

HENRY EDUARDO VACACELA AJILA

**INNOVATING THE MANAGEMENT OF MITES ON STRAWBERRY CROPS**

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  
2018

**Ficha catalográfica preparada pela Biblioteca Central da Universidade  
Federal de Viçosa - Câmpus Viçosa**

T

V112i  
2018 Vacacela Ajila, Henry Eduardo, 1983-  
Innovating the management of mites on strawberry crops /  
Henry Eduardo Vacacela Ajila. – Viçosa, MG, 2018.  
xi, 105 f. : il. (algumas color.) ; 29 cm.

Texto em inglês.

Orientador: Angelo Pallini Filho.

Tese (doutorado) - Universidade Federal de Viçosa.

Inclui bibliografia.

1. *Phytonemus pallidus*. 2. *Tetranychus urticae*. 3.  
*Neoseiulus californicus*. 4. *Phytoseiulus macropilis*. 5. Ácaros -  
Alimentos. 6. Radiação ultravioleta. 7. Morango - Doenças e  
pragas - Controle. I. Universidade Federal de Viçosa.  
Departamento de Biologia Animal. Programa de Pós-Graduação  
em Entomologia. II. Título.

CDD 22. ed. 595.42

HENRY EDUARDO VACACELA AJILA

**INNOVATING THE MANAGEMENT OF MITES ON STRAWBERRY CROPS**

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: 03 de dezembro de 2018.



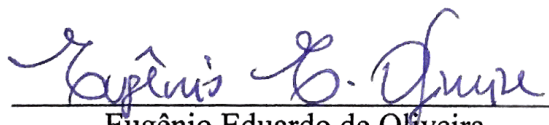
---

André Lage Perez



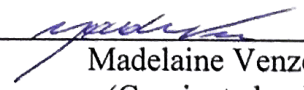
---

Mateus Chediak




---

Eugênio Eduardo de Oliveira  
(Coorientador)



---

Madelaine Venzon  
(Coorientadora)



---

Angelo Pallini Filho  
(Orientador)

## AGRADECIMENTOS

A Deus, por me dar força, saúde e sobre tudo valentia para vencer os obstáculos encontrados ao longo deste caminho e assim conquistar o título de Doutor.

A Universidade Federal de Viçosa e ao Programa de Pós-Graduação em Entomologia, pela oportunidade e realização do curso. Aos professores do programa de Pós-Graduação em Entomologia, obrigado pelos conhecimentos, conselhos e amizade.

Ao Programa Estudante Convênio de Pós-Graduação (PEC-PG), à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e a Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) pela bolsa de estudos e o financiamento do projeto de pesquisa.

Agradeço de forma especial ao meu orientador Professor Angelo Pallini, pelas orientações e conselhos durante todo o doutorado. Por sempre me dar um espaço para conversar e resolver meus problemas... muito obrigado!

Aos meus coorientadores Madelaine Venzon, Eugênio Oliveira e Felipe Colares pelas sugestões, conselhos e orientação. Sua participação foi valiosa para o desenvolvimento deste trabalho.

Ao meu orientador em Kansas State University KSU, JP. Michaud, pela oportunidade de fazer uma parte do meu doutorado nesta instituição. Sou grato por todos os conselhos, sugestões no trabalho e sobre tudo pelos conhecimentos transmitidos. Foi uma experiência única que acrescentou muito no meu crescimento científico e pessoal... Obrigado JP!.

A todos os que tiveram uma participação nas coletas de campo, manutenção de criações no laboratório, execução de experimentos, análises estatísticas. De forma especial agradeço aos estagiários Pedro Hermano, Eliza Franklin, Manoel Gleizer. Foram

horas e horas que dedicamos ao trabalho dentro do laboratório ou na casa de vegetação, mesmo aos finais de semana. Obrigado pela sua dedicação e compromisso, sem vocês não teria sido possível executar o meu projeto. Agradeço também aos colegas do laboratório Willian do Vale, Cleber Oliveira, Felipe Lemos, Cleide Dias, Marcus Duarte. Obrigado pelos conselhos, discussões de experimentos, análises estatísticas, etc. Sua participação foi muito importante.

Aos amigos do Programa de Pós-Graduação em Entomologia e do Laboratório de Acarologia pela amizade e ajuda que sempre me deram para a realização deste trabalho.

Agradeço em especial, a minha mãe Elsa Ajila, ao meu pai Segundo Vacacela, aos meus irmãos Nancy, Wilmer, Franklin, Mirian, Jaime e a meus sobrinhos e sobrinhas. Obrigado pelo apoio e carinho que tiveram comigo em todo momento.

A todos meus amigos dos diferentes países que tive a oportunidade de conhecer no Brasil.

## BIOGRAFIA

Henry Eduardo Vacacela Ajila, filho de Elsa Leonila Ajila Cuenca e Segundo Juan Vacacela Belduma, nasceu em Loja, Província de Loja, Equador em 26 de novembro de 1983.

Formou-se como Engenheiro Agrônomo pela Universidade Nacional de Loja (UNL) em abril de 2008 sob a orientação do professor Toño Jaramillo. Durante a graduação foi estagiário no laboratório de fitopatologia do Serviço Equatoriano de Sanidade Agropecuária SESA atualmente AGROCALIDAD sob orientação do professor Toño Jaramillo. Durante o ano 2010 e 2012 trabalhou no Ministério de Agricultura, Pecuária, Aquicultura e Pesca MAGAP na área de transferência de tecnologia.

Em agosto de 2013 iniciou o curso de Mestrado em Entomologia pela Universidade Federal de Viçosa, sob orientação do pesquisador Angelo Pallini Filho e coorientação de João Alfredo Marinho Ferreira e Madelaine Venzon. Em fevereiro de 2015 obteve o título de *Magister Scientiae* em Entomologia pela Universidade Federal de Viçosa.

Em março de 2015 iniciou o curso de Doutorado em Entomologia pela Universidade Federal de Viçosa, também sob orientação do professor Angelo Pallini e coorientação de Eugênio Eduardo Oliveira, Madelaine Venzon e Felipe Colares. Em dezembro de 2018 submeteu-se a defesa da tese tendo como membros da banca, além dos pesquisadores já citados, André Lage Perez e Mateus Chediak.

## CONTENTS

ABSTRACT .....	viii
RESUMO .....	x
GENERAL INTRODUCTION .....	1
REFERENCES .....	6
CHAPTER 1 .....	12
A new record of a pest mite on strawberry: <i>Phytonemus pallidus</i> (Banks) (Acari: Tarsonemidae) arrives in Minas Gerais, Brazil .....	12
ACKNOWLEDGMENTS .....	15
SUMMARY .....	15
SUMÁRIO .....	16
REFERENCES CITED .....	17
FIGURE CAPTION .....	19
Figure 1 .....	20
Figure 2 .....	21
CHAPTER 2 .....	22
Supplementary food for <i>Neoseiulus californicus</i> boosts biological control of <i>Tetranychus urticae</i> on strawberry .....	22
ABSTRACT .....	23
1 INTRODUCTION .....	24
2 MATERIALS AND METHODS .....	26
2.1 Plants .....	26
2.2 Mites .....	26
2.3 Supplementary foods .....	27
2.4 Experimental procedures .....	28
2.4.1 Suitability of supplementary foods for <i>N. californicus</i> .....	28
2.4.2 Development and reproduction of <i>N. californicus</i> on <i>T. urticae</i> and cattail pollen .....	29
2.4.3 Population dynamics of <i>T. urticae</i> and predatory mites on strawberry plants in the greenhouse .....	30
3. RESULTS .....	31
3.1 Suitability of supplementary foods for <i>N. californicus</i> .....	31
3.2 Development and reproduction of <i>N. californicus</i> on <i>T. urticae</i> and cattail pollen .....	32
3.3 Population dynamics of <i>T. urticae</i> and predatory mites on strawberry plants in the greenhouse .....	32
4 DISCUSSION .....	33
ACKNOWLEDGEMENTS .....	37
AUTHOR CONTRIBUTIONS .....	37
CONFLICT OF INTEREST .....	37
INFORMED CONSENT .....	38
REFERENCES .....	38
FIGURE LEGENDS .....	44
Figure 1. ....	45
Figure 2. ....	46
Figure 3. ....	47

Figure 4. ....	48
Figure 5. ....	49
Table 1. ....	50
CHAPTER 3 .....	51
Flipping advantages to adversity: lime sulfur side effects on predatory mites of natural occurrence in strawberry plants .....	51
ABSTRACT .....	52
KEY MESSAGE .....	53
1 INTRODUCTION .....	54
2 MATERIALS AND METHODS .....	56
2.1 Production of plants .....	56
2.2 Mite rearing .....	56
2.3 Lime sulfur .....	58
2.4 Concentration-response bioassays .....	59
2.5 Sublethal effects on oviposition and egg viability .....	60
2.6 Repellent effect of the lime sulfur .....	61
2.7 Statistical analyses .....	63
3 RESULTS .....	63
3.1 Concentration-response bioassays .....	63
3.2 Sublethal effects on oviposition and egg viability .....	64
3.2.1 Repellent effect of the lime sulfur .....	65
4. DISCUSSION .....	66
AUTHOR'S CONTRIBUTIONS .....	69
ACKNOWLEDGEMENTS .....	69
COMPLIANCE WITH ETHICAL STANDARDS .....	70
Conflict of interest .....	70
Informed consent .....	70
Research involving human participants and/or animals .....	70
REFERENCES .....	70
FIGURE LEGENDS .....	75
Figure 1. ....	76
Figure 2. ....	77
Figure 3. ....	78
Figure 4. ....	79
CHAPTER 4 .....	80
Ultraviolet (UV) irradiation causes sterility in <i>Tetranychus urticae</i> (Koch): a technique to improve the release and commercialization of predatory mites .....	80
ABSTRACT .....	81
INTRODUCTION .....	82
MATERIALS AND METHODS .....	84
Jack bean plants .....	84
Mite rearing .....	84
Cohorts .....	85
Ultraviolet exposure .....	86
Experimental procedures .....	86
Ultraviolet (UV) exposure and its effects on egg and females of <i>T. urticae</i> .....	86
Ultraviolet (UV) exposure and its indirect effects on predatory mites .....	88
RESULTS .....	89

Ultraviolet (UV) exposure and its effects on egg and females of <i>T. urticae</i> .....	89
Ultraviolet (UV) exposure and its indirect effects on predatory mites .....	90
DISCUSSION .....	92
AUTHOR 'S CONTRIBUTIONS .....	94
ACKNOWLEDGEMENTS .....	95
REFERENCES .....	95
FIGURE LEGENDS .....	98
Figure 1. ....	100
Figure 2. ....	101
Figure 3. ....	102
Figure 4. ....	103
GENERAL CONCLUSIONS .....	104

## ABSTRACT

VACACELA AJILA, Henry Eduardo, D.Sc., Universidade Federal de Viçosa, December, 2018. **Innovating the management of mites on strawberry crops.** Adviser: Angelo Pallini Filho. Co-advisers: Eugênio Eduardo de Oliveira, Madelaine Venzon and Felipe Colares.

