two spotted spider mites. These population characteristics make the spider mite control difficult to be managed (Zemek and Nachman, 1999).

Chemical control is the most used control for spider mite. Prophylactic pesticide applications are used widely in commercial fields to manage spider mites. However, increasing concerns about the pesticides impact on the environment and human safety have made pesticides less desirable and in some cases have resulted in governmental regulation that prohibit the use of certain pesticides (Stark and Banks, 2003). In addition, few acaricides of different chemical classes are available and a spraying sequence with one active ingredient bears the risk of fast resistance development (Sechser et al., 2002). Thus, there is the need of implementation of other forms for spider mite control. Biological control of this herbivore using predatory mite is the main alternative to chemical pesticides.

Predatory mites are the most important biological control agents of spider mite affecting many crops in different parts of the world (Helle and Sabelis, 1985). In some agroecosystems these natural enemies may drive spider mite numbers below economically damaging levels (Nirop et al., 1998). Infested plants with *T. urticae* produce volatiles that are used by the predator in both long range and local foraging (Sabelis & Dicke, 1985). At long range, volatiles may attract a predator to the source or cause a predator to search more intensively around a prey-infested plant (Janssen, 1999). Once on a plant, these same plant volatiles may be important in local arrestment (Sabelis et al., 1984; Sabelis & Afman, 1994).

Phytoseiulus persimilis Athias-Henriot (Acari-Phytoseiidae) is the predatory mite most used against spider mite and normally this predator is mass reared in laboratory and then released in the field. However, biological control using naturally occurring predators has been attempted (Waite, 1987; García-Marí and González-Zamora, 1999). In Brazil, field studies showed that the indigenous predaceous mite *Phytoseiulus macropilis* (Banks) is constantly found associated with spider mites in strawberry crops in the Minas Gerais state, Brazil (Fadini et al., 2004). *P. macropilis* life cycle is well known (Prasad, 1967) and it is shorter than its prey, the spider mite *T. urticae* (Fadini et al., 2004) (Figure 1). This predatory mite occurrence is high on abandoned strawberry fields, but on the plants that are yet commercially explored and that are sprayed with acaricides the occurrence of *P. macropilis* is less frequent. So, spider mite control is done through acaricides application in the area studied. The most applied acaricides in the area are abamectin and dimethoate. Although, studies are done concerning sublethal effects of pesticides on population growth rate of other beneficial organisms (Stark and Banks, 2003, Teodoro et al., 2005), little is known about the susceptibility of *P. macropilis* to these acaricides.

In this thesis, it was studied if spider mite local population can be controlled by the predator mite *P. macropilis*. In chapter 2, the capacity of *P. macropilis* to control local population of *T. urticae* was investigated. This trait was accessed through the predation and oviposition rates of the predators when fed on all prey stages. *P. macropilis* had high rates of predation and oviposition when fed on spider mite and showed capacity to control local population of this pest in five days.

In chapter 3, was studied the lethal and sublethal effects of the acaricides abamectin and dimethoate on *T. urticae* and *P. macropilis.* In this chapter, sublethal effects were accessed through growth rate population study of both mite species. Acaricides caused lethal and sublethal effects on *T. urticae* and *P. macropilis*. However, *P. macropilis* was more affected by acaricides application.

In chapter 4, by using olfactory experiment, acaricides effects on the capacity of the *P. macropilis* to detect volatiles from strawberry infested plants with *T. urticae* was studied. *P. macropilis* that was exposed to abamectin and dimethoate was able to survive, but was unable to discern between volatiles that were from infested and non infested plants.

Concluding, the predatory mite *P. macropilis* is able to control *T. urticae* population. Its reproduction and growth rates are higher than *Phytoseiulus persimilis* Banks (Acari: Phytoseiidae), which is the most used predatory mite on biological control programs worldwide. However, acaricides application is the source of the negative effects on *P. macropilis*. Predators that survived the acaricide application have lower population growth rate than *T. urticae* when exposed to the same acaricide dose. Besides, surviving predators became unable to detect volatiles from infested plants with *T. urticae*.

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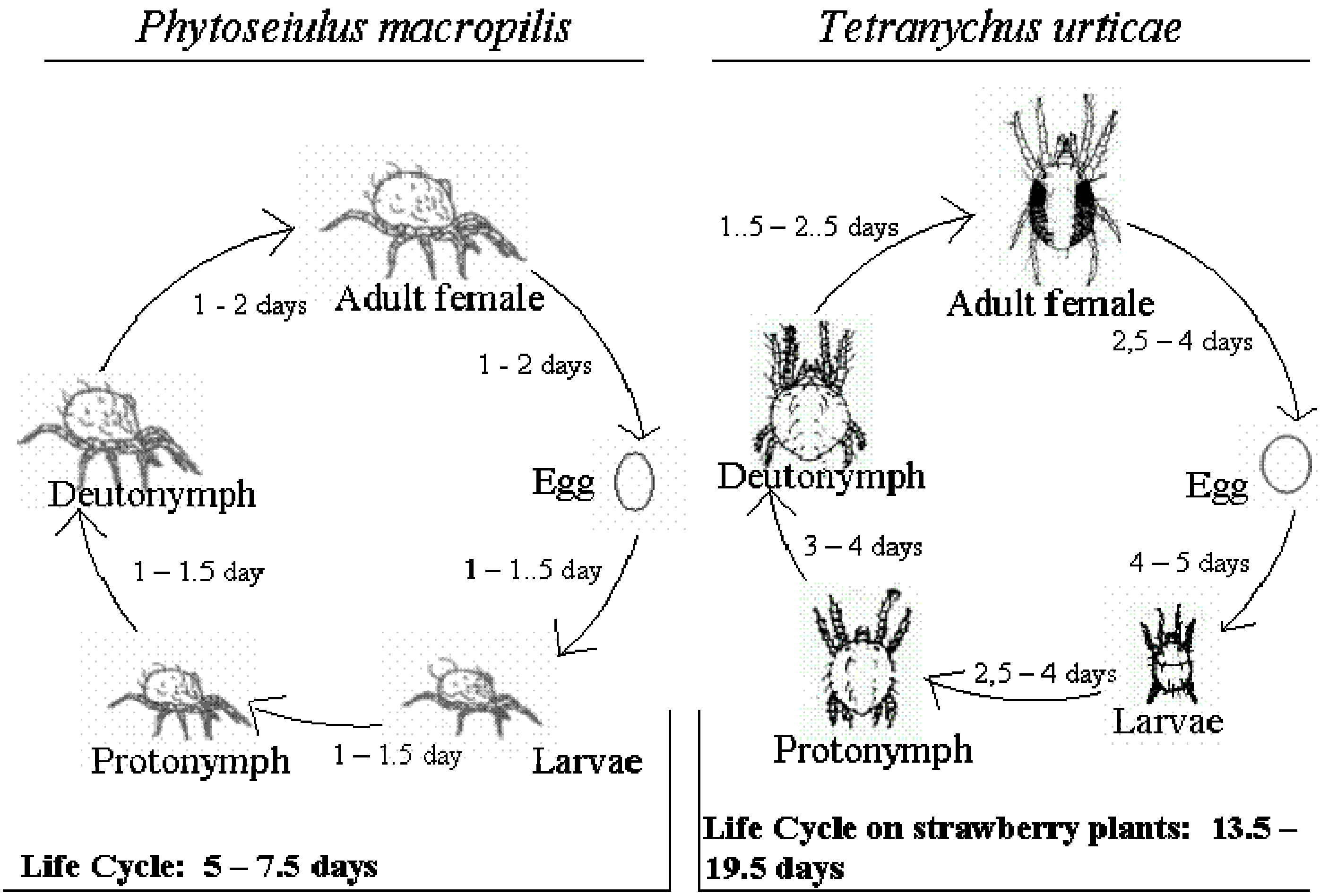
Figure Legend

Figure 1: Life cycle of predatory mite *P. macropilis* and of *Tetranychus urticae* on Strawberry plants.

Figure :1


Phytoseiulus macropilis
Adult female
1 - 2 days
1 - 2 days
Egg
1 – 1..5 day
Larvae
1 – 1.5 day
Protonymph
1 – 1.5 day
Deutonymph
Life Cycle: 5 – 7.5 days
Tetranychus urticae
Adult female
2,5 – 4 days
Egg
4 – 5 days
Larvae
2,5 – 4 days
Protonymph
3 – 4 days
Deutonymph
1..5 – 2..5 days
Life Cycle on strawberry plants: 13.5 – 19.5 days

CHAPTER 2: A PHYTOSEIID PREDATOR FROM THE TROPICS AS POTENTIAL BIOLOGICAL CONTROL AGENT FOR THE SPIDER MITE *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE)"

Abstract

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pests of many crops in temperate and tropical climates. Chemical control is the main method of combating this mite, but it is less preferred due to the development of resistance as well as environmental problems associated with the use of pesticides. Biological control of spider mites has been tried as a logical alternative method for chemical control. Studies have been conducted in different countries to assess the effect and potential of natural enemies for controlling the pest. The predatory mite *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) was found associated with spider mites in strawberry crops in the state of Minas Gerais, Brazil. Earlier studies suggested that *P. macropilis* was able to control spider mites, but predation and oviposition rates have not been measured so far. We measured predation and oviposition on strawberry with spider mites as prey in the laboratory. The predator fed on all prey stages and showed capacity to control local prey populations on leaf disks. The oviposition rate of *P. macropilis* is similar of the most used predatory mite *Phytoseiulus persimilis* and the predation rate is higher than that of the latter. Our results suggest that *P. macropilis* is a promising candidate to control two-spotted spider mites in the tropics and other areas.

Key words: *Phytoseiulus macropilis*, predatory mites, local prey population, strawberry plants, prey stages

1. Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pests of many crops in temperate and tropical climates (Skirvin and Williams; 1999; García-Marí and Gozález-Zamora, 1999). Spider mites can colonize plants shortly after crops have been planted and damaging outbreaks usually occur later during the growing season (Badii al., 2004). The number of spider mites can increase up 40% per day. This exponential population growth usually ends abruptly due to overexploitation of the host plant (Krips et al., 1998).

Chemical control is the main method of combating spider mites. Due to the excessive use of pesticides and the associated problems of pesticide resistance and environmental pollution, there is an increasing demand for sustainable, environmental-friendly control methods. Biological control of spider mites has been tried as an alternative method to chemical control. Several species of natural enemies have been reported to prey on spider mites and studies have been conducted in different countries to assess the potential of natural enemies for controlling the pest without the use of pesticides and without economic damage to the crop (Opit et al., 2005).

Predatory mites from the family Phytoseiidae have been used to suppress *T. urticae* populations (Escudero and Ferragut, 2005). One species of phytoseiid mite that is widely used with success and is commercially available is *Phytoseiulus persimilis* Athias-Henriot. However, in Mediterranean countries, *P. persimilis* has been less successful, possibly because it is not well adapted to prevailing climatic conditions (Bakker et al., 1993). Skirvin and Fenlon (2003) reported that temperatures above 25 °C may be detrimental to the predatory efficiency of this species. Therefore, the lack of successful control of *T.*

urticae in warmer areas has resulted in a search for predators that are adapted to such climatic conditions (Prasad, 1967; Shih et al., 1979).