Two species of pest mites are responsible for causing great losses in strawberry production. The main one, the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), may cause losses up to 80%. On the other hand, the cyclamen mite *Phytonemus pallidus* (Banks) (Acari: Tarsonemidae) is also an important pest in strawberry. Although it has not previously been reported in Brazil, this pest is commonly associated with strawberry crops in several countries in the world. Therefore, in this study, initially, we report the occurrence of this new pest on strawberry in Minas Gerais-Brazil. Subsequently, we examined the suitability of different pollen sources as supplementary food for the predatory mite *N. californicus* (McGregor) (Acari: Phytoseiidae) when the population of its prey *T. urticae* is low. Additionally, we investigated the relative suitability of different pollen types for *N. californicus* survival and reproduction, and the life history impacts of a mixed diet of pollen and prey. Then, we assessed the population dynamics of *T. urticae* on strawberry plants in the presence of the *N. californicus*, with or without pollen, and in comparison to the specialist predatory mite *P. macropilis* (Banks) (Acari: Phytoseiidae). Further, we evaluated the toxicity of the lime sulfur to the phytophagous mite *T. urticae* and its natural enemies *P. macropilis* and *N. californicus*. In the same study, we evaluated the effect of sublethal concentrations of lime sulfur over oviposition, egg viability and foraging behaviour of those three mite species. Lastly, we studied the effect of ultraviolet (UV) irradiation on female mortality and egg sterility of *T. urticae*. We investigated whether inactive mites (females and eggs of *T. urticae*) may have negative effects on the biological parameters of the predatory mites *N. californicus* and *P. macropilis*. The main results of this study showed that *P. pallidus* was found in organic strawberry crops in two municipalities in the state of Minas Gerais. We confirmed that *N. californicus* is an efficient biological control agent of *T. urticae* in strawberry crop and the provision of supplementary food could retain them in the crop prior to the arrival of the pest or in periods of prey scarcity. Additionally, we found that lime sulfur exhibited higher toxicity to the predators *N. californicus* and *P. macropilis* than to *T. urticae*. On the other hand, the exposure to field-applied concentrations resulted in higher reductions on the oviposition rate of *T. urticae*

than on the predatory mites. Interestingly, although the egg viability of *P. macropilis* was less affected by such lime sulfur exposures, these mites were unable to avoid lime sulfur-treated areas. Finally, we proved that UV irradiation has a negative effect on the phytophagous mite *T. urticae* causing sterility of the eggs and the mortality of the females. Both predatory mites *N. californicus* and *P. macropilis* can feed on females and sterile eggs of *T. urticae* without affecting their biological parameters. So, this process can improve mass rearing and could be used in several biological control systems.

## RESUMO

VACACELA AJILA, Henry Eduardo, D.Sc., Universidade Federal de Viçosa, dezembro de 2018. **Inovando o manejo de ácaros na cultura do morango**. Orientador: Angelo Pallini Filho. Coorientador: Eugênio Eduardo de Oliveira, Madelaine Venzon e Felipe Colares.

Na cultura do morango duas espécies de ácaros praga são as responsáveis por provocar grandes prejuízos na produção. A principal, o ácaro rajado *Tetranychus urticae* Koch (Acari: Tetranychidae), pode provocar perdas acima do 80% quando não é controlada de uma forma adequada. Por outro lado, o ácaro *Phytonemus pallidus* (Banks) (Acari: Tarsonemidae) também constitui uma praga de importância no morango. Apesar de não ter sido anteriormente reportada no Brasil, esta praga está comumente associada ao morango em diversos países no mundo. Portanto, nesse trabalho, inicialmente, foi reportado a presença desta nova praga na cultura de morango em Minas Gerais-Brasil. Posteriormente, nós examinamos a adequabilidade de diferentes tipos de pólen como alimento suplementar para o ácaro predador *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) quando a população de sua presa *T. urticae* é baixa. Também foi investigado a adequabilidade de diferentes tipos de pólen na sobrevivência e reprodução de *N. californicus* e os efeitos de uma dieta mista de pólen e presa na história de vida do predador. Adicionalmente, nós testamos a dinâmica populacional de *T. urticae* em plantas de morango na presença de *N. californicus*, com e sem pólen, e em comparação ao ácaro predador especialista *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae). Além disso, nós avaliamos a toxicidade da calda sulfocálcica no ácaro fitófago *T. urticae* e nos inimigos naturais *P. macropilis* e *N. californicus*. No mesmo estudo, foi avaliado o efeito subletal de concentrações da calda sulfocálcica na oviposição, viabilidade dos ovos e no comportamento de forrageamento das três espécies de ácaros. Por fim, nós estudamos o efeito da irradiação ultravioleta (UV) na mortalidade das fêmeas e esterilidade dos ovos de *T. urticae*. Foi investigado se ácaros “inativos” (fêmeas e ovos de *T. urticae*) podem provocar efeitos negativos nos parâmetros biológicos dos ácaros predadores *N. californicus* e *P. macropilis*. Os principais resultados do presente estudo indicam que *P. pallidus* foi encontrado no cultivo de morango orgânico em dois municípios do estado de Minas Gerais. Também, foi demonstrado que *N. californicus* é um eficiente agente de controle biológico de *T. urticae* e que a provisão de alimento suplementar pode manter os predadores no cultivo de morango antes que a praga se estabeleça na cultura ou em

períodos de escassez da mesma. Adicionalmente, foi verificado que a calda sulfocálcica exibiu uma alta toxicidade para os predadores *N. californicus* e *P. macropilis* em comparação de *T. urticae*. Contrariamente, concentrações de calda sulfocálcica usadas em campo provocaram uma maior redução na oviposição de *T. urticae* do que na dos predadores. Curiosamente, embora a viabilidade de ovos de *P. macropilis* tenha sido menos afetada devido as exposições da calda sulfocálcica, esses ácaros foram incapazes de evitar áreas tratadas. Finalmente, foi demonstrado também que a irradiação UV provocou efeito negativo no ácaro fitófago *T. urticae* causando a esterilidade dos ovos e a mortalidade das fêmeas. Sendo assim, os ácaros predadores *N. californicus* e *P. macropilis* podem se alimentar das fêmeas e ovos “estéreis” de *T. urticae* sem afetar seus parâmetros biológicos. Desta maneira, esse processo pode melhorar as técnicas de criações massais usadas no controle biológico de pragas.

## GENERAL INTRODUCTION

Strawberry is a very important crop in Brazil due to the economic return it generates (Costa et al. 2015; Cerutti et al. 2018). The leading producing states are Minas Gerais, Rio Grande do Sul and São Paulo (Antunes et al. 2007; Henz 2010). Among the several organisms that damage the strawberry plants, phytophagous mites stand out (Overmeer 1985; Fadini et al. 2007) due to their high reproductive capacity to increase their population and to reduce productivity in the field (Tomczyk and Kropczynska 1985).

In several regions of the world *Tetranychus urticae* Koch (Acari: Tetranychidae) is considered the main pest on strawberry crop (Chiavegato and Mischan 1981; Edge and James 1986; García-Marí and Enrique González-Zamora 1999). In Brazil, there are records of losses up to 80% caused due to inefficient control of this herbivore (Chiavegato and Mischan 1981). Infested plants show white-silver stain and chlorotic areas that later on turns brown on the upper surface of leaves with web on their underside (Sato et al., 2002). Subsequently, leaves will dry and fall (Flechtmann 1972; Sances et al. 1982; Fadini et al. 2004a).

*Phytonemus pallidus* (Banks) (Acari: Tarsonemidae) is another phytophagous mite that is commonly associated with strawberry crops (Alford 1972; Easterbrook et al. 2001; Gobin and Bangels 2008). It usually infests young leaves, flowers and fruits (Smith and Goldsmith 1936; Alford 1972; Easterbrook et al. 2001; Petrova et al. 2002; Berglund et al. 2007; Gobin and Bangels 2008; Tuovinen and Lindqvist 2010). Severe infestations of *P. pallidus* lead to a reduction in the development of petioles and consequently the size of plants. The leaves become brown or brittle (Croft et al. 1998). Fruits are reduced and develop a brownish color making them unsuitable for harvest (Pallini et al. 2002; Gobin and Bangels 2008). Estimates are that *P. pallidus* populations could cause up to

22-50% reduction in strawberry yields (Alford 1972; Stenseth and Nordby 1976). However, reports already show 100% of loss due to this pest in Minas Gerais (Vacacela et al. 2018).

There are few registered pesticides for the control of pest mites making them difficult to manage (Easterbrook et al. 2001). Abamectin is the active ingredient in many commercial products in Brazil (Agrofit 2018), and the selection of populations resistant to common acaricides makes the situation even more complex, especially with *T. urticae* (Easterbrook et al. 2001; Sato et al. 2007). Ecological pest control is an essential alternative to reduce dependence on agrochemicals and support sustainable production (Fadini et al. 2004b). This approach prioritizes the use of alternative phytosanitary products to regulate the population of pests without negatively impacting natural enemy population (Fadini et al. 2004b; Madail et al. 2007). Beyond that, non-chemical alternatives can be used in the management of this pest as the use of biological control agents (predatory mites) (Edge and James 1986; Easterbrook et al. 2001; Greco et al. 2005; Rhodes and Liburd 2006; Oliveira et al. 2007, 2009; Fraulo and Liburd 2007). Furthermore, ultraviolet (UV) irradiation (Suzuki et al. 2009) gives also opportunities for control that should not be ignored.

Lime sulfur is an alternative product commonly used in organic production both in the control of diseases and to reduce the populations of pests (Russell 2005; Venzon et al. 2006, 2013; Tuelher et al. 2014; Andrezza et al. 2017). The lime sulfur acts on insects when air passes through a solution of calcium carbonate and sulfur releasing gases such as sulfur dioxide and hydrogen sulphide. The insecticidal effect is generally attributed to the latter (Abbott 1945). In many crops, depending on the dose the lime sulfur is efficient in controlling phytophagous mites without increasing mortality in natural enemies (Soto et al. 2010; Tuelher et al. 2014). Some examples of successful use of lime sulfur in pest

control are on *Oligonychus ilicis* (McGregor) in coffee (Tuelher et al. 2014), *Tetranychus evansi* Baker & Pritchard in tomato (Soto et al. 2010) and *Polyphagotarsonemus latus* (Banks) in chili pepper (Venzon et al. 2006, 2013).

Another alternative for the reduction of agrochemical application is biological control through the release of predatory mites such as *Phytoseiulus persimilis* (Simmonds 1970; Rhodes and Liburd 2006), *Phytoseiulus macropilis* (Oliveira et al. 2007, 2009), *Neoseiulus californicus* (García-Marí and Enrique González-Zamora 1999; Easterbrook et al. 2001; Greco et al. 2005; Rhodes and Liburd 2006; Fraulo and Liburd 2007; Ahn et al. 2010; Gugole Ottaviano et al. 2015; Soltaniyan et al. 2018) and *Neoseiulus cucumeris* (Easterbrook et al. 2001), among others. These mites have been successfully used to control phytophagous species such as *T. urticae* (García-Marí and Enrique González-Zamora 1999; Easterbrook et al. 2001; Greco et al. 2005; Rhodes and Liburd 2006; Sato et al. 2007; Fraulo and Liburd 2007; Oliveira et al. 2009; Ahn et al. 2010; Gugole Ottaviano et al. 2015) and *P. pallidus* in strawberry (Simmonds 1970; Croft et al. 1998; Easterbrook et al. 2001). However, specialist species such as *P. persimilis* may exhibit some limitations. They may have low tolerance to pesticides, regional specificity (Easterbrook et al. 2001) or low persistence capacity under environment with low prey density (McMurtry and Croft 1997; Gerson et al. 2003; Oliveira et al. 2009; McMurtry et al. 2013), which could decrease their effectiveness as sustainable biological control agents.

Other species belonging to the genus *Neoseiulus* are generalists on their feeding habits (McMurtry and Croft 1997; McMurtry et al. 2013). These mites prefer tetranychid mites but may also feed on thrips, pollen (Van Rijn and Tanigoshi 1999; Delisle et al. 2015b, a; Duarte et al. 2015) and other species of phytophagous mites (Gerson et al. 2003). *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) is one of these

generalist species that is an outstanding biological control agent of a major pest of strawberry worldwide, *T. urticae* (García-Marí and Enrique González-Zamora 1999; Easterbrook et al. 2001; Greco et al. 2005; Rhodes and Liburd 2006; Sato et al. 2007; Fraulo and Liburd 2007; Oliveira et al. 2009; Ahn et al. 2010; Gugole Ottaviano et al. 2015). In Brazil, *N. californicus* occurs naturally in strawberry (Ferla et al. 2007) and is sometimes augmented by growers in combination with *P. macropilis* for control of *T. urticae* (Sato et al. 2007; Oliveira et al. 2007). Provisioning pollen as a supplemental food source could improve biological control of *T. urticae* through retention of *Neoseiulus californicus* as a preventative control even in the absence of the primary pest.

Different types of plants produce pollen of high nutritional value suitable for predatory mites (Reis and Alves 1997; Broufas and Koveos 2000; Lofego and Moraes 2005; Goleva and Zebitz 2013; Khanamani et al. 2016). Some examples are cherry, peach, apricot, poppy (Broufas and Koveos 2000) and castor bean (*Ricinus communis* L.) (Yamamoto and Gravena 1996; Reis and Alves 1997; Rodríguez-Cruz et al. 2013) and cattail pollen (*Typha* sp.) (Park et al. 2011; Pijnakker et al. 2016; Ghasemzadeh et al. 2017; Lee and Zhang 2018). Castor bean and cattail pollen are widely available in Brazil and bee pollen is commercially available worldwide. However, the nutritional content of each type of pollen is highly variable and can directly affect the performance of predatory mites.

Apart from biological control, the ultraviolet (UV) irradiation could be used like a technique to improve the release and commercialization of predatory mites. UV irradiation in certain doses can promote the mortality (Ohtsuka and Osakabe 2009), escape (Barcelo and Calkins 1980; Barcelo 1981; Ohtsuka and Osakabe 2009; Tachi and Osakabe 2012) and inhibition of oviposition (Barcelo 1981; Ohtsuka and Osakabe 2009; Sakai et al. 2012; Suzuki et al. 2014) of *T. urticae* (Suzuki et al. 2009). UV irradiation

directly damages DNA (Suzuki et al. 2009) and other important cell components such as lipids, lipid membranes, nucleic acids, and proteins (Jurkiewicz and Buettner 1994; Shindo et al. 1994). Furthermore, UV irradiation on *T. urticae* could be used to improve mass rearing and commercialization, inducing the sterility of the pest and using them as food for predatory mites.

In this thesis, in chapter one we reported the presence of a new pest mite (*P. pallidus*) in the strawberry crop in Minas Gerais-Brazil. Then, in Chapter two we examined the suitability of different pollen sources as supplementary foods for the predatory mite *N. californicus* when populations of *T. urticae* are low. We investigated the relative suitability of different pollen types for *N. californicus* survival and reproduction and the life history impacts of a mixed diet of pollen and prey. In a separate experiment, we assessed the population dynamics of *T. urticae* on strawberry plants in the presence of *N. californicus*, with and without pollen, and in comparison to the specialist predatory mite *P. macropilis*.

In the third Chapter, we evaluated the toxicity of the lime sulfur to the phytophagous mite *T. urticae* and its natural enemies *P. macropilis* and *N. californicus*. Additionally, we evaluated the effect of sublethal concentrations of lime sulfur on oviposition, egg viability and foraging behaviour of those three mite species. Finally, in the last chapter, we determined UV irradiation time necessary for female mortality and egg sterility of *T. urticae*. We investigated whether 'inactive' mites (females and eggs of *T. urticae*) may have negative effects on the biological parameters of the predatory mites *N. californicus* and *P. macropilis*.

## REFERENCES

- Abbott C (1945) The toxic cases of lime-sulfur. *J Econ Entomol* 38:618–620
- Agrofit (2018) Sistema de agrotóxicos fitossanitários. In: Ministério da Agricultura, Pecuária e Abastecimento
- Ahn JJ, Kim KW, Lee JH (2010) Functional response of *Neoseiulus californicus* (Acari: Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae) on strawberry leaves. *J Appl Entomol* 134:98–104
- Alford D (1972) The effect of *Tarsonemus fragariae* Zimmermann (Acarina: Tarsonemidae) on strawberry yields. *Ann Appl Biol* 70:13–18
- Andreazza F, Vacacela Ajila HE, Haddi K, et al (2017) Toxicity to and egg-laying avoidance of *Drosophila suzukii* (Diptera: Drosophilidae) caused by an old alternative inorganic insecticide preparation. *Pest Manag Sci* 74:861–867
- Antunes L, Duarte Filho J, Calegario F, et al (2007) Produção integrada de morango (PIMo) no Brasil. *Morango Conquistando Novas Front Inf Agropecuário Belo Horiz* 28:34–39
- Barcelo JA (1981) Photoeffects of visible and ultraviolet radiation on the two-spotted spider mite, *Tetranychus urticae*. *Photochem Photobiol* 33:703–706
- Barcelo JA, Calkins J (1980) The kinetics of avoidance of simulated solar UV radiation by two arthropods. *Biophys J* 32:921–929
- Berglund R, Svensson B, Nilsson C (2007) Evaluation of methods to control *Phytonemus pallidus* and *Anthonomus rubi* in organic strawberry production. *J Appl Entomol* 131:573–578
- Broufas GD, Koveos DS (2000) Effect of different pollens on development, survivorship and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae). *Environ Entomol* 29:743–749
- Cerutti PH, dos Santos M, Gemeli MS, et al (2018) Desafios do cultivo de morangueiro no Brasil. *Rev Científica Rural* 20:236–252
- Chiavegato L, Mischán M (1981) Efeito do acaro *Tetranychus* (T.) *urticae* (Koch, 1836) Boudreaux & Dosse, 1963 (Acari, Tetranychidae) na produção do morangueiro (*Fragaria* spp.) cv. 'Campinas'. *Científica* 9:257–266
- Costa AF, Leal NR, Ventura JA, et al (2015) Adaptability and stability of strawberry cultivars using a mixed model. *Acta Sci Agron* 37:435–440
- Croft B, Pratt P, Koskela G, Kaufman D (1998) Predation, reproduction, and impact of phytoseiid mites (Acari: Phytoseiidae) on cyclamen mite (Acari: Tarsonemidae) on strawberry. *J Econ Entomol* 91:1307–1314