The predatory mite *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) was first found in Florida and it is the most common predatory mite in this region (Saba, 1974). It was also found in Europe and tropical areas in Africa and South America (Moraes et al., 2004, Rosa et al., 2005). In Brazil, *P. macropilis* was found associated with spider mites in strawberry crops in the state of Minas Gerais (Fadini et al., 2004) where the annual average temperature is about 25°C. The reproductive biology of *P. macropilis* when fed on *T. urticae* mites at various temperatures has already been studied. Its net reproductive rate was highest at 28°C and the intrinsic rate of increase also peaked at 28°C (Ali, 1998). However, the efficacy of *P. macropilis* to control populations of *T. urticae* has never been tested. We evaluated the oviposition and predation rates on each stage of *T. urticae*, as well as the capacity of the predator to control small prey populations on strawberry leaves.

2. Materials and methods

2.1 General procedures

Strawberry plants were grown in 500 ml pots in a greenhouse (13-38 °C, 40-60 RH and 13:12 L:D). Part of these plants was used to rear *T. urticae*. Adults of *T. urticae* were transferred from these plants through detached strawberry leaves and then placed on moistened cotton pads on top of a sponge (3.0 cm thick) in a plastic box (15 x 25 x 5 cm) that was used as rearing unit. Water was added to the rearing units when necessary to keep the cotton moist. To rear predators, arenas with *T. urticae* were taken from the culture and a predator was released on this arena. Cultures were kept inside a climate box at 25 ± 1°C, 60

± 5% RH and at 13:11 light: dark cycle, corresponding to the conditions in the region where mites were collected. When leaves were deteriorating, they were taken from the rearing unit and placed on top of a new arena to allow the mites to move onto the new arena. Arenas were examined once per day. When over-population of the predators was observed, predators were transferred to new arenas infested with prey.

2.2 Predation rates and reproduction on different prey stages

The predation and oviposition rate of *P. macropilis* were measured on a diet consisting of either *T. urticae* eggs, larvae, protonymphs, deutonymphs or adult female prey. In order to obtain prey eggs, strawberry leaf discs (Ø = 2 cm) were infested with 45-50 adult females for 24 h. Subsequently, females were removed and the eggs were counted. We did not manipulate egg densities because this would also damage the web produced by the spider mites. The other stages were collected from a rearing unit and placed on clean strawberry leaf discs with a fine brush. A single gravid female of *P. macropilis* was added immediately after releasing the prey. Hence, in the experiments with juvenile and adult stages there was almost no spider-mite web on the leaf disc. Fifteen replicates were done for each stage, and the densities per arena were 98.60 ± 7.40 eggs, 78.00 ± 5.29 larvae, 72.53 ± 3.54 protonymphs, 77.13 ± 3.09 deutonymphs and 46.47 ± 0.84 adult females. These numbers are high enough to ensure sufficient food for the predator (Oliveira, pers. obs). As a control for natural prey mortality, five arenas per stage with the same mean number of prey were incubated without predator. The number of consumed and alive prey as well as survival and oviposition of the predators was recorded every day during three days. Because predation and oviposition rates on the first day are affected by the previous food source (Nomikou et al., 2001), we analyzed data only from the second and third day. Daily oviposition and predation rates were analyzed using ANOVA followed by posthoc Tukey tests (α = 0.05) when necessary.

2.2 Time needed to control a local prey population

In order to test the capacity of *P. macropilis* to control local prey populations in presence of web produced by prey, strawberry leaf discs (Ø = 2 cm) were infested with 50 adult female of *T. urticae*, which were allowed to oviposit for 24h. Subsequently, the number of adults and eggs was assessed. One gravid female predator was released on each leaf disc, and all stages of prey and predators were counted daily. The experiment was terminated when numbers of prey eggs were too low (14.7 eggs per arena) to sustain the predators. The control consisted of five arenas infested with 50 adult female prey without predators. Data were analyzed using ANOVA followed by post hoc Tukey tests α = 0.05 when necessary.

3. Results

3.1 Predation rate and reproduction on different prey stages

Predators were able to feed on all prey stages (Figure 1). Predation was higher on eggs than on the later stages (F = 221.98; df = 4; p<0.00001) (Figure 1). Because the number of prey offered largely exceeded the maximum predation rate observed, it is reasonable to assume that predators were not limited by food and that the results reflect the maximum predation rates.

Predators oviposited when feeding on each prey stage, however, oviposition rates varied with the prey stage offered (F = 14.5; df = 4; p<0.001) (Figure 2). The highest oviposition rate was observed when predators fed on eggs and on adult females and eggs (Figure 2).

3.2 Time needed to control a local prey population

The time needed for a predator to control a local prey population was determined until the prey numbers remaining on the arenas were too low to feed the predator. The numbers of adult female prey and eggs reached such low levels in five days (Figure 3 and 4), whereas the numbers of predators increased (Figure 5), showing that the predators were capable of controlling small local prey populations.

4. Discussion

Adult females of the predatory mite *P. macropilis* fed on all stages of the spider mite *T. urticae*, but predation was higher on eggs than on the other stages. The differences in predation of the various stages could be due to differences in the ability of spider mites to escape and because of the preference of predators. The predation rate on spider mite eggs was about 40 eggs per day, which is much higher than that of the widely used control agent *P. persimilis*. Ashihara et al. (1978) reported that *P. persimilis* fed on 28.1 spider mite eggs per day.

Predators oviposited when feeding on each prey stage, but the highest oviposition rate of *P. macropilis* was observed when predators fed on eggs and on adult females more eggs. The oviposition rate is similar to that of *P. persimilis* (M.W. Sabelis & A. Janssen, unpubl. data). Probably, the high oviposition rate shown by *P. macropilis* when feeding on adult females was partly caused by predators feeding on eggs produced by the adult female prey.

The capacity of *P. macropilis* to control spider mites was confirmed by the finding that predators controlled small prey populations on leaf disks after as little as five days. The populations on these discs reflected the natural structure of the prey population; spider mites are usually concentrated in a relatively small area of a plant, and deposit eggs close to were they feed. Most offspring do not move very far from where they hatch, dispersing only far enough to find undamaged leaf tissue near the parent cluster (Kondo and Takafuji, 1985; Mayland et al., 2000), resulting in small prey colonies with high densities.

An important characteristic of *P. macropilis* is that it is well adapted to warm climates and may be more efficient in controlling *T. urticae* in Mediterranean areas than are the currently available natural enemies (Bakker et al., 1993; Escudero and Ferragut, 2005). Moreover, it can probably also be used in greenhouses, where temperatures can easily exceed 25°C, particularly in the summer months. In contrast, the predation rate of the commonly used control agent *P. persimilis* declines at temperatures above 25 °C (Skirvin and Fenlon, 2003).

If *P. macropilis* can suppress *T. urticae* populations, the question remains why this pest reaches high numbers in crops in Brazil, such as strawberry, tomatoes, cucumbers and roses, where the predator does occur. This may be because of the frequency of pesticide applications, including acaricides, which will kill large numbers of the predators, whereas spider mites may develop resistance (Oliveira et al. in prep.). If this is the case, a reduction of pesticide application would probably result in increased control of the spider mite pest.

Summarizing, the predatory mite *P. macropilis* is a promising potential biological control agent of the two-spotted spider mite, especially in warmer areas. Other studies suggested that *P. macropilis* was able to control spider mites (Prasad, 1967; Shih et al., 1979; Ali, 1998; Rosa et al., 2005) however, none of these studies measured predation and oviposition rates as showed here. It remains to be investigated how this predatory mite controls groups of local spider mite populations (metapopulations).

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Figure legends.

Figure 1: Average (±1 SE) predation rate of *P. macropilis* females on different stages of the prey *T. urticae* under laboratory conditions. Different characters above the bars indicate significant differences in predation rate ($p < 0.05$, Tukey HSD test).

Figure 2: Average (±1 SE) oviposition rate of *P. macropilis* females on different stages of the prey *T. urticae* under laboratory conditions. Different characters above the bars indicate significant differences in oviposition rate ($p < 0.05$, Tukey HSD test).

Figure 3: Average numbers of *T. urticae* females as function of time on leaf discs without *P. macropilis* (open circles) or with *P. macropilis* (open triangles). Data shown are means ± 1 SE. Different characters above the symbols indicate significant differences in numbers of adult females with and without predators ($p < 0.05$ by Tukey HSD test).

Figure 4: Average numbers of *T. urticae* eggs as function of time on leaf discs without *P. macropilis* (open circle) and with *P. macropilis* (open triangle). Data shown are means ± 1 SE. Different characters above the symbols indicate significant differences in numbers of adult females with and without predators ($p < 0.05$ by Tukey HSD test).

Figure 5: Average numbers of mobile stages of *P. macropilis* as function of time on leaf discs with adult females and eggs of *T. urticae* as food.

Figure 1:

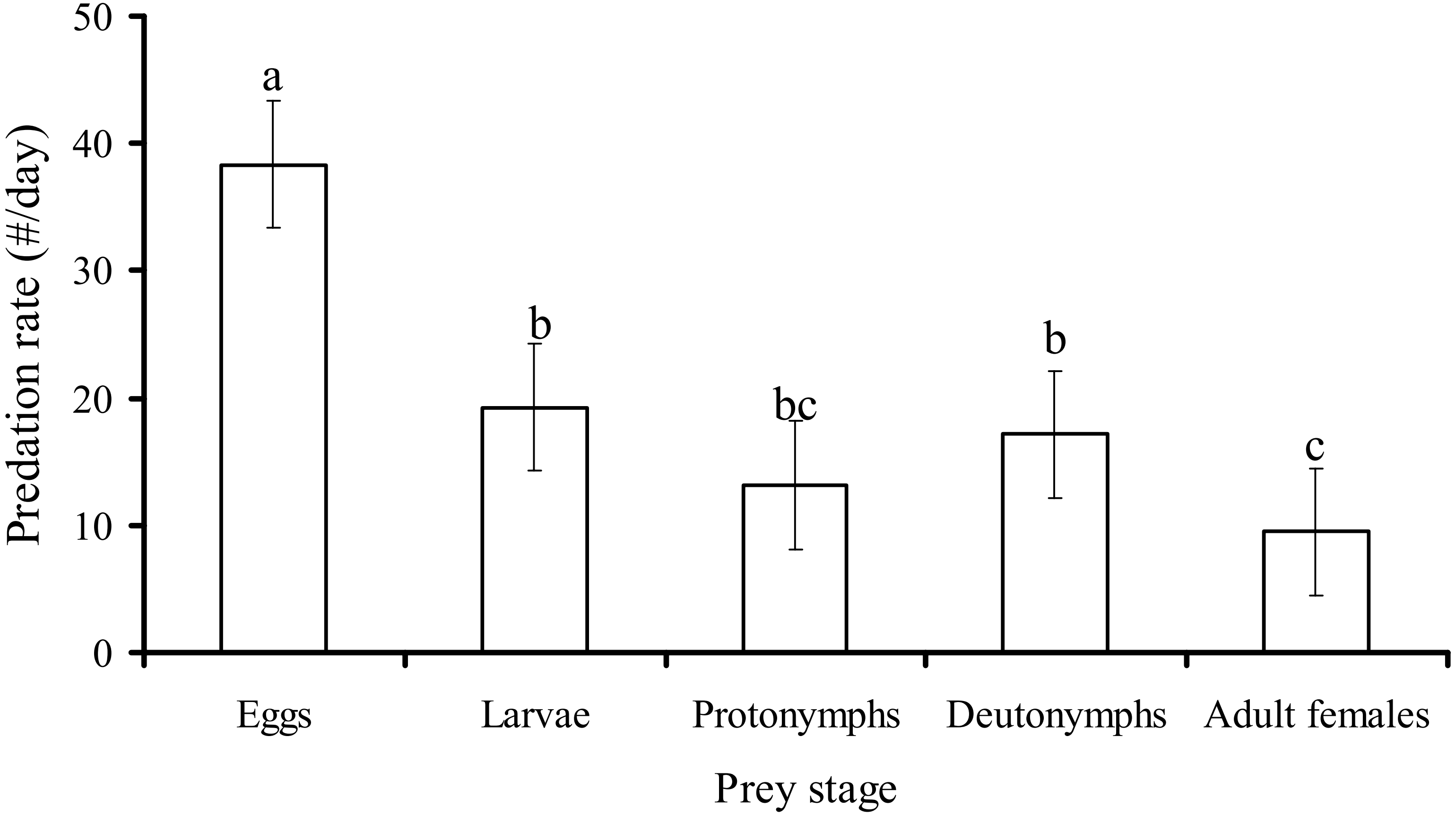

50
40
30
20
10
0
Predation rate (#/day)
a
b
bc
b
c
Eggs
Larvae
Protonymphs
Deutonymphs
Adult females
Prey stage