- Delisle JF, Brodeur J, Shipp L (2015a) Evaluation of various types of supplemental food for two species of predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae). *Exp Appl Acarol* 65:483–494
- Delisle JF, Shipp L, Brodeur J (2015b) Apple pollen as a supplemental food source for the control of western flower thrips by two predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae), on potted chrysanthemum. *Exp Appl Acarol* 65:495–509
- Duarte MVA, Venzon M, Bittencourt MC de S, et al (2015) Alternative food promotes broad mite control on chilli pepper plants. *BioControl* 60:817–825
- Easterbrook MA, Fitzgerald JD, Solomon MG (2001) Biological control of strawberry tarsonemid mite *Phytonemus pallidus* and two-spotted spider mite *Tetranychus urticae* on strawberry in the UK using species of *Neoseiulus* (*Amblyseius*) (Acari: Phytoseiidae). *Exp Appl Acarol* 25:25–36
- Edge VE, James DG (1986) Organo-tin Resistance in *Tetranychus urticae* (Acari: Tetranychidae) in Australia. *J Econ Entomol* 79:1477–1483
- Fadini MA, Oliveira HG, Venzon M, et al (2007) Distribuição espacial de ácaros fitófagos (Acari: Tetranychidae) em morangueiro. *Neotrop Entomol* 36:783–789
- Fadini MAM, Lemos WP, Pallini A, et al (2004a) Herbivoria de *Tetranychus urticae* Koch (Acari: Tetranychidae) induz defesa direta em morangueiro? *Neotrop Entomol* 33:293–297
- Fadini MAM, Pallini A, Venzon M (2004b) Controle de ácaros em sistema de produção integrada de morango. *Ciênc Rural* 34:1271–1277
- Ferla NJ, Marchetti MM, Gonçalves D (2007) Ácaros predadores (Acari) associados à cultura do morango (*Fragaria sp.*, Rosaceae) e plantas próximas no Estado do Rio Grande do Sul. *Biota Neotropica* 7:1–8
- Flechtmann CH (1972) Ácaros de importância agrícola. Livraria Nobel, São Paulo, Brasil
- Fraulo AB, Liburd OE (2007) Biological control of twospotted spider mite, *Tetranychus urticae*, with predatory mite, *Neoseiulus californicus*, in strawberries. *Exp Appl Acarol* 43:109
- García-Marí F, Enrique González-Zamora J (1999) Biological control of *Tetranychus urticae* (Acari: Tetranychidae) with naturally occurring predators in strawberry plantings in Valencia, Spain. *Exp Appl Acarol* 23:487–495
- Gerson U, Smiley RL, Ochoa R (2003) Mites (Acari) for pest control. Blackwell Science, Oxford, England
- Ghasemzadeh S, Leman A, Messelink GJ (2017) Biological control of *Echinothrips americanus* by phytoseiid predatory mites and the effect of pollen as supplemental food. *Exp Appl Acarol* 73:209–221

- Gobin B, Bangels E (2008) Field control of strawberry mite *Phytonemus pallidus*. IOBCWPRS Bull 39:97–100
- Goleva I, Zebitz CPW (2013) Suitability of different pollen as alternative food for the predatory mite *Amblyseius swirskii* (Acari, Phytoseiidae). Exp Appl Acarol 61:259–283
- Greco NM, Sánchez NE, Liljeström GG (2005) *Neoseiulus californicus* (Acari: Phytoseiidae) as a potential control agent of *Tetranychus urticae* (Acari: Tetranychidae): effect of pest/predator ratio on pest abundance on strawberry. Exp Appl Acarol 37:57–66
- Gugole Ottaviano MF, Cédola CV, Sánchez NE, Greco NM (2015) Conservation biological control in strawberry: effect of different pollen on development, survival, and reproduction of *Neoseiulus californicus* (Acari: Phytoseiidae). Exp Appl Acarol 67:507–521
- Henz GP (2010) Desafios enfrentados por agricultores familiares na produção de morango no Distrito Federal. Horti Bras 28:260–265
- Jurkiewicz BA, Buettner GR (1994) Ultraviolet light-induced free radical formation in skin: an electron paramagnetic resonance study. Photochem Photobiol 59:1–4
- Khanamani M, Fathipour Y, Talebi AA, Mehrabadi M (2016) Linking pollen quality and performance of *Neoseiulus californicus* (Acari: Phytoseiidae) in two-spotted spider mite management programmes. Pest Manag Sci 73:452–461
- Lee MH, Zhang Z-Q (2018) Assessing the augmentation of *Amblydromalus limonicus* with the supplementation of pollen, thread, and substrates to combat greenhouse whitefly populations. Sci Rep 8:12189
- Lofego A, Moraes G (2005) Taxa de oviposição dos predadores *Amblyseius acalyphus* e *Amblyseius neochiapensis* (Acari: Phytoseiidae) com diferentes tipos de alimento. Arq Inst Biol 72:379–382
- Madail JCM, Antunes LE, Belarmino LC, et al (2007) Avaliação econômica dos sistemas de produção de morango: convencional, integrado e orgânico. Pelotas: Embrapa Clima Temperado (Comunicado técnico, 181) 4 p
- McMurtry JA, Croft BA (1997) Life-styles of phytoseiid mites and their roles in biological control. Annu Rev Entomol 42:291–321
- McMurtry JA, Moraes GJD, Sourassou NF (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Syst Appl Acarol 18:297–320
- Ohtsuka K, Osakabe M (2009) Deleterious effects of UV-B radiation on herbivorous spider mites: they can avoid it by remaining on lower leaf surfaces. Environ Entomol 38:920–929
- Oliveira H, Fadini MAM, Venzon M, et al (2009) Evaluation of the predatory mite *Phytoseiulus macropilis* (Acari: Phytoseiidae) as a biological control agent of the

- two-spotted spider mite on strawberry plants under greenhouse conditions. *Exp Appl Acarol* 47:275–283
- Oliveira H, Janssen A, Pallini A, et al (2007) A phytoseiid predator from the tropics as potential biological control agent for the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Biol Control* 42:105–109
- Overmeer W (1985) Toxicological methods. In: Helle & Sabelis, Spider mites their biology, natural enemies and control. Elsevier, Amsterdam, New York, pp 183–189
- Pallini A, Fadini M, Lemos W, Venzon M (2002) Manejo integrado de ácaros em fruteiras tropicais e subtropicais. In: Zambolin; L. Manejo integrado-fruteiras tropicais: Doenças e pragas. Viçosa: Universidade Federal de Viçosa, pp 579–614
- Park H-H, Shipp L, Buitenhuis R, Ahn JJ (2011) Life history parameters of a commercially available *Amblyseius swirskii* (Acari: Phytoseiidae) fed on cattail (*Typha latifolia*) pollen and tomato russet mite (*Aculops lycopersici*). *J Asia-Pac Entomol* 14:497–501. doi: 10.1016/j.aspen.2011.07.010
- Petrova V, Cudare Z, Steinite I (2002) The efficiency of the predatory mite *Amblyseius cucumeris* (Acari: Phytoseiidae) as a control agent of the strawberry mite *Phytonemus pallidus* (Acari: Tarsonemidae) on field strawberry. In: *Acta Horticulturae*. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp 675–678
- Pijnakker J, Arijs Y, de Souza A, et al (2016) The use of *Typha angustifolia* (cattail) pollen to establish the predatory mites *Amblyseius swirskii*, *Iphiseius degenerans*, *Euseius ovalis* and *Euseius gallicus* in glasshouse crops. *IOBC WPRS Bull* 120:47–54
- Reis PR, Alves EB (1997) Criação do ácaro predador *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae) em laboratório. *Soc Entomol Bras* 26:565–568
- Rhodes EM, Liburd OE (2006) Evaluation of predatory mites and acramite for control of twospotted spider mites in strawberries in North Central Florida. *J Econ Entomol* 99:1291–1298
- Rodríguez-Cruz FA, Venzon M, Pinto CMF (2013) Performance of *Amblyseius herbicolus* on broad mites and on castor bean and sunnhemp pollen. *Exp Appl Acarol* 60:497–507
- Russell PE (2005) A century of fungicide evolution. *J Agric Sci* 143:11–25
- Sakai Y, Sudo M, Osakabe M (2012) Seasonal changes in the deleterious effects of solar ultraviolet-B radiation on eggs of the twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *Appl Entomol Zool* 47:67–73
- Sances FV, Toscano NC, Oatman ER, et al (1982) Reductions in plant processes by *Tetranychus urticae* (Acari: Tetranychidae) feeding on strawberry. *Environ Entomol* 11:733–737

- Sato ME, Da Silva MZ, De Souza Filho MF, et al (2007) Management of *Tetranychus urticae* (Acari: Tetranychidae) in strawberry fields with *Neoseiulus californicus* (Acari: Phytoseiidae) and acaricides. *Exp Appl Acarol* 42:107–120
- Shindo Y, Witt E, Han D, Packer L (1994) Dose-Response effects of acute ultraviolet irradiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. *J Invest Dermatol* 102:470–475
- Simmonds SP (1970) The possible control of *Steneotarsonemus pallidus* on strawberries by *Phytoseiulus persimilis*. *Plant Pathol* 19:106–107
- Smith LM, Goldsmith EV (1936) The cyclamen mite, *Tarsonemus pallidus*, and its control on field strawberries. *Hilgardia* 10:53–94
- Soltaniyan A, Kheradmand K, Fathipour Y, Shirdel D (2018) Suitability of pollen from different plant species as alternative food sources for *Neoseiulus californicus* (Acari: Phytoseiidae) in comparison with a natural prey. *J Econ Entomol* 111:2046–2052. doi: 10.1093/jee/toy172
- Soto A, Venzon M, Oliveira RM, et al (2010) Alternative control of *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on tomato plants grown in greenhouses. *Neotrop Entomol* 39:638–644
- Stenseth C, Nordby ALF (1976) Damage, and control of the strawberry mite *Steneotarsonemus pallidus* (Acarina: Tarsonemidae), on strawberries. *J Hortic Sci* 51:49–24
- Suzuki T, Watanabe M, Takeda M (2009) UV tolerance in the two-spotted spider mite, *Tetranychus urticae*. *J Insect Physiol* 55:649–654
- Suzuki T, Yoshioka Y, Tsarsitalidou O, et al (2014) An LED-based UV-B irradiation system for tiny organisms: System description and demonstration experiment to determine the hatchability of eggs from four *Tetranychus* spider mite species from Okinawa. *J Insect Physiol* 62:1–10
- Tachi F, Osakabe M (2012) Vulnerability and behavioral response to ultraviolet radiation in the components of a foliar mite prey–predator system. *Naturwissenschaften* 99:1031–1038
- Tomczyk A, Kropczynska D (1985) Effects on the host plant. In: *Spider mites: their biology, natural enemies and control*. Elsevier, Amsterdam, pp 317–327
- Tuelher ES, Venzon M, Guedes RNC, Pallini A (2014) Toxicity of organic-coffee-approved products to the southern red mite *Oligonychus ilicis* and to its predator *Iphiseiodes zuluagai*. *Crop Prot* 55:28–34
- Tuovinen T, Lindqvist I (2010) Maintenance of predatory phytoseiid mites for preventive control of strawberry tarsonemid mite *Phytonemus pallidus* in strawberry plant propagation. *Biol Control* 54:119–125

- Vacacela HE, Lemos F, Colares F, et al (2018) A new Record of a pest mite on strawberry: *Phytonemus pallidus* (Banks) (Acari: Tarsonemidae) arrives in Minas Gerais, Brazil. Fla Entomol 101:529–532
- Van Rijn PC, Tanigoshi LK (1999) Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history. Exp Appl Acarol 23:785–802
- Venzon M, Oliveira RM, Perez AL, et al (2013) Lime sulfur toxicity to broad mite, to its host plants and to natural enemies. Pest Manag Sci 69:738–743
- Venzon M, Rosado M da C, Pinto CMF, et al (2006) Potencial de defensivos alternativos para o controle do ácaro-branco em pimenta “Malagueta.” Horti Bras 24:224–227
- Yamamoto PT, Gravena S (1996) Influência da temperatura e fontes de alimento no desenvolvimento e oviposição de *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae). Soc Entomol Bras 25:109–115

## CHAPTER 1

# **A new record of a pest mite on strawberry: *Phytonemus pallidus* (Banks) (Acari: Tarsonemidae) arrives in Minas Gerais, Brazil**

Henry E. Vacacela Ajila<sup>1,\*</sup>, Felipe Lemos<sup>2</sup>, Felipe Colares<sup>2</sup>, João A. M. Ferreira<sup>2</sup>,  
Antônio C. Lofego<sup>3</sup>, Angelo Pallini<sup>1</sup>

*Florida Entomologist* (2018) 101:529-532  
DOI 10.1653/024.101.0330

Address:

<sup>1</sup> *Department of Entomology, Section Acarology, Federal University of Viçosa, Viçosa-MG, 36570-900, Brazil, E-mail: [henryeduar2@hotmail.com](mailto:henryeduar2@hotmail.com), [pallini@ufv.br](mailto:pallini@ufv.br)*

<sup>2</sup> *Econtrole Research & Consulting Ltda., Technological Center of Regional Development of Viçosa (CenTev), Federal University of Viçosa, Viçosa-MG, 36570-900, Brazil, E-mail: [felipelemosufv@yahoo.com.br](mailto:felipelemosufv@yahoo.com.br), [fcolaesbatista@yahoo.com.br](mailto:fcolaesbatista@yahoo.com.br), [joao.ferreira@outlook.com](mailto:joao.ferreira@outlook.com)*

<sup>3</sup> *Department of Zoology and Botany, São Paulo State University, São José do Rio Preto-SP, 15054-000, Brazil. E-mail: [aclofego@ig.com.br](mailto:aclofego@ig.com.br)*

\*Corresponding author:  
[henryeduar2@hotmail.com](mailto:henryeduar2@hotmail.com)  
Tel. (55)(31) 3899-2472

*Phytonemus pallidus* (Banks) (Acari: Tarsonemidae), also known as cyclamen mite, is a phytophagous mite commonly associated with strawberry crops in several countries, where it infests young leaves, flowers, and fruits of the plants (Smith & Goldsmith 1936; Alford 1972; Easterbrook et al. 2001; Petrova et al. 2002; Berglund et al. 2007; Gobin & Bangels 2008; Tuovinen & Lindqvist 2010). Severe infestations of *P. pallidus* lead to a reduction in the development of petioles, which become brown or brittle, and results in dwarfing of the strawberry plants (Smith & Goldsmith 1936; Croft et al. 1998; Fadini et al. 2004). The infested fruits present a reduced size and a brownish color, and are unsuitable for harvest (Pallini et al. 2002; Gobin & Bangels 2008).

In Brazil, strawberry is a very important crop due to the economic return it generates (Antunes et al. 2007). The leading producing states are Minas Gerais, Rio Grande do Sul, and São Paulo (IBGE 2006; Antunes et al. 2007; Henz 2010). There are several pests and diseases that attack strawberry crops in Brazil; however, to date, there are no reports of *P. pallidus* in this crop, although it has been reported on grapes and cyclamen (Moraes & Flechtmann 2008; Oliveira Andrade-Bertolo et al. 2013).

In two strawberry-producing localities in the municipalities of Ervália and Canaã (latitude: 20.842778°S - 42.675306°W; 20.706853 °S - 42.648578 °W), we found strawberry plants with the typical symptoms caused by *P. pallidus*: brownish leaves and fruits and an apparent reduction in size (Fig. 1 B). We monitored the plants in greenhouses at both localities for several weeks (from November 2014 until March of 2015). The estimated losses caused by *P. pallidus* ranges from 22-50% of strawberry yields (Alford 1972; Stenseth & Nordby 1976); however, we observed losses of 100% in production due to the attack of this pest in Minas Gerais (personal observations) (Fig. 1 A).

During the monitoring period, we collected plant material (leaves and fruits) and packed it into paper bags for transporting to the laboratory and performed further analysis. Under a stereomicroscope (Stemi 2000 Zeiss, Göttingen, Germany) (at 5x) the fruits and leaves were examined. We observed mites in different stages of development (eggs, juveniles and adults) on both leaves and fruits, with more individuals observed on the latter (Fig. 2 A, B and C). Additionally, adults were observed walking on the surface and in the crevices of the fruits.

To confirm the species identity, individuals were collected, mounted on slides, and sent to a specialist. After analysis by a taxonomist (A. C. Lofego) based on morphological characteristics, it was identified as *P. pallidus*. Diagnostic features observed in females were: stigmata located post laterally and very close to seta *v1*; sejugal apodeme not visible; post-sternal apodeme reduced; seta *pl*" (spike) absent in tarsus II; Apodeme 4 extending postero-lateral relative to the base of seta 3b; Tergite setae (*c1*, *c2*, *d*, *e*, *f*, *h*) with similar sizes, between 8 and 12µm, except for *c1*, which is thin smooth, the others are slightly thickened and serrated (Fig. 2 D, E and F).

The failure of *P. pallidus* to be reported earlier in the principal strawberry-producing areas in Brazil does not necessarily mean it was not present. Here we report the presence of this mite infesting and causing severe losses to growers of Minas Gerais. We believe that from now on more attention should be paid to the presence and possible economic losses caused by *P. pallidus* on other producing areas of strawberry in Brazil.

The severity of the attack of *P. pallidus* to strawberry crops around the world is unquestionable. The key management approach for *P. pallidus* management is the acquisition and planting of strawberry plants that are free of the pest. Once *P. pallidus* is already established on strawberry, its suppression is more complex due to its tendency to seek refuge under the calyxes or within the crevices of the fruits, where they are less

conspicuous and somewhat protected from insecticides. Furthermore, no commercial acaricide is registered for the control of *P. pallidus* in Brazil. For this reason, the search for alternative methods of control that are compatible with organic farming systems should be considered as a priority measure. The use of natural enemies for the management of *P. pallidus* on strawberry crop in Brazil could constitute a sustainable alternative, as this approach is already practiced in other countries (Smith & Goldsmith 1936; Huffaker & Spitzer 1951; Huffaker & Kennett 1956; Stenseth & Nordby 1976; Croft et al. 1998; Easterbrook et al. 2001; Petrova et al. 2002; Tuovinen & Lindqvist 2010).