Figure 2:

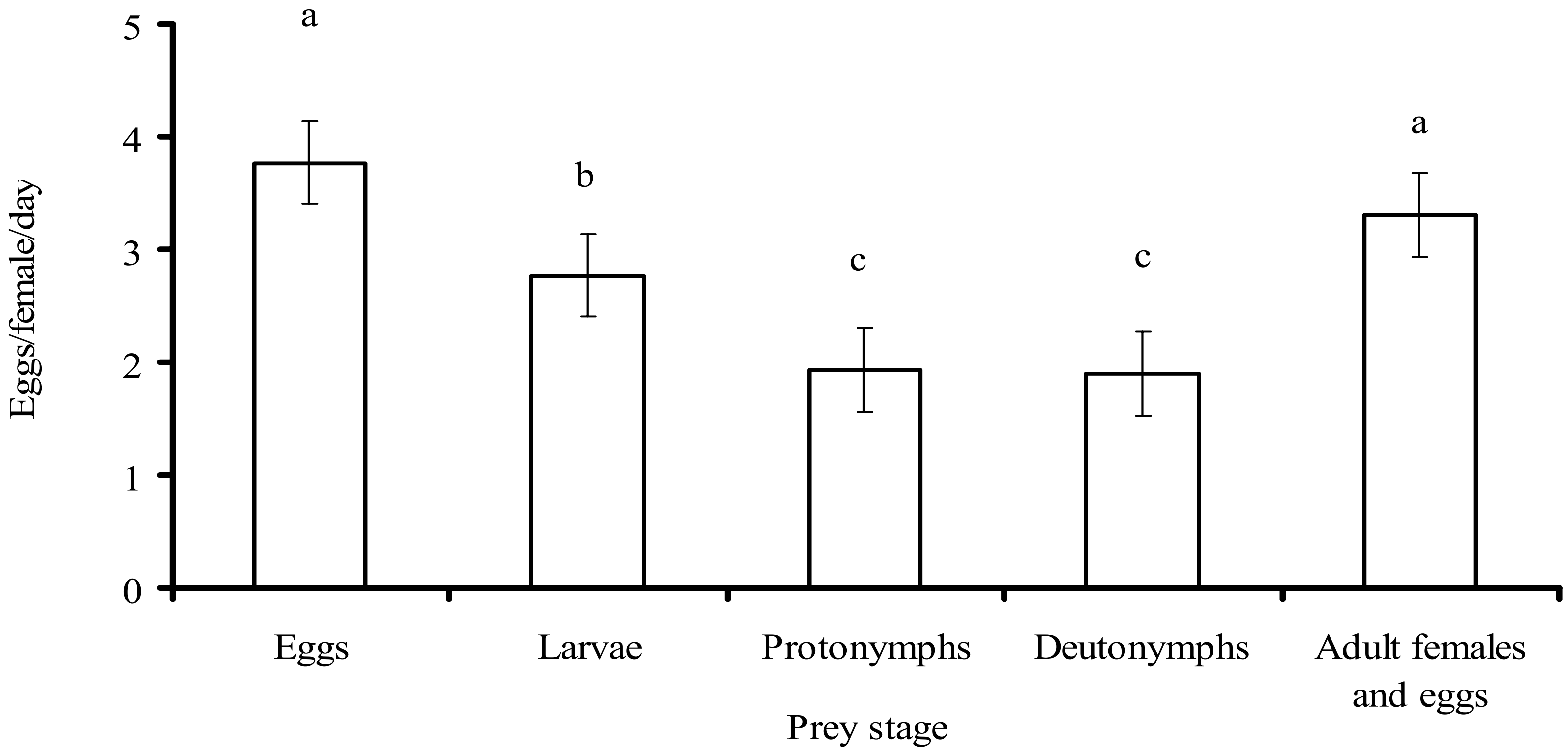
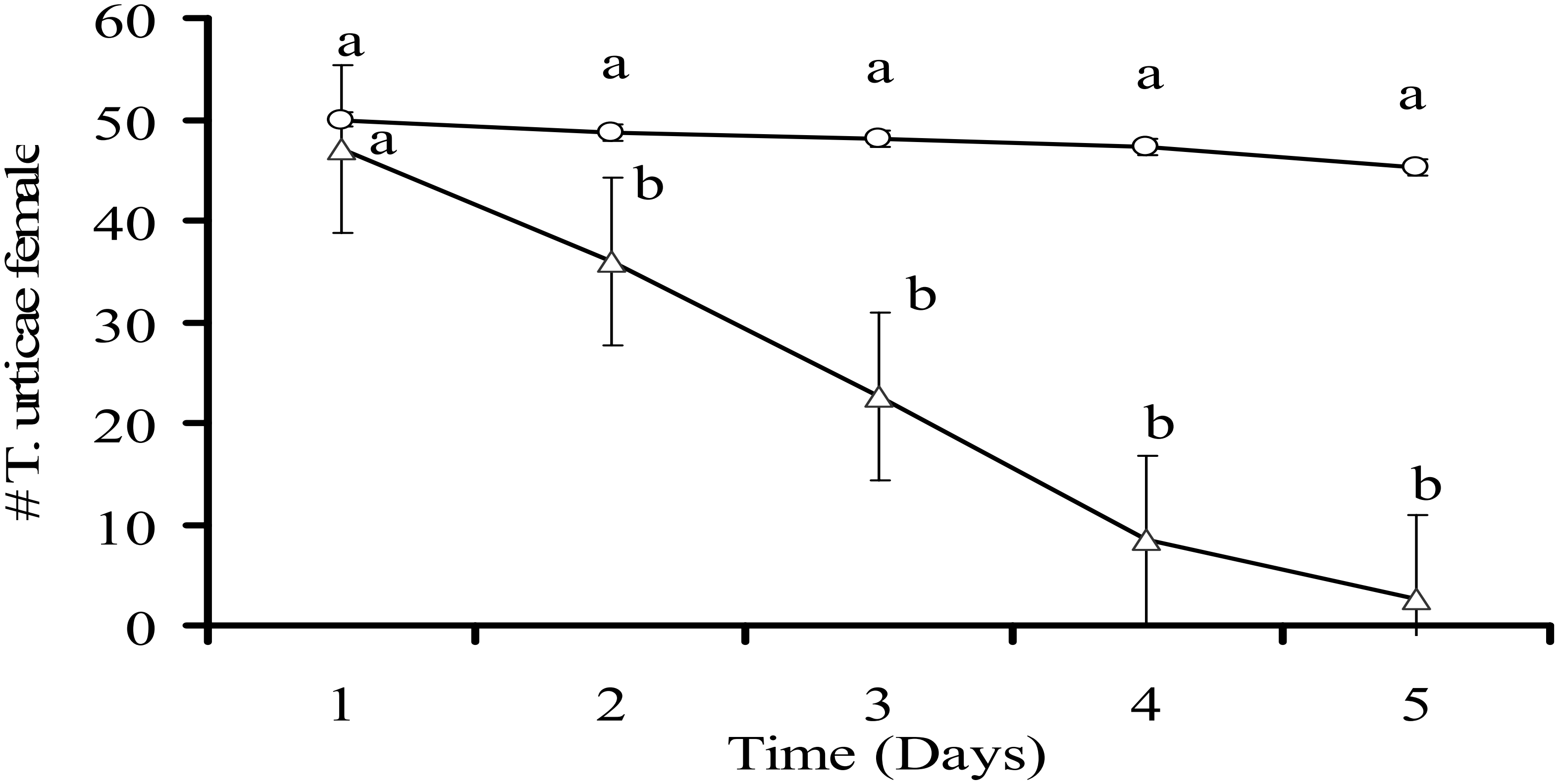
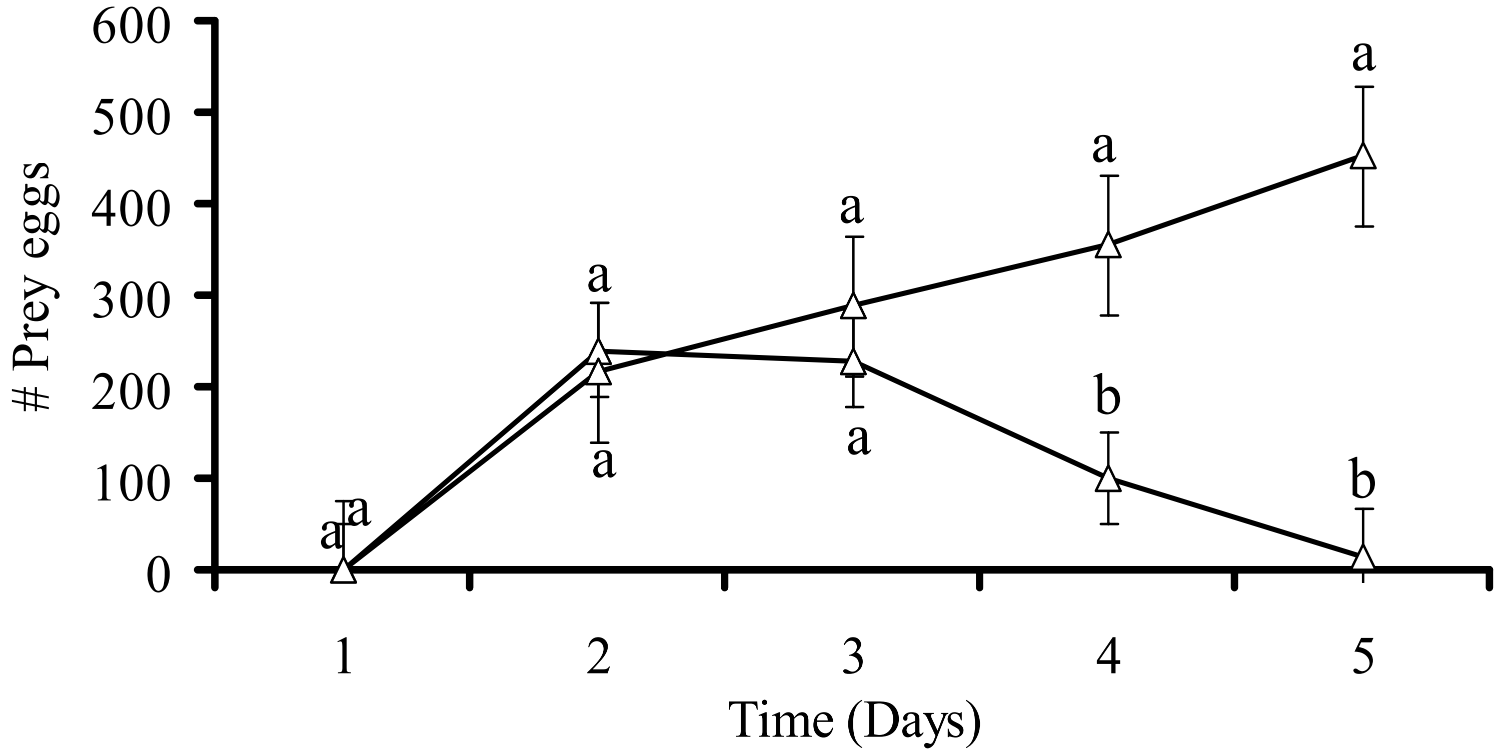

Eggs/female/day
0
1
2
3
4
5
a
b
c
c
a
Eggs
Larvae
Protonymphs
Deutonymphs
Adult females and eggs
Prey stage

Figure 3:



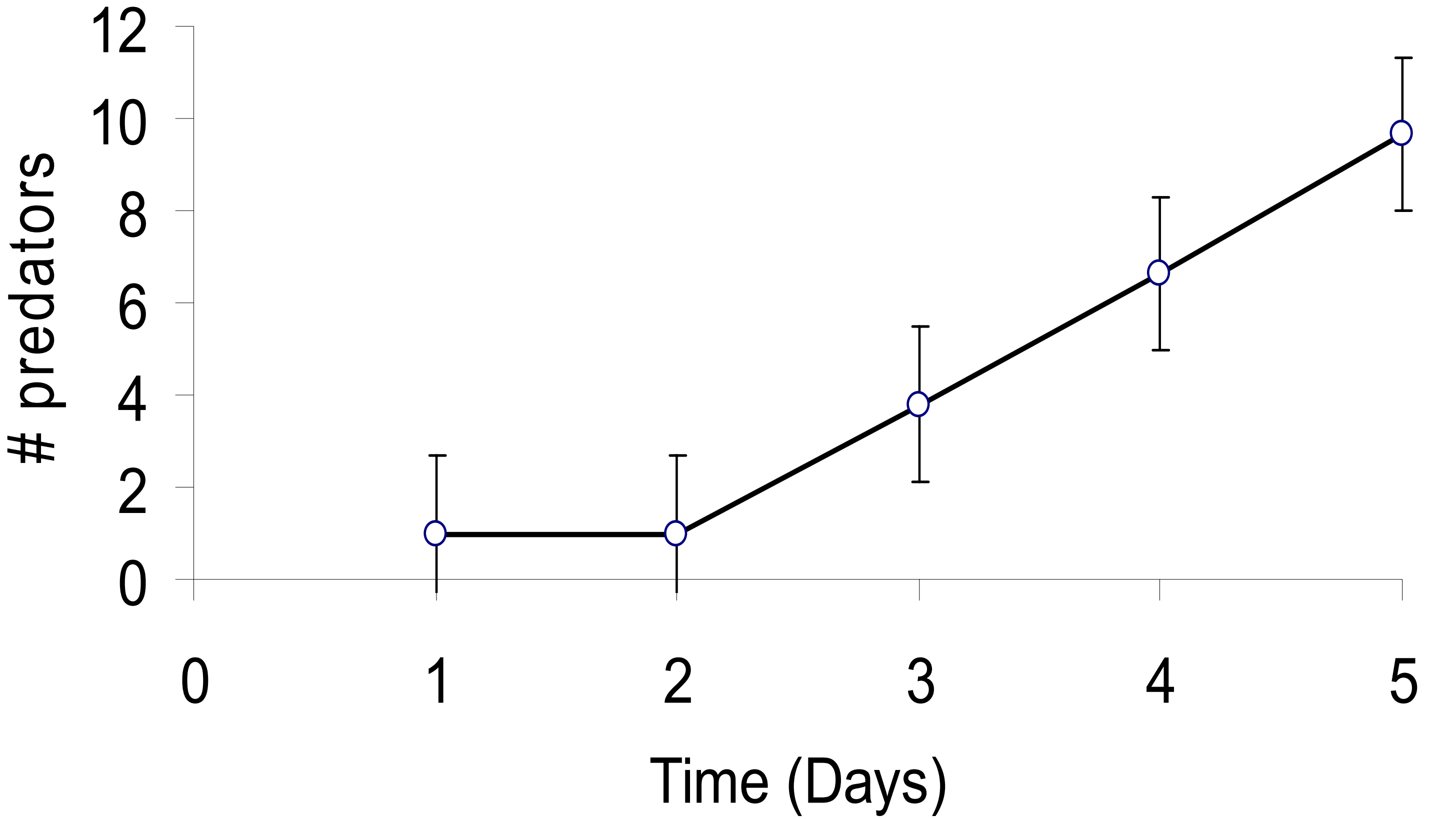
60
50
40
30
20
10
0
T. urticae female
a
a
a
a
a
a
b
b
b
b
1
2
3
4
5
Time (Days)

Figure 4:



600
500
400
300
200
100
0
Prey eggs
1
2
3
4
5
Time (Days)
a
a
a
a
a
a
a
a
a
b
b

Figure: 5



CHAPTER: 3 ABAMECTIN AND DIMETHOATE ARE MORE HARMFUL TO THE PREDATOR *PHYTOSEIULUS MACROPILIS* THAN TO ITS PREY *TETRANYCHUS URTICAE*

Abstract

Recent studies in laboratory showed that the predatory mite *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae) is efficient in to control spider mite *Tetranychus urticae* (Acari: Phytoseiidae, Tetranychidae) population. However, in Brazilian strawberry field, one of the various cultures when the predatory mite was found, spider mite is the major pest and it control is done by acaricides application. In contradiction, splayed plants are continually damaged by spider mite and predatory presence is low on those plants. After production season, plants are no more splayed with acaricides and predatory mite is found in high number on those and all spider mite population is controlled. Thus, we formulated the hypotheses that acaricide application is more harmful for predatory mite that for the pest spider mite. Acaricides more used on strawberry plants in Brazil are abamectin and dimethoate. We addressed the lethal and sublethal effects by determining the relative toxicity of the acaricides abamectin and dimethoate to the predaceous mite *P. macropilis* and to its prey, the spider mite *T. urticae* (Acari: Phytoseiidae, Tetranychidae). The spider mite was 2.58x and 4.21x more tolerant than the predator to abamectin and dimethoate, respectively. The instantaneous rate of increase of spider mites and predatory mites linearly decreased with increasing concentrations of both acaricides. However, the instantaneous rate of increase of the predator decreased more rapidly with increasing acaricide concentrations than that of the pest, resulting in extinction of predator populations while the pest persisted. Because *P. macropolis* is a promising candidate for biological control of spider mites, farmers may achieve better control by not spraying, but managing the populations of this predator.

Key words: Pesticides, biological control, Integrated Pest Management, lethal and sublethal doses.

Introduction

The phytophagous spider mite *Tetranychus urticae* Koch has a broad host-plant range and is a serious pest of various crops worldwide as is described by various studies (Easterbrook et al., 2001; Sato et al., 2002; Kishimoto, 2002; Fadini et al., 2004,). Strawberry plants are often heavily infested and suffer severe damage by *T. urticae* (Sato et al., 2001). The mite mainly feeds on leaves; usually on the underside, but can also cause direct economic damage when feed on fruits, flowers and on tips of shoots (Lourenção et al., 2000). The typical symptoms of spider mite feeding are small, light colored punctures (Alford, 1972).

Prophylactic pesticide applications are widely used in commercial greenhouses to control spider mites on strawberry plants (Easterbrook et al., 2001). However, increasing concerns about the impact of pesticides on the environment and human safety have made pesticides less desirable and resulted in more restrictive governmental regulation regarding their use (Opit et al., 2001). Besides, economical damage levels of spider mites have been reported even when acaricides were applied (Wilson et al., 1991). Thus, there is a need to implement other, more sustainable methods for spider mite control. Biological control with predatory mites is the main alternative to chemical pesticides (Shrewsbury and Hardin, 2003).

Several species of natural enemies have been reported to prey on *T. urticae*, and studies have been conducted in different countries to assess the potential of natural enemies for controlling the pest without the use of pesticides and without economic damage to the crop. Two approaches have been developed for using biological control. These are the mass release of *Phytoseiulus persimilis* (Acari: Phytoseiidae) and other predatory mites (Oatman *et al*., 1968; Simmonds, 1971; Benuzzi and Nicoli, 1991) and the increase of native populations of predators. The first option has been the most common strategy, but biocontrol of spider mites by native predators has also been attempted, mainly with phytoseiids (Fournier *et al*., 1985; Waite, 1988, García-Marí and Gonzáles-Zamora, 1999).

In field surveys, the native predaceous mite *Phytoseiulus macropilis* (Banks) was found associated with spider mites in strawberry crops in the State of Minas Gerais, Brazil (Fadini et al., 2004). Probably *P. macropilis* that are found on strawberry plants is originated in local vegetation that there is near strawberry fields. Laboratory and greenhouse experiment showed that *P. macropilis* has high predation rate on *T. urticae* and can control local population of this pest in few days (Oliveira et al., unpubl. data).

Despite the presence of this predatory mite in strawberry fields, spider mites are predominantly controlled through chemical pesticides. Two acaricides, abamectin and dimethoate, are frequently used in Brazilian strawberry fields (Sato et al., 2002). Applications of these acaricides are timed to coincide with the appearance of spider mite after its presence is noted by farmer observation and follows a calendar base. At this same time, *P. macropilis* is present in strawberry fields. Thus, an overlap occurs between the acaricide applications against spider mites and the presence of *P. macropilis.* However, nothing is known about the susceptibility of *P. macropilis* to these acaricides. We noticed that acaricides splaying causes a contradiction because splayed plants are continually damaged by spider mite and predatory presence turns low or absent on those plants. After production season, plants remained in the field and are no more splayed with acaricides. In this time predatory mite is found in high number on those plants and all spider mite population is controlled. Thus, we formulated the hypotheses that acaricides application are more harmful for predatory mite that for the pest spider mite. The study was done by determination of the lethal and sublethal effects of abamectin and dimethoate on *P. macropilis* and its prey *T. urticae*.

Material and methods

Adult phytophagous and predatory mites from strawberry field in south state of Minas Gerais, Brazil were brought for the lab. Phytophagous were reared on arenas made of strawberry leaves that were put on moistened cotton pads on top of a sponge (3.0 cm thick) in a plastic box (15 cm x 25 cm x 5 cm). Predatory mites were reared on these spider mites arenas. Arenas with predators and with phytophagous mites were kept in separate climate boxes at 25 ± 1°C, 60 ± 5% RH and 13 h of light. Water was added to the rearing units when necessary to keep the cotton moist. Arenas were examined every day and when a high population of predators was observed, the predators were transferred to new arenas infested with the pest.

Concentration–response bioassays

Concentration–response bioassays were carried out for both acaricides using adult female predatory mites (4–5 days old), or *T. urticae* (14-15 days old) at the beginning of their reproductive period. The acaricides were sprayed on strawberry leaf disks (3.0 cm diameter) using a Potter tower. Spraying was carried out at 0.34 bar (= 3.44/104 kPa) with a 2.5 ml spray aliquot in accordance with recommendations of the International Organization for Biological Control of Noxious Animals and Plants (Hassan et al. 1994).