### **Acknowledgments**

We are grateful to Minas Gerais Research Support Foundation - FAPEMIG (Project number APQ-00479-16), Coordination for the Improvement of Higher Education Personnel (CAPES), and National Council of Science and Technological Development (CNPq), Econrole Research & Consulting Ltda., and to the strawberry growers José Vanderley Rezende Ferreira and Henrique Leandro Machado. HEVA was supported by the Students-Postgraduate Agreement Program - PEC-PG, CAPES / CNPq – Brazil and AP by FAPEMIG and CNPq.

### **Summary**

*Phytonemus pallidus* was found in organic strawberry crops in two municipalities at the state of Minas Gerais. The little information about this pest in the country indicated that *P. pallidus* had been observed previously only on grapes in southern Brazil. The presence of this pest in the country is relatively new, although in the USA and in some European countries it is already well known due to the economic losses it causes. The report of the

occurrence of this herbivore, as well as the affected crops, is essential for the official institutions to take appropriate measures to prevent dispersion to other states. Future studies should be directed to the search for effective alternatives for the management of this pest because currently there are no insecticides registered for control it in Brazil. An alternative that could be considered is biological control.

**Keywords:** cyclamen mite, first report, organic strawberry crops, phytophagous mite.

### **Sumário**

*Phytonemus pallidus* foi encontrado em cultivos de morango em dois municípios pertencentes à zona da Mata Mineira. A pouca informação existente sobre esta praga indicava que *P. pallidus* estava presente apenas na cultura da videira no sul do Brasil. A presença desta praga no país é relativamente recente, já nos EUA e em alguns países da Europa ela é bem conhecida devido às perdas econômicas que pode ocasionar. O relato da ocorrência deste herbívoro nas diferentes regiões do país, bem como das culturas afetadas, é fundamental para que as instituições responsáveis tomem as medidas cabíveis para evitar a sua dispersão para outros estados da federação onde também se cultiva morango. Futuros estudos deveriam estar direcionados na busca de alternativas efetivas para o manejo adequado desta praga, uma vez que não há acaricidas registrados para o seu controle até o momento. Uma alternativa que poderia ser considerada é o controle biológico.

**Palavras chaves:** Ácaro do enfezamento; primeiro relato; cultivos de morango orgânico, ácaro fitófago.

## References Cited

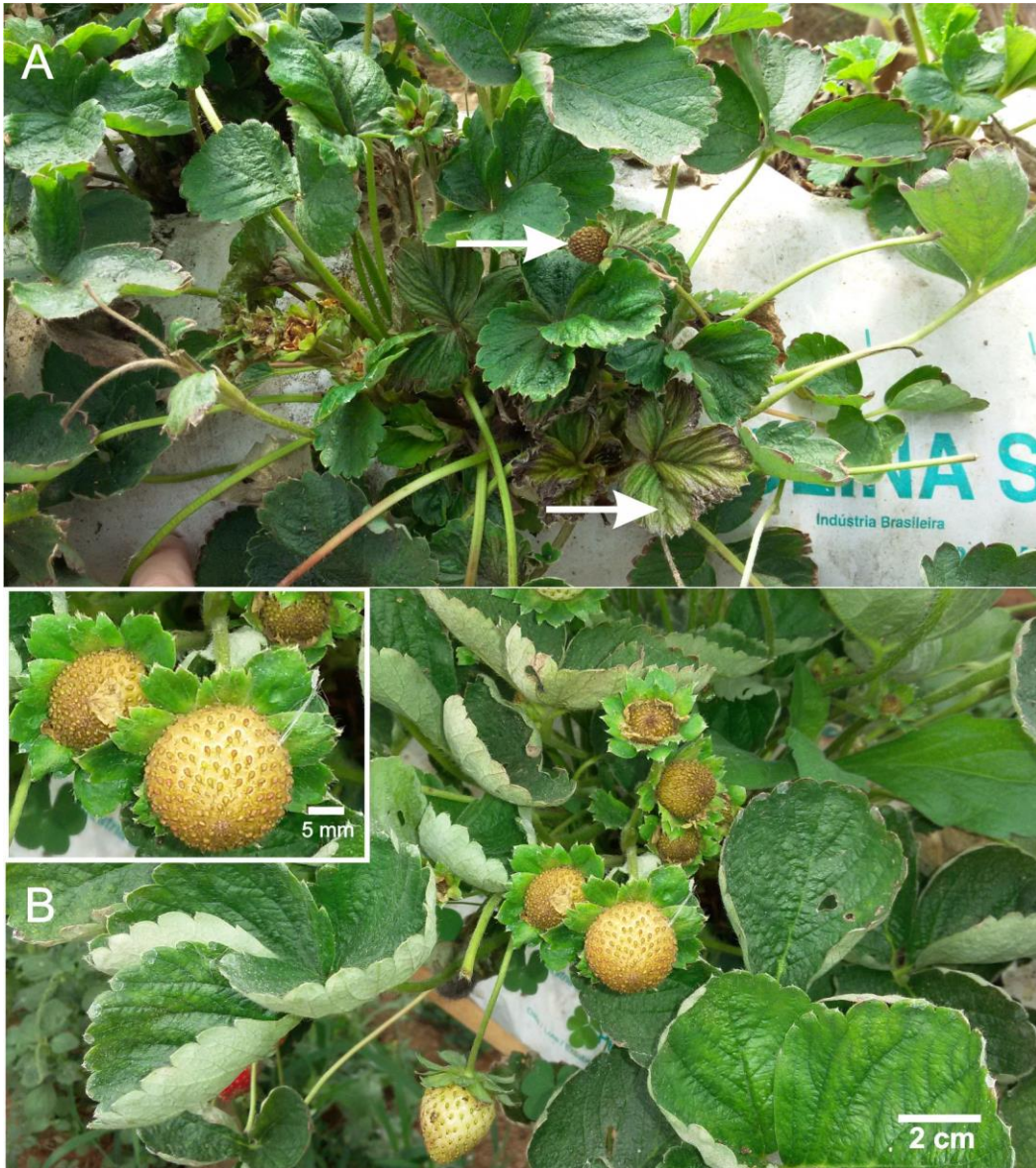
- Alford D. 1972. The effect of *Tarsonemus fragariae* Zimmermann (Acarina: Tarsonemidae) on strawberry yields. *Annals of Applied Biology* 70: 13–18.
- Antunes L, Duarte Filho J, Calegario F, Costa H, Reisser Junior C. 2007. Produção integrada de morango (PIMo) no Brasil. *Morango: Conquistando novas fronteiras. Informe Agropecuário: Belo Horizonte* 28: 34–39.
- Berglund R, Svensson B, Nilsson C. 2007. Evaluation of methods to control *Phytonemus pallidus* and *Anthonomus rubi* in organic strawberry production. *Journal of Applied Entomology* 131: 573–578.
- Croft B, Pratt P, Koskela G, Kaufman D. 1998. Predation, reproduction, and impact of phytoseiid mites (Acari: Phytoseiidae) on cyclamen mite (Acari: Tarsonemidae) on strawberry. *Journal of Economic Entomology* 91: 1307–1314.
- Easterbrook MA, Fitzgerald JD, Solomon MG. 2001. Biological control of strawberry tarsonemid mite *Phytonemus pallidus* and two-spotted spider mite *Tetranychus urticae* on strawberry in the UK using species of *Neoseiulus* (*Amblyseius*) (Acari: Phytoseiidae). *Experimental and Applied Acarology* 25: 25–36.
- Fadini MAM, Venzon M, Pallini A, Oliveira HG. 2004. Manejo ecológico de ácaros fitófagos na cultura do morangueiro. 2º Simpósio Nacional do Morango 1º Encontro de Pequenas Frutas e Frutas Nativas: 80.
- Gobin B, Bangels E. 2008. Field control of strawberry mite *Phytonemus pallidus*. *IOBC/WPRS Bulletins* 39: 97–100.
- Henz GP. 2010. Desafios enfrentados por agricultores familiares na produção de morango no Distrito Federal. *Horticultura Brasileira* 28: 260–265.
- Huffaker CB, Kennett CE. 1956. Experimental studies on predation: predation and cyclamen-mite populations on strawberries in California. *Hilgardia* 26: 191–222 pp.
- Huffaker CB, Spitzer CH Jr. 1951. Data on the natural control of the cyclamen mite on strawberries. *Journal of Economic Entomology* 44: 519–522.
- IBGE. 2006. Censo agropecuário 2006 - Brasil, grandes regiões e unidades da federação. 777pp.
- Moraes GJ, Flechtmann CHW. 2008. Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil. *Holos*.
- Oliveira Andrade-Bertolo F, Moura RB, Matioli AL, Ott AP. 2013. New records of mites (Acari: Prostigmata) on *Vitis sp.*(L.) in Brazil. *Comunicata Scientiae* 4: 414–418.

- Pallini A, Fadini M, Lemos W, Venzon M. 2002. Manejo integrado de ácaros em fruteiras tropicais e subtropicais, pp. 579–614 *In* Zambolin; L. Manejo integrado-fruteiras tropicais: Doenças e pragas. Viçosa: Universidade Federal de Viçosa.
- Petrova V, Cudare Z, Steinite I. 2002. The efficiency of the predatory mite *Amblyseius cucumeris* (Acari: Phytoseiidae) as a control agent of the strawberry mite *Phytonemus pallidus* (Acari: Tarsonemidae) on field strawberry, pp. 675–678 *In* Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium.
- Smith LM, Goldsmith EV. 1936. The cyclamen mite, *Tarsonemus pallidus*, and its control on field strawberries. *Hilgardia* 10: 53–94.
- Stenseth C, Nordby ALF. 1976. Damage, and control of the strawberry mite *Steneotarsonemus pallidus* (Acarina: Tarsonemidae), on strawberries. *Journal of Horticultural Science* 51: 49–24.
- Tuovinen T, Lindqvist I. 2010. Maintenance of predatory phytoseiid mites for preventive control of strawberry tarsonemid mite *Phytonemus pallidus* in strawberry plant propagation. *Biological Control* 54: 119–125.

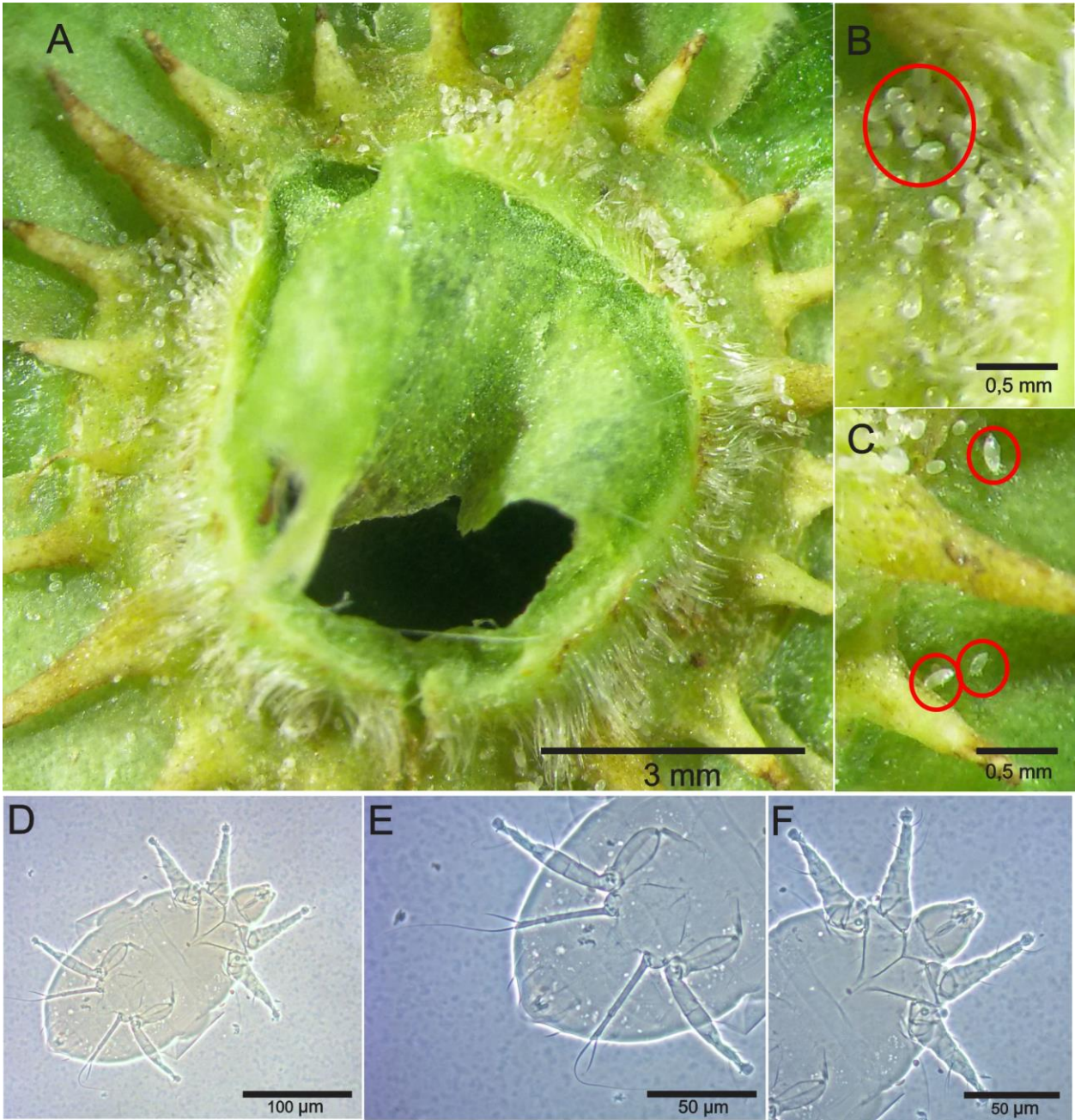
## Figure Caption

**Fig. 1.** Strawberry plant damaged by *Phytonemus pallidus* in the field. Arrows indicate typical "tanning" damage in leaves and fruits (A); strawberry plants infested by the phytophagous mite *P. pallidus* (B). Inset, a magnification showing the tanning on the fruits.

**Fig. 2.** Calyx of strawberry fruit infested with *Phytonemus pallidus* mite (A); *P. pallidus* eggs (B); juveniles and adults on the surface of the calyx (C); ventral view of a *P. pallidus* female under microscope (20x) (D); idiosoma (40x) (E); proterosoma (40x) (F).



**Figure 1.**



**Figure 2.**

## CHAPTER 2

# **Supplementary food for *Neoseiulus californicus* boosts biological control of *Tetranychus urticae* on strawberry**

Henry E. Vacacela Ajila<sup>1,\*</sup>, Felipe Colares<sup>2</sup>, Felipe Lemos<sup>2,4</sup>, Pedro H. Marques<sup>1</sup>, Eliza C. Franklin<sup>1</sup>, Willian Santos do Vale<sup>1</sup>, Eugênio E. Oliveira<sup>1</sup>, Madelaine Venzon<sup>3</sup>,  
Angelo Pallini<sup>1</sup>

*Pest Management Science* (2018)  
(Submitted)

Address:

<sup>1</sup>*Department of Entomology, Section Acarology, Federal University of Viçosa, Viçosa, MG, 36570-900, Brazil*

<sup>2</sup>*Econtrole Research & Consulting Ltda., Technological Center of Regional Development of Viçosa (CenTev), Federal University of Viçosa, Viçosa-MG, 36570-900, Brazil*

<sup>3</sup>*Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), Vila Gianetti 46, 36570-000, Viçosa, Minas Gerais, Brazil*

<sup>4</sup>*Federal University for Latin American Integration (UNILA), Foz do Iguaçu, Brazil*

**Running title:** *N. californicus: supplementary food and population dynamics*

\*Corresponding author:  
Henry E. Vacacela Ajila  
Tel. (55)(31) 3899-2472  
E-mail: henryeduar2@hotmail.com

## ABSTRACT

**BACKGROUND:** A wide range of alternative food resources can be offered to generalist predatory mites to improve their survival and reproduction and enhance their persistence in the environment. We investigated the relative suitability of different pollen types for the survival and reproduction of *Neoseiulus californicus* and its life history on a mixed diet of pollen and prey. Also, we evaluated the population dynamics of *Tetranychus urticae* in the presence of *N. californicus* with or without pollen, and in comparison to *Phytoseiulus macropilis*.

**RESULTS:** Survival and reproduction of *N. californicus* adults were satisfactory on exclusive diets of *T. urticae*, cattail pollen, palm pollen and bee pollen. However, mites did not reproduce when fed only pollen during both immature and adult stages. Both predatory mites successfully controlled *T. urticae*. Although the specialist *P. macropilis* was more efficient in suppressing the pest population in the short term, it abandoned plants at low prey densities, whereas *N. californicus* performed better and persisted longer under prey scarcity.

**CONCLUSION:** *N. californicus* is an efficient biological control agent of *T. urticae* and the provision of supplementary food could retain them in the crop prior to the arrival of the pest or in periods of prey scarcity.

**Keywords:** generalist predator, development, mixed diet, food sources, pollen

## 1 INTRODUCTION

Predatory arthropods may use a variety of alternative or supplementary food resources to aid their survival and reproduction in the absence of their primary prey.<sup>1-5</sup> Important plant-based resources include pollen and nectar<sup>6-8</sup>, and these have the potential to be manipulated by humans to favour natural enemies and improve the biological control of agricultural pests.<sup>9</sup>

Alternative food resources can be nutritionally limiting for predators<sup>2,5,10-14</sup>, or they can support high rates of development and reproduction.<sup>2,3,5,6,10,12,14-23</sup> Some supplementary foods may enable only predator survival while prey are unavailable.<sup>2,13,24-26</sup> Moreover, different life stages may have different nutritional requirements, rendering certain foods differentially suitable for immatures and adults.<sup>11,27</sup> Furthermore, some generalist predators may require a mixture of primary and supplementary foods to achieve optimal biological performance.<sup>10,15,28</sup>

The family Phytoseiidae contains most of the predatory mite species used to control herbivorous mites and insects.<sup>3,18,19,21,29,30</sup> Most phytoseiid mites do not restrict their diet to a single type of food.<sup>2,5,7,12-14,31-33</sup> Although most species are generalist predators and many are omnivores, feeding on pollen, nectar and even leaf tissue, some species are exclusively carnivorous. The predatory mites *Phytoseiulus persimilis* (Athias-Henriot) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) are often employed to control outbreaks of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) on various crops around the world.<sup>34-38</sup> Despite a high capacity to reduce pest populations, these predators persist poorly at low prey densities.<sup>30,38-40</sup> Generalist or omnivorous natural enemies able to feed on a broader range of foods may not be so constrained.

Predatory mites of the genus *Neoseiulus* prefer phytophagous mites as prey but may also feed on thrips and pollen.<sup>4-6,10,16</sup> One species, *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae), is an outstanding biological control agent of *T. urticae*, one of the major pests of strawberry worldwide.<sup>5,13,41-46</sup> *Neoseiulus californicus* occurs naturally on strawberry plants in Brazil<sup>47</sup> and is sometimes augmented by strawberry growers, in combination with *P. macropilis*, for control of *T. urticae*.<sup>37,48</sup> However, the efficiency of *T. urticae* biological control might be improved if *N. californicus* could be retained in the crop in the absence of the primary pest, possibly by provisioning pollen as an alternative food source.