The sprayed leaf disks and acetone-sprayed controls were air-dried for 1 h. Subsequently, five adult female predators or 10 adult female spider mites were placed on each disk. Five replicates were used per acaricide concentration for each mite species. During the experiment, other strawberry leaf disks where sprayed with the same acaricide concentration and with spider mites were incubated on this disc. These mites were used for feeding the predators whenever necessary during the experiment, in order to better reflect field conditions because in splayed crops, predatory mite will prey only splayed spider mite. Were maintained 200 or more prey per arena per day as food for predatory mite.

Treated leaf discs with mites were maintained at 25 ± 2 ^{0}C, 60 ± 10% R.H. and 13 h light in a climate box. The concentrations used in the bioassays were selected after preliminary tests with a broad concentration range, allowing selection of lower and upper limits of the concentration that causes minimum and maximum mite mortality for each acaricide and mite species. Technical grade acaricides (> 84% pure) were obtained from BASF and CHEMINOVA Chemical Companies. Acaricides were dissolved in acetone. A control treatment in which only acetone was sprayed was used to correct the data for mortality that was not caused by application of acaricides. Mite mortality was assessed after 72 h exposure and mites were considered dead if they did not move after disturbing them with a fine brush (Stark et al. 1997). Concentration–mortality curves were estimated using probit analysis (SAS Institute 1997). For each acaricide, the index of differential selectivity was obtained by dividing the LC_{50} of spider mites by the LC_{50} of predatory mites, and confidence limits (95%) were calculated following Robertson and Preisler (1992).

Demographic bioassays

The instantaneous rate of increase (r_i) was estimated using the equation $r_i=[\ln(N_f/N_o)]/\Delta T$, where N_f is the final number of living mites, N_o is the initial number of living mites and ΔT is the time interval (days) elapsed between the start and the end of the bioassay (Stark et al. 1997). The experimental procedures and acaricides were the same as those used for concentration–mortality bioassays with daily progeny assessments up to seven days following initial exposure. Either five adult female predators or ten adult female spider mites were used per replicate.

The mites were kept in rearing chambers under the same conditions as the concentration–response bioassays. Spider mite females were provided daily as food source for the predatory mites. These spider mites where obtained from strawberry leaf disks sprayed with the same concentration as that of the leaf disks with predatory mites. The acaricide concentrations for the demographic assays were based on the previously obtained concentration–mortality curves. Controls were sprayed with acetone only. Regression analyses were carried out to assess the effect of acaricide concentrations on the instantaneous rate of increase of each mite species.

Results

The spider mite *T. urticae* was more tolerant to both acaricides than its predator *P. macropilis* (Table 1).

The instantaneous population growth rate of *T. urticae* and *P. macropilis* decreased linearly with increasing concentrations of both acaricides (Figures 1 and 2). Spider mite populations that were exposed to concentrations of abamectin around LC_{50} (i.e., 0.31 mg a.i./L) showed positive values of r_i (0.10 ± 0.08 day^{-1}), indicating population growth after seven days of exposure to this acaricide (Figure 1). Extinction of spider mite populations occurred only at concentrations ≥ 0.42 mg a.i./L, close to the LC_{60}. Abamectin showed a more drastic effect on the population growth of the predator, which declined at concentrations as low as 0.27 mg a.i./Ll (r_i = -0.10 ± 0.04 day $^{-1}$) after seven days of exposure (Figure 1). Spider mites had a positive population growth rate at this concentration (r_i = 0.12 ± 0.09 day^{-1}) after seven days of exposure.

Results for dimethoate also showed more drastic effects for predatory mites than for spider mites. Predator extinction occurred at 4.25 mg a.i./L (r_i = -0.013 ± 0.00 day^{-1}) whereas for spider mites, extinction occurred at 6.0 mg a.i./L (r_i = -0.024 ± 0.001 day^{-1}) after seven days of exposure (Figure 2).

Discussion

The spider mite *T. urticae* was more tolerant to the acaricides abamectin and dimethoate than its natural enemy, the predatory mite *P. macropilis*. Contrary results were reported by Kim and Yoo (2002) that showed that the acaricides bifenazate, acequinocyl, chlorfenapyr and fenbutatin oxide were much less toxic to *Phytoseiulus persimilis* Koch adult females than to *T. urticae* adult females. Dekeyser et al. (1996) also reported that the acaricide bifenazate shows high activity on phytophagous mites, such as *Tetranychus*, *Eutetranychus*, *Oligonychus* and *Panonychus* species, whereas it is harmless to predacious mites, such as the phytoseiids *Amblyseius fallacis* Garman, *Galendromus occidentalis* Nesbitt and *Zetzellia mali* Ewing.

In the areas were these mites used in this study where collected, the farmers apply acaricides twice per week. This acaricide application is done for more than 10 years ago. Strawberry culture is cultivated for eight months per year. Under this environment acaricide resistance is likely to occur, as observed elsewhere (Sato et al., 2004). Population of spider mite resistant to dimethoate and abamectin was reported in Brazil on various cultures (Sato et al., 2000, 2002, 2005). Failures in chemical control of spider mites caused by resistance have been reported for various compounds, such as organophosphates, dicofol, organotins, hexythiazox, clofentezine and abamectin, only a few years after the introduction of such products (Stumpf and Nauen 2001). The rapid development of resistance in *T. urticae* is favoured by its high reproductive potential, extremely short life cycle and its arrhenotokous mating system, and it is increased due to the relative isolation of mite populations that normally coupled in the path, the extended growing season and the frequency of spraying (Cranham and Helle, 1985).

Considerable research efforts have been devoted to finding alternative strategies for suppression of *T. urticae* populations (Kim and Yoo, 2001). Phytoseiid predators are the most important biological control agents of phytophagous mites in integrated mite management programs of outdoors and of greenhouse crops (Van Lenteren and Woets, 1988). Until recently, *P. macropilis* was not recognized as a potentially effective biocontrol agent of *T. urticae*, and it was therefore not considered in decisions of acaricide applications. This predator species is found in strawberry fields mainly at the end of the growing season, when acaricides are no longer applied (Fadini et al., 2004). Thus, interruption of acaricide applications is probably the main cause of predator presence at end of the season, and consequently their absence on sprayed plants, as confirmed in the present study.

As an effective biological control agent, predaceous mites must be the center of concern for the development of sustainable integrated management of phytophagous mites and their native occurrence should be incremented (Amano and Haseeb, 2001). In particular, acaricide use is not compatible with management of populations of *P. macropilis* because the recommended field concentration of abamectin is 0.135 mg a.i./L (Andrei, 1999), close to the LC_{55} of the predator, but corresponding to the estimated LC_{25} for spider mites. Spraying would thus cause a small decrease in the population growth rate of spider mites and have a much larger negative effect on predator populations. The recommended field concentration of dimethoate (4.8 g a.i./L) (Andrei, 1999) corresponds to the LC_{60} of spider mites, but did not cause spider mite extinction in our assays, whereas this concentration corresponds to the LC_{90} of *P. macropilis* and led to its extinction in the present study. This shows that the recommended acaricide doses do not exterminate spider mite populations, but cause severe reductions of predatory mite populations.

The lethal and sub-lethal assessments presented here indicate that the use of abamectin and dimethoate at recommended concentrations for the control of spider mites are more harmful for predators than for the pest species. Pest control programs depending on application of broad-spectrum pesticides often produce various detrimental side effects (Kongchuensin and Takafuji, 2006). The use of such acaricides should therefore be avoided where predators are present. Some chemicals, such as these used in this study can reduce control of pests in agroecosystem by destroying natural enemies such as phytoseiids.

Our results support the hypothesis that the capacity of *P. macropilis* to control spider mites is impaired by acaricide applications because these acaricides are more detrimental to the predator than to the spider mites. As *P. macropilis* has high potential to control spider mite populations, we believe that the control of spider mite populations may be improved by abandoning acaricide applications.

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Which predatory mite can control both a dominant mite pest, *Tetranychus urticae*, and a latent mite pest, *Eotetranychus asiaticus*, on strawberry?

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Table 1: Table 1: Toxicity of abamectin and dimethoate to the predatory mite *Phytoseiulus macropilis* and its prey *Tetranychus urticae.*

Acaricide	Mite species	Number of mites tested	LC50 (95% c.i.)[1]	LC95 (95% c.i.)	χ^2	Index of differential selectivity (95% c.i.)
Abamectin	*T. urticae*	850	0.31 (0.27 - 0.38)	5.62 (3.09-11.81)	5.85	2.58 (2.45-2.71)
	P. macropilis	425	0.12 (0.11-0.14)	1.21 (0.83-2.09)	3.07	-
Dimethoate	*T. urticae*	670	3.73 (3.27-4.21)	5.71 (12.49-21.75)	6.8	4.21 (3.82-5.16)
	P. macropilis	375	1.16 (0.96-1.36)	5.28 (9.93-29.62)	12.21	-

In mg a.i./L;

Figure Legends

Figure 1. Instantaneous rate of increase (r_i, day^{-1}) of *Phytoseiulus macropilis* (open dots) (Y = -1.8187x + 0.4414) and *Tetranychus urticae* (open squares) (Y = -0.8597x + 0.3543) exposed to various concentrations of abamectin.

Figure 2. Instantaneous rate of increase (r_i, day^{-1}) of *Phytoseiulus macropilis* (open dots) (Y = -0.0835x + 0.3587) and *Tetranychus urticae* (open squares) (Y = -0.0586x + 0.3643) exposed to various concentrations of dimethoate.

Figure 1:

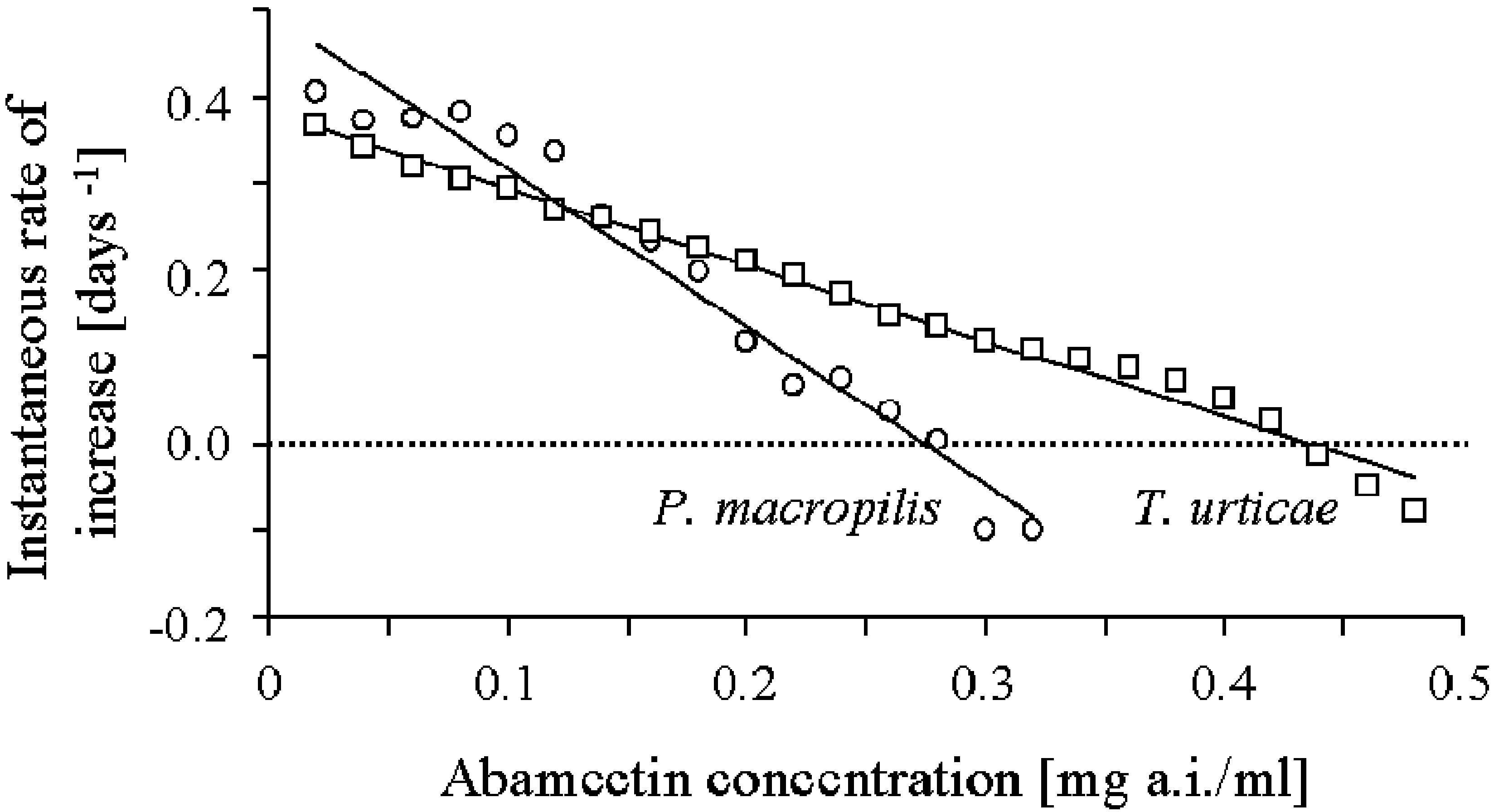
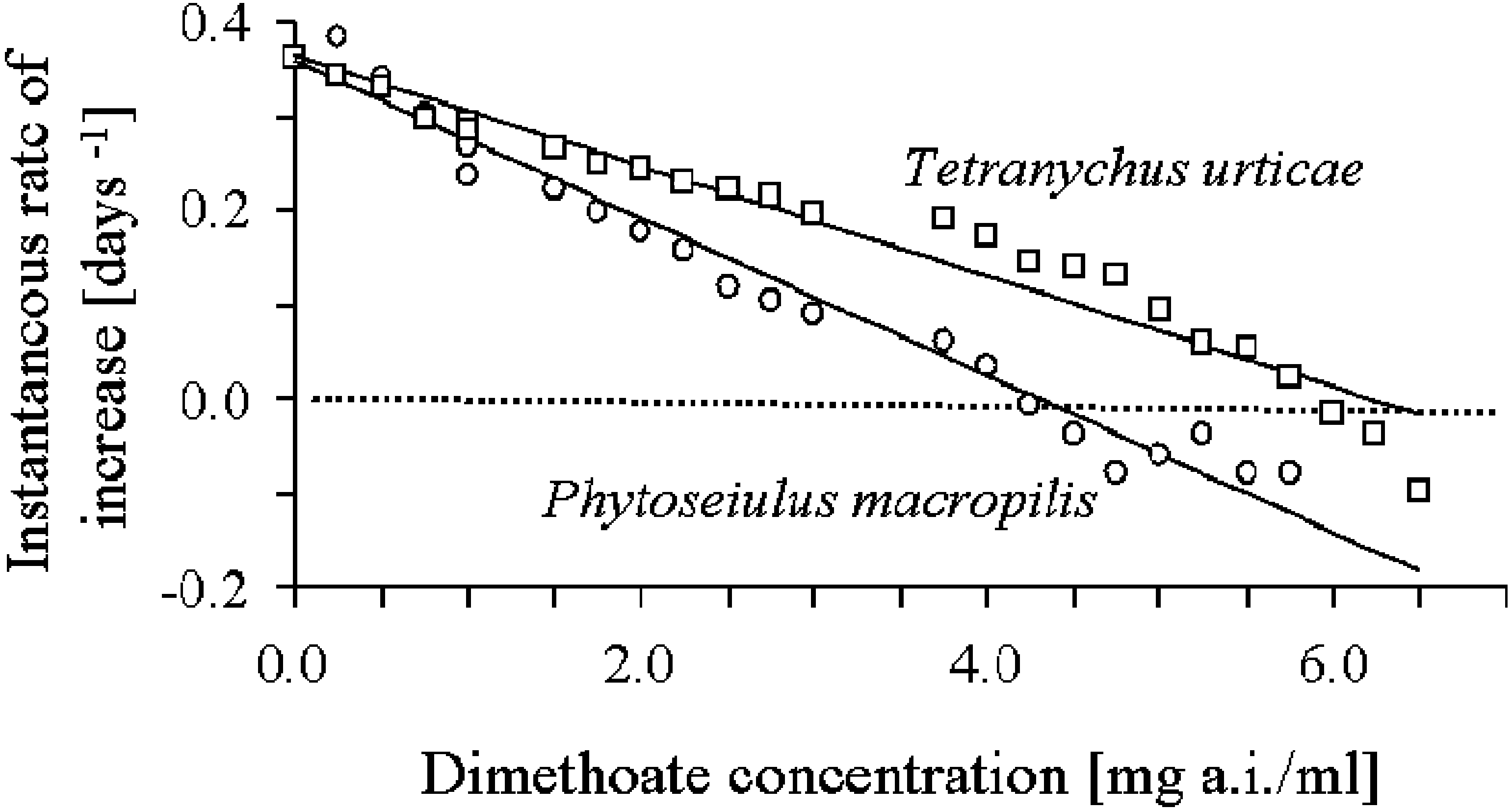

Instantaneous rate of increase [days $^{-1}$]
0.4
0.2
0.0
-0.2
0
0.1
0.2
0.3
0.4
0.5
Abamectin concentration [mg a.i./ml]
P. macropilis
T. urticae

Figure 2:


Instantaneous rate of increase [days -1]
0.4
0.2
0.0
-0.2
0.0
2.0
4.0
6.0
Dimethoate concentration [mg a.i./ml]
Tetranychus urticae
Phytoseiulus macropilis

CHAPTER 4: ACARICIDE IMPEDES THE OLFACTORY RESPONSE OF A PREDATORY MITE TO PLANTS INFESTED WITH ITS PREY

Abstract

It is well-known that natural enemies use plant volatiles that are induced by herbivores to detect plants with their prey. These studies have shown that the response of predators to infested plants can be affected by several factors, such as plant species, plant cultivar, plant developmental stage, herbivore species, and herbivore developmental stage. In this paper we investigated the sublethal effects of the acaricides abamectin and dimethoate on the olfactory response of predatory mites, *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae), to volatiles from strawberry plants infested with spider mites, their prey. Predators that were exposed to residues of abamectin (0.01 and 0.02 mg a.i./ml) and dimethoate (1 and 2 mg a.i. /ml) did not discriminate between odours from plants infested with spider mites and clean plants. Pesticides can be very harmful to beneficial organisms, but usually only the lethal effects are studied. This study shows that sublethal effects of pesticides can be very important for the foraging efficiency of predatory mites.

Key words: *Phytoseiulus macropilis, Tetranychus urticae*, induced defence, sublethal effects, biological control

1 - **Introduction**

Plants produce volatiles in response to herbivore damage (Dicke, 1994), and these volatiles thus provide information about the presence of herbivores (Sabelis and Dicke, 1985; Sabelis et al., 1998). It has been documented that carnivorous arthropods can exploit these volatiles to locate herbivorous prey (Dicke, 1999a,b; Turlings et al. 1990a,b; 1991a,b; Turlings and Tumlinson 1992; Moraes et al., 1998; Kessler and Baldwin 2001). Assuming that there are no more than three trophic levels in the food chain (i.e. plants, herbivores, and predators), emission of infochemicals will benefit plants in two separate ways. First, these volatiles signal to other herbivores that the quality of the plant is reduced because it is already under attack and the defense system of plants has been switched on. Second, they signal to the predators of herbivores that their prey is in the vicinity.

The response of predators and parasitoids to these volatiles can be affected by several factors (Maeda et al., 2000; Maeda and Liu, 2006). For example, the olfactory response of the predatory mite *P. persimilis* to plant volatiles depends upon plant species, plant cultivars, plant developmental stage and herbivore species (Takabayashi et al., 1994) and on predator experience (Drukker et al., 2000). Exposure to pesticides may also change the response of natural enemies to volatiles, and thus impede searching for prey. For example, prey consumption by *Pardosa amentata* was significantly reduced after its exposure to cypermethrin (Shaw et al., 2006). Exposure of coccinellids to insecticide residues on plant surfaces and through the consumption of contaminated prey may lead to mortality, or sub-lethal effects resulting in altered foraging and searching patterns, and thus altered effectiveness of coccinellids as aphid predators (Singh et al., 2001, 2004). The effects of topical and surface exposure to pesticides on carabids are described as decreasing activity (Mauchline et al., 2004) and changing locomotion (Jensen et al., 1997). In honeybees, sublethal doses of insecticides have been shown to disturb the homing-flight (Vandame et al.,

1995) and learning of odours (Abramson et al., 1999; Decourtye and Pham-Delegue, 2002). Orientation to sex pheromone in the pink bollworm moth Pectinophora gossypiella is disturbed by permethrin (Haynes and Baker, 1985). Komeza et al. (2001) demonstrated that the behavioural response of the parasitoid *Leptopilina boulardi* to host chemicals could be disturbed by a low dose of chlorpyrifos. This insecticide has also been shown to alter sex pheromone communication in *Trichogramma* (Delpuech et al., 1998). An increased response to host habitat odour after exposure to chlorpyrifos was also described by Rafalimanana et al. (2002). Deltamethrin at a sublethal dose increased the arrestment behaviour of treated *Trichogramma* males responding to female pheromone (Delpuech et al., 1999). *Trissolcus basalis* females exposed to a low dose of this insecticide reduced their walking speed and the time spent on host-patches (Salerno et al., 2002). As was described above, individuals that survive toxicant exposure may still sustain significant damage, which can be manifested as a reduction in population growth rate and changes in behaviour such as feeding. Thus, pesticides exert subtle as well as overt effects on beneficials organisms and on natural enemies that must be considered when examining their impact.

In this paper, we show that exposure of a natural enemy to sublethal doses of two acaricides impedes their response to odours produced by plants with its prey.

The study system

In Brazilian strawberry fields, the predator mite *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae) is found preying on *Tetranychus urticae* (Fadini et al., 2004), on which it has a high predation rate (Oliveira et al., pers. obs.). Despite the presence of this natural enemy in strawberry fields, spider mites are predominantly controlled through toxic pesticides such as abamectin and dimethoate. Abamectin is part of the avermectins group, which is composed of closely related macrocyclic lactones isolated from the fungus *Streptomyces avermitilis*. The basic structural motif of the avermectins (they are macrocyclic lactones) is evident in the natural product avermectin B_{1a}, which is the principal constituent of the insecticide abamectin. In arthropods poisoned by avermectins, ataxia and paralysis are the major signs of intoxication, with little or no hyperexcitation. The avermectins block electrical activity in nerves and muscles by increasing the membrane conductance to chloride ions and its action is essentially irreversible (Clark et al., 1995). Hence, this acaricide could well affect the response of predatory mites to volatiles.

Dimethoate is an organophosphorous insecticide that is used to control a variety of insect pests on several food crops and is often used as a toxic standard in risk assessment studies of pesticide effects on non-target arthropods in the field (Kennedy et al., 2001). Organophosphates are usually non-persistent in the environment and do not bioaccumulate (Chambers & Levi, 1992). However, they have high acute toxicity and often lack physiological selectivity. This means that there is a high risk of accidental poisoning of natural enemies and non-target organisms.

Experiments show that these acaricides are more harmful to the predatory mites than to the spider mites against which they are applied (Oliveira et al., unpubl. data). In addition to their lethal effect, pesticide residues can alter behaviour through their neurotoxic activity, even at low doses (Pham-Delegue et al., 1990). However, effects of the acaricides abamectin and dimethoate on the olfactory response of predatory mites were never studied. Sublethal effects of the pesticides could possibly further affect the efficiency of the predatory mites through affecting their searching behaviour. In this paper we investigated the sublethal effects of abamectin and dimethoate on the olfactory capacity of *P. macropilis* to detect volatiles from infested strawberry plants with spider mites.

Interactions among plants, spider mites and predatory mites are normally studied using a Y-tube olfactometer (Sabelis and van de Baan, 1983) to detect the olfactory response of predatory mites to induced plant volatiles. For instance, lima bean plants, *Phaseolus lunatus*, infested with the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) emit a blend of volatiles that attract the predatory mite *Phytoseiulus persimilis* (Dicke et al., 1999).

2 - Materials and methods

Rearing methods.

Spider mites were collected from strawberry plants in a commercial strawberry field in Minas Gerais State, Brazil, in June 2005. They were reared on strawberry plants in a climate room (25°C; and 13h of light). Plants were grown in a separate room until they were three months old. Subsequently, some plants were infested with spider mites.

Plants were incubated in this way for seven days. In each experiment, infested and clean plants were of the same age, having four fully developed leaves. The number of spider mites on infested plants ranged from 1400 to over 2000 mites per plant. Predatory mites (*Phytoseiulus macropilis*) from a mass rearing in a greenhouse on lima been plants infested with spider mites were collected with a fine paintbrush and were reared at 25°C on strawberry plants infested with prey in a climate room. New plants with spider mites were added to the predatory mite culture once per week.

Concentration–response bioassays

Concentration–response bioassays were carried out for abamectin and dimethoate using adult female predators at the beginning of their reproductive period (4–5 days old). In order to obtain prey eggs and subsequent stages, strawberry leaf discs (Ø = 3 cm) were infested with 45-50 adult female spider mites for 48 h. Subsequently, the discs with the spider mites and their eggs were sprayed with acaricides dissolved in acetone with a Potter tower (Potter 1952). Acaricide spraying was carried out at 0.34 bar (=3.44/104 kPa) with a 2.5 ml spray aliquot in accordance with the International Organization for Biological Control of Noxious Animals and Plants/West

Paleartic Regional Section (IOBC/WPRS) recommendations (Hassan et al., 1994). This process was repeated every day for each acaricide concentration. Two concentrations for each acaricide were used (0.01 and 0.02 mg/ml for abamectin and 1.0 and 2.0 mg/ml for dimethoate). These concentrations were selected after preliminary tests with broad concentration ranges, allowing the selection of concentrations that had lethal and sublethal effects on *P. macropilis* (Table 1). The sprayed leaf disks plus acetone sprayed controls were let to air dry for 1 h before 10 young adult female predatory mites were placed on them. Discs with predatory mites were subsequently incubated at 25 ± 2°C, 60 ± 10% R.H. and 13 h of light. Mite mortality was assessed every day and surviving predatory mites were tested in an olfactometer seven days after exposure. Mites were scored as dead if they did not move when touched by fine paintbrush. Concentration–mortality curves were estimated by probity analysis (SAS Institute 1997).

Olfactometer experiments

A Y-tube olfactometer was used to study the response of adult female predatory mites to volatiles emanating from strawberry plants infested with spider mites (Sabelis & van de Baan, 1983). It consists of a glass tube in the form of a Y, with a black Y-shaped metal wire in the middle to railroad the mites. The tube base was connected to a pump that produced an airflow from the arms of the tube to the base. Potted strawberry plants were placed in a tray (l x w x h = 50 x 35 x 42 cm) that was put inside another water-containing tray (60 x 38 x 4 cm). Subsequently, a Plexiglas container was put over the plants so that it rested in the outer, water-containing tray. In this way a water barrier was created to prevent spider mites from escaping, furthermore the water served as an airtight seal. The container had an air inlet and outlet (diam. 4 cm) in the opposing walls, covered with mite-proof gauze. A container was connected to each of the two arms of the olfactometer with plastic hose.

A hot-wire anemometer was used to measure wind speeds in both arms of the olfactometer. Wind speeds were calibrated using valves that were inserted into the hoses connecting the containers (one with three clean plants and one with three infested plants) and the arms of the olfactometer and was used to calibrate the incoming air speed in each arm of the olfactometer. When wind speeds in both arms are equal (0.4 m/s), the odours form two neatly separated fields in the base of the Y-tube with the interface coinciding with the metal wire (Sabelis And van de Baan, 1983).

Adult female predatory mites were starved for one hour prior to the experiments. Mites were introduced one at a time by disconnecting the pump and putting one female on the metal wire at the base of the Y-tube. After reconnecting the pump, the female started moving upwind to the junction of the wire, where she had to choose one of the two arms. Each individual was observed until she had reached the end of the arm or for a maximum of five minutes and was subsequently removed (Janssen, 1999). Depending on the mite supply, we tested 16 to 23 predators per replicate, and the odour sources were switched to the opposite arm of the olfactometer after each five mites tested to correct for any unforeseen asymmetry in the experimental set-up. Four replicates were done, using a different set of plants and group of predators for each replicate. Mites used in this experiment were the same age.

We tested the number of responding predatory mites of each repetition against an expected 50:50 distribution with a G-test. The results of the different treatments were compared with a generalized linear model with binomial error distributions (R Development Core Team, 2004). Contrasts between treatments were assessed through model simplification (Crawley, 2002).

3 - Results

Olfactometer experiments showed that *P. macropilis* that were incubated on leaf discs treated with acetone (control) had a significant preference for odours from strawberry plants infested with *T. urticae* relative to clean plants (Figure 1, $G_P = 25.03$, $p = 0.00005$). The results were not significantly heterogeneous ($G_H = 0.27$; $p = 0.86$). In contrast, *P. macropilis* that were exposed to residues of abamectin (Figure 2) and to dimethoate (Figure 3) did not discriminate between odours from infested and clean plants.

There was a significant effect of treatment on the response of the predators ($F_{4,10} = 5.99$, $p < 0.001$). Model simplification showed that none of the differences among pesticide treatments was significant (all p's > 0.2), but the control differed significantly from the pesticide treatments ($F_{1,13} = 22.3$, $p < 0.001$).

4 - Discussion

Predatory mites that had been incubated on leaf discs with acetone were significantly attracted to strawberry plants infested with spider mites. Similar high responses were found for the closely related predatory mite *P. persimilis* (Sabelis and van de Baan, 1983; Takabayashi et al., 1994a,b; Janssen, 1999). Predatory mites are well known for their ability to use volatile infochemicals for distant spider mite location (Sabelis and van de Baan, 1983; Sabelis and Dicke, 1985; Dicke, 1994; Janssen, 1999). In contrast, predatory mites exposed to abamectin and dimethoate residues were not attracted by infested strawberry plants. This shows that exposure to acaricide residues impedes the response of predators to herbivore-induced plant volatiles. In contrasts, predatory mites that survived exposure to residues of abamectin and dimethoate were able to reproduce, at least under laboratory conditions (Oliveira, pers. obs.).

Recently, studies have been done on sublethal effects of pesticides on beneficial organisms (Stark and Banks, 2003; Teodoro et al., 2005). Sublethal effects may range from decrease in lifespan to decreases in fertility and changes in sex ratio, feeding and oviposition behaviour (Starke and Banks, 2003). Besides assessment of toxicity, population growth rates and especially the intrinsic rate of increase have been recommended for studies on sublethal effects to provide a more accurate estimate of population level effects of toxic compounds (Stark et al., 1997). The effects of sublethal doses of pesticides on behaviour have received somewhat less attention.

Sublethal effects are particularly expected on the behaviour of arthropods exposed to neurotoxic pesticides. Predatory mites spend a significant part of their lives searching for prey, and this searching behaviour involves orientation to infested plants using herbivore-induced plant volatiles. Obviously, olfaction depends on neural transmission, which is targeted by neurotoxic insecticides through different modes of action. The most common targets are the voltage-sensitive sodium channel on neuron membranes, whose openings are prolonged by pyrethroids (Soderlund and Bloomquist, 1989) and acetylcholinesterase, which is inhibited by organophosphorous pesticides (Padilla, 1995). In the field, exposure to low doses of pesticides residues is highly probable due to the widespread use of pesticides and the induction of sublethal effects in contaminated surviving beneficials is therefore expected (Desneux et al., 2004.

This paper is the first to show that sublethal doses of pesticides can affect the capacity of predatory mites to respond plants with prey. We recommend that searching behaviour is also included in assessment of effects of pesticides on beneficial arthropods. The pesticides used in the current study are commonly used to control spider mites in strawberry in Brazil. Besides being more toxic to the predatory mites than to the spider mites, they also impede the searching behaviour of the predators. Hence, the pest may ultimately benefit from the application of pesticides because they kill their natural enemies or impede their searching behaviour.

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Table 1: Treatments applied to predatory mites *Phytoseiulus macropilis* and its lethal and sublethal effects on predator populations.

Treatments	Dose applied (g a.i./L)	Instantaneous growth rate (day-1)	Mortality (%)
Control (acetone)		0.39	00.0
Abamectin	0.10	0.26	40.0
	0.20	0.18	70.0
Dimethoate	1.0	0.27	45.0
	2.0	0.18	58.0

Figure legends

Figure 1: Response of *P. macropilis* that were previously incubated on acetone-treated leaf discs (control) to odours from plants infested with *T. urticae* (left) and odours from clean strawberry plants (right) in an olfactometer. Numbers inside the bars refer to the number of females that chose either of two odour sources. Results of the test for each replica are given inside each bar. There was no significant heterogeneity among replicates (G test, G_H = 0.27; d.f. = 2, P = 0.86). Overall, there was a significant attraction by plants with *T. urticae* (G-test on pooled results: G_P = 25.03; d.f. = 1, P = 0.00005).

Figure 2: Response of *P. macropilis* that were previously exposed to the acaricide abamectin (A: 0.01 mg/L; B: 0.02 mg/L) to odours from plants infested with *T. urticae* (left) and odours from clean strawberry plants (right) in an olfactometer. Numbers inside the bars refer to the number of females that chose either of two odour sources. Results of the G-test for each replica are given inside each bar. There was no significant heterogeneity among replicates (A: G test, G_H = 0.14; d.f. = 2, P = 0.89; B: G_H = 0.38; d.f. = 2, P = 0.82). Overall, there was also no significant attraction by plants with *T. urticae* (G-test on pooled results: A: G_P = 0.01; d.f. = 1, P = 0.28; B: G_P = 0.15; d.f. = 1, P = 0.69).

Figure 3: Response of *P. macropilis* that were previously exposed to the acaricide abamectin (A: 1.0 mg/L; B: 2.0 mg/L) to odours from plants infested with *T. urticae* (left) and odours from clean strawberry plants (right) in an olfactometer. Numbers inside the bars refer to the number of females that chose either of two odour sources. Results of the G-test for each replica are given inside each bar. There was no significant

heterogeneity among replicates (A: G test, $G_H = 1.92$; d.f. = 2, P = 0.37; B: $G_H = 1.91$; d.f. = 2, P = 0.38). Overall, there was also no significant attraction by plants with *T. urticae* (G-test on pooled results: A: $G_P = 1.93$; d.f. = 1, P = 0.16; B: $G_P = 1.03$; d.f. = 1, P = 0.26).

Figure 1:

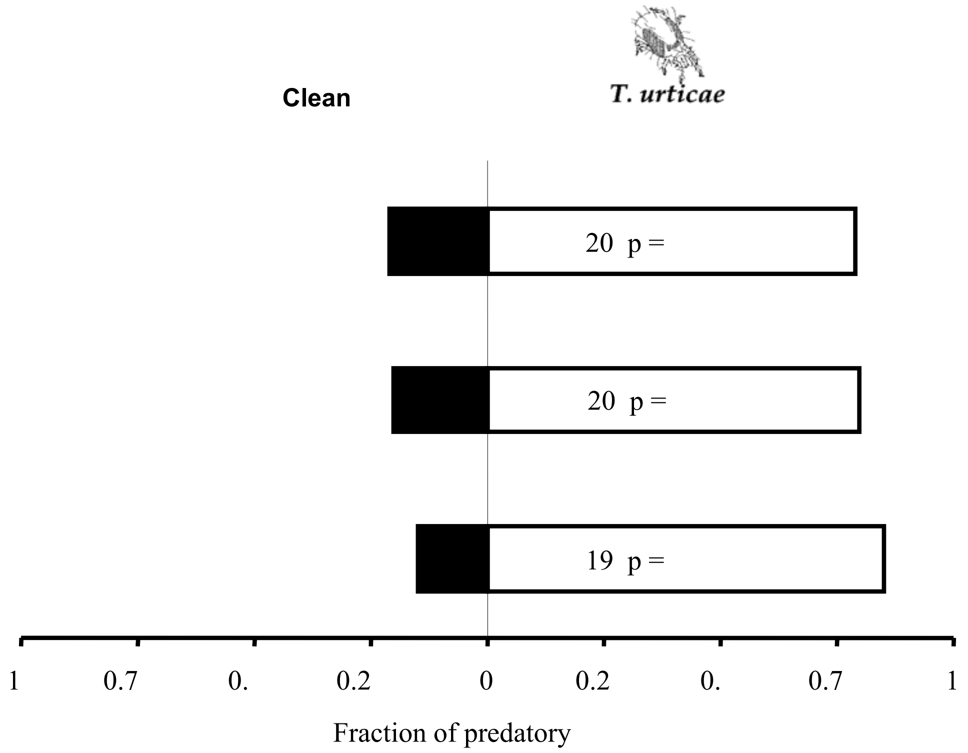

Clean
T. urticae
20 p =
20 p =
19 p =
1
0.7
0.
0.2
0
0.2
0.
0.7
1
Fraction of predatory

Figure 2:

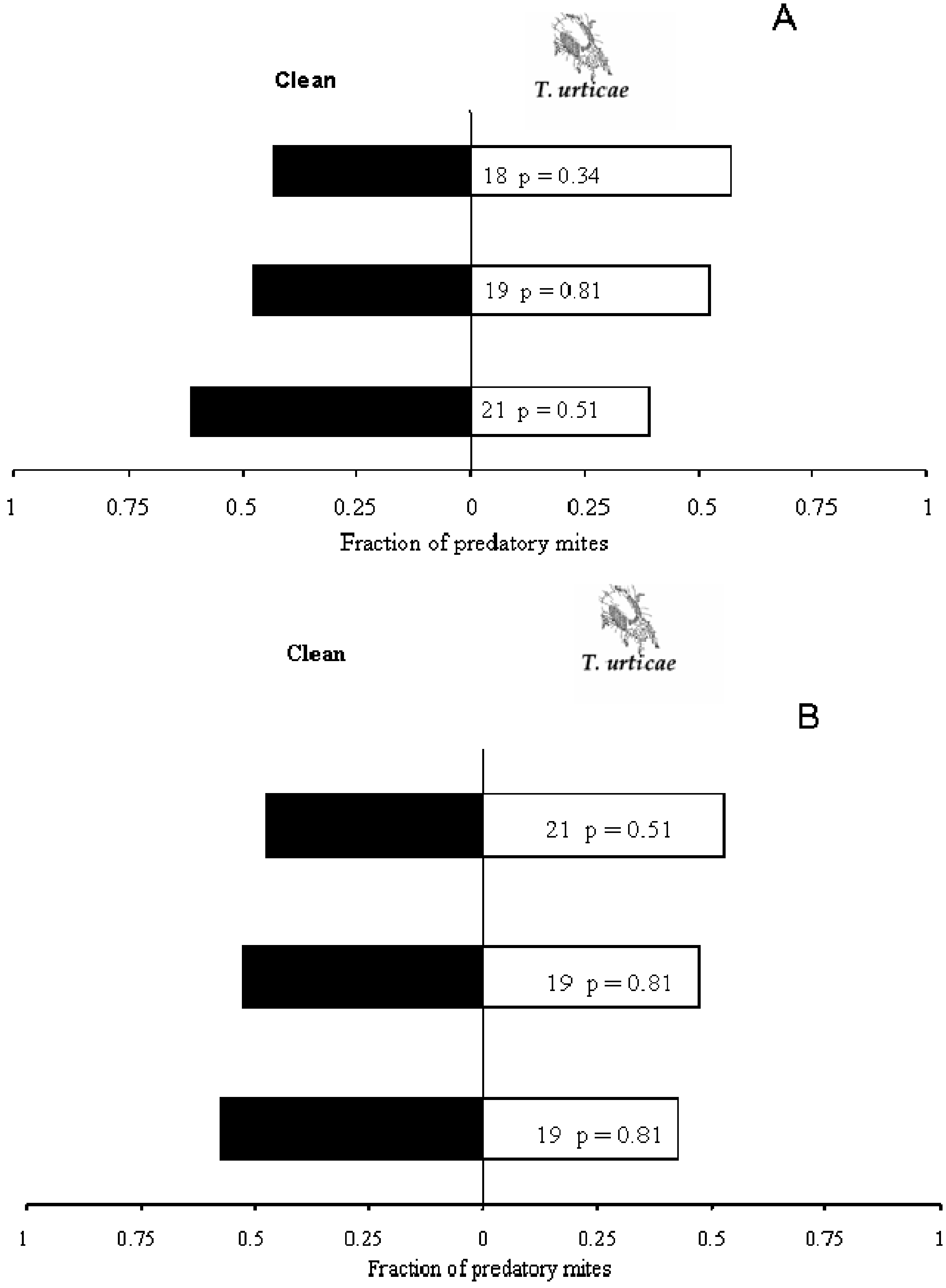
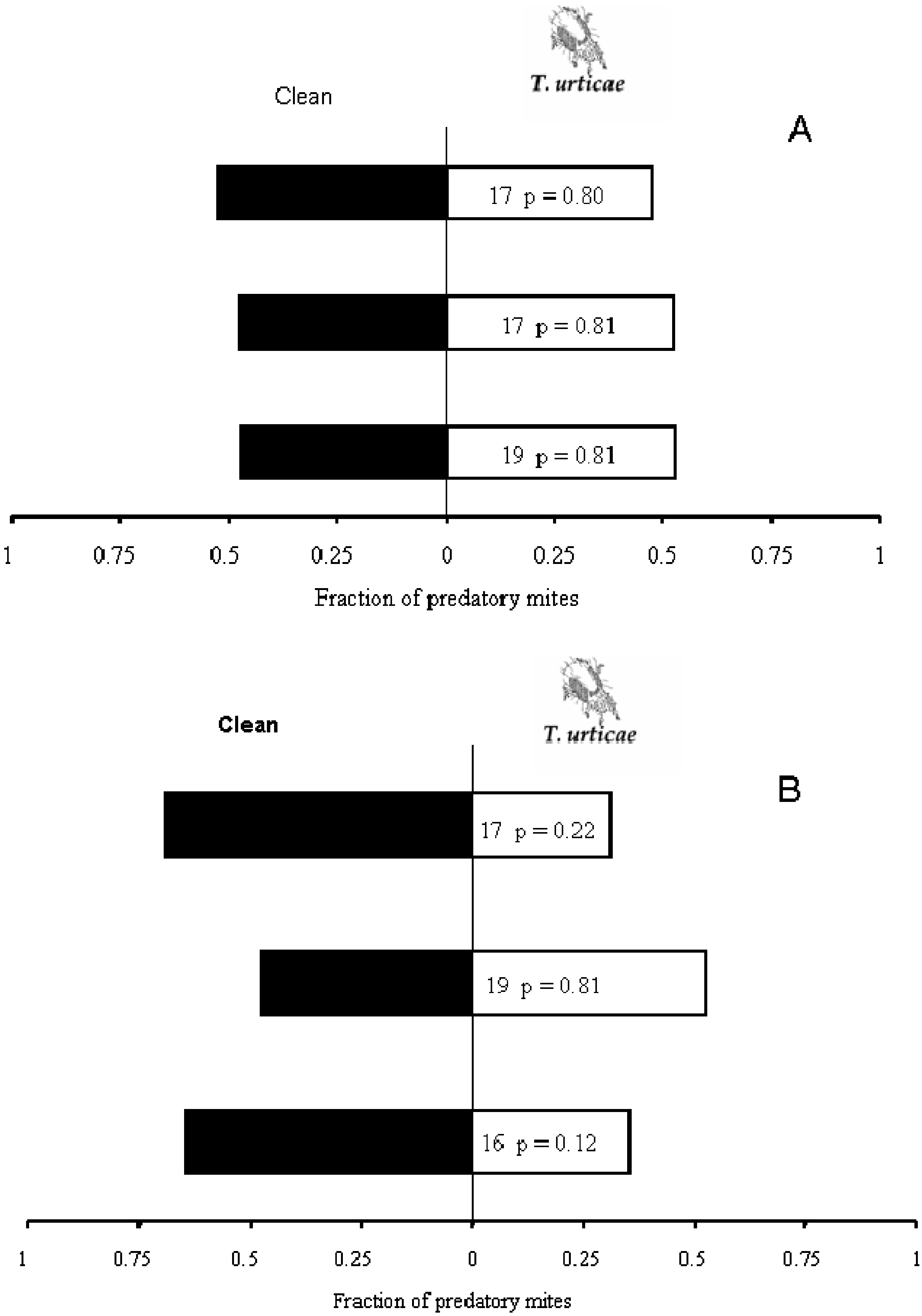

A
Clean
T. urticae
18 p = 0.34
19 p = 0.81
21 p = 0.51
1
0.75
0.5
0.25
0
0.25
0.5
0.75
1
Fraction of predatory mites
Clean
T. urticae
B
21 p = 0.51
19 p = 0.81
19 p = 0.81
1
0.75
0.5
0.25
0
0.25
0.5
0.75
1
Fraction of predatory mites

Figure 3:


Clean
T. urticae
A
17 p = 0.80
17 p = 0.81
19 p = 0.81
1
0.75
0.5
0.25
0
0.25
0.5
0.75
1
Fraction of predatory mites
Clean
T. urticae
B
17 p = 0.22
19 p = 0.81
16 p = 0.12
1
0.75
0.5
0.25
0
0.25
0.5
0.75
1
Fraction of predatory mites