Many plants produce abundant pollen of high nutritional value that is consumed by predatory mites when prey is absent.<sup>12,33,49,50</sup> Important plant species in this regard are cherry, peach, apricot, walnut, poppy<sup>33</sup>, castor bean (*Ricinus communis* L.)<sup>49,51,52</sup> and cattail (*Typha* sp.).<sup>19-21,23,26</sup> Bee pollen, which usually is a mixture of pollen from different plant species, has often been utilized in laboratory cultures.<sup>50,53,54</sup> In Brazil, castor bean, cattail and bee pollen are widely available in nature, and bee pollen is commercially available worldwide. However, phytoseiid performance can vary greatly among pollen sources, as these can vary widely in nutritional content.<sup>2,6,12,39,40,52</sup>

The present study examined the suitability of different pollen sources as supplementary foods for the predatory mite *N. californicus* when populations of *T. urticae* are low. We investigated the relative suitability of different pollen types for *N. californicus* survival and reproduction, and the life history impacts of a mixed diet of pollen and prey. In a separate experiment, we assayed the population dynamics of *T. urticae* on strawberry plants in the presence of the *N. californicus*, with or without pollen, and in comparison to the specialist predatory mite *P. macropilis*.

## 2 MATERIALS AND METHODS

### 2.1 Plants

Seedlings of the commercial strawberry cultivar “Portola” were transplanted to plastic pots (3 L) filled with commercial potting soil (Bioplant®) and kept in a greenhouse at  $25 \pm 7^\circ\text{C}$ ,  $70\% \pm 20\%$  RH. The plants were watered twice a day and fertilised with ammonium sulphate (21%; 0.83 g/plant) and potassium chloride (60%; 0.42 g/plant) every fifteen days and N-P-K (3-17-0; 6 g/plant) every thirty days. Leaves of these plants were collected for use in experiments. Seeds of jack bean (*Canavalia ensiformis*) were sown in plastic pots in the same way and watered twice daily. No plants were ever exposed to any pesticides.

### 2.2 Mites

All mite colonies were held in a climatically-controlled breeding room at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and 12:12 (L:D) photoperiod. Twelve days after germination, jack bean plants were transferred to the room and inoculated with *T. urticae*-infested leaves. Bean leaves with symptoms of mite feeding were selected for harvest from these plants and used to feed the predatory mites *N. californicus* and *P. macropilis*, or to supply spider mites used in experiments.

A laboratory population of *N. californicus* was initiated with individuals collected from pesticide-free strawberry plantations in the municipality of Ervália in the state of Minas Gerais, Brazil ( $20^\circ50'34.0''\text{S}$  -  $42^\circ40'31.1''\text{W}$ ). Leaves and fruits of strawberry plants with symptoms of phytophagous mite infestation were collected, packed in paper

bags, and transported to the laboratory where they were inspected under a stereomicroscope. Predatory mites were collected using a camel hair brush and confined in plastic trays (44 x 30 x 8 cm) containing leaves of jack bean infested with *T. urticae*. The plastic trays were set inside larger trays (60 x 40 x 10 cm) containing a solution of water plus detergent to preventing mite escape. Colonies of predators were fed three times per week, each tray receiving three leaves of *T. urticae*-infested jack bean.

Individuals of *P. macropilis* were obtained from an established stock-culture held in the Laboratory of Acarology at the Federal University of Viçosa, Minas Gerais-Brazil, and fed with *T. urticae* in the same manner as described above for *N. californicus*.

Synchronous cohorts of predatory mites for use in experiments were obtained by collecting adult females from the colonies and transferring them to rectangular arenas (7 x 8 cm) cut from jack bean leaves infested with all life stages of *T. urticae*. The arenas with leaves were placed on a black plastic sheet (11 x 19 cm), which had previously been placed over a sponge soaked in water inside a plastic tray (15.5 x 22 cm). The edges of the plastic sheets were covered with wet paper tissues to prevent mite escape. The females were removed after 24 h, and the arenas with eggs were provisioned with *T. urticae*-infested jack bean leaves every other day until the mites reached adulthood.

### **2.3 Supplementary foods**

Three supplementary foods were tested with *N. californicus*. Cattail pollen was collected from *Typha* sp. plants in Viçosa, MG. Palm pollen was collected from *Elaeis* sp. plants on the campus of the Federal University of Viçosa. The cattail pollen was dried for 24 hours at 60°C in a laboratory incubator (411/FDP Ethik Technology), and pollen of both plant species was stored in the freezer at -20°C for a maximum of ca. one year prior to

use in experiments. Commercial bee pollen (Santa Bárbara® dehydrated pollen, Santa Bárbara, MG) was obtained that consisted of a mixture of pollens from *Eucalyptus* sp., *Bidens pilosa* L., *Citrus* sp., *Angycus* sp. and *Ricinus* sp.

## 2.4 Experimental procedures

### 2.4.1 Suitability of supplementary foods for *N. californicus*

In this experiment, females of *N. californicus* were fed on different diets and their biological performance compared by recording their oviposition and survival. The experiment was performed on arenas consisting of strawberry leaf discs (2.8 cm Ø), each placed over an agar/water solution (1 g agar in 10 mL of water) in a Petri dish (6.0 x 1.5 cm). Immediately after the Petri dish was filled with the solution, a strawberry leaf disc was floated in the center of the dish. After the solution solidified, a moat was formed around each leaf disc by removing agar and filling the space with distilled water to prevent mite escape and keep the leaf disc hydrated and turgid. Next, ten female *T. urticae* were placed into each arena and arenas were assigned to one of 6 treatments: (i) *T. urticae* + cattail pollen (n = 20), (ii) *T. urticae* (n = 21), (iii) cattail pollen (n = 23), (iv) palm pollen (n = 21), (v) bee pollen (n = 16) and (vi) no food (starvation) (n = 19). Each arena was then infested with a single, mated, 14 d-old *N. californicus* female. Pollen was renewed every 48 hours by placing ca. 0.412 mg on each leaf disc and leaf discs were changed whenever deterioration became apparent. The only mixed diet tested was *T. urticae* + cattail pollen because preliminary tests indicated this pollen was highly suitable for *N. californicus*. The numbers of eggs laid per female were recorded for eight consecutive

days, discarding data of the first oviposition day to remove any effect of the previous diet.<sup>55</sup> Female survival was monitored for 16 days.

The fecundity of *N. californicus* on the different treatments was analyzed by Generalized Linear Model (GLM) with quasi-Poisson error distribution.<sup>56</sup> Treatment means were separated by stepwise deletion of nonsignificant terms in the model.<sup>57</sup> Survival data was submitted to Kaplan-Meier survival analysis and curves were contrasted by comparison of their means.<sup>56</sup>

#### **2.4.2 Development and reproduction of *N. californicus* on *T. urticae* and cattail pollen**

Because cattail pollen emerged as the most suitable supplementary food for *N. californicus*, we examined the development and reproduction of *N. californicus* when fed on *T. urticae* (n = 17), on cattail pollen (n = 13), or a combination of both (n = 16). The experiment was conducted on the same leaf disc arenas as described above. Two mated, 12 d-old females of *N. californicus* were released on each leaf disc and removed after six hours to leave only one egg of *N. californicus*; leaf discs where females laid no eggs within this period were discarded. After eclosion, arenas were observed three times daily to record developmental times and survival of the mites until they molted to adults. Immature transitions were inferred by the presence of exuviae on leaf discs.

Female *N. californicus* were collected from the colony and released on arenas (as described above) containing jack bean leaves infested with *T. urticae*. After 24 hours, eggs were gently transferred to new arenas of jack bean leaves which were each assigned to one of three diet treatments: *T. urticae*, cattail pollen, or a mixture of *T. urticae* and cattail pollen. After the immature mites reached adulthood (approximately eight days

later), females of each treatment were isolated on strawberry leaf discs (2.8 cm Ø), *T. urticae* n = 15, cattail pollen n = 15, and mixed diet n = 23, and a male from the colony was added to each arena. The adult *N. californicus* received the same diet that they received during immature development. The number of eggs laid by each female was tallied daily for ten consecutive days. Because females receiving only pollen did not lay eggs within this period, we provisioned them with 10 *T. urticae* females each and tallied oviposition for five more days to verify that these females could restore their fecundity once prey was obtained. The development rate (days) and the number of eggs laid by *N. californicus* were compared between treatments using Generalized Linear Models (GLM) with Poisson error distribution. Treatments means were separated by stepwise deletion of terms in the model. All analyses were performed using the R statistical software.<sup>56</sup>

### **2.4.3 Population dynamics of *T. urticae* and predatory mites on strawberry plants in the greenhouse**

This experiment assayed the suppression of *T. urticae* by *N. californicus*, with and without pollen, in comparison to the specialist predator *P. macropilis* using four treatments: T1) *N. californicus*; T2) *P. macropilis*; T3) *N. californicus* + cattail pollen; and T4) *T. urticae* alone (control). Strawberry plants (n = 20 per treatment) ca. 45 days old (with 3 to 4 fully expanded leaves) were each infested with 100 mated females of *T. urticae*. These mites were collected from the stock colony using a fine brush and scattered over each plant (about 25-30 females per leaf). After seven days, spider mites numbers were assessed by cutting the leaves of four strawberry plants from each treatment group and counting all mite life stages under a binocular microscope. Then, four 14 d-old, mated, female predatory mites were released on each strawberry plant. Due to the reported

efficiency of *P. macropilis* as a biological control agent of *T. urticae*<sup>38</sup>, this species was used as a positive control. Destructive evaluations were made weekly by selecting four strawberry plants at random from each treatment and counting all developmental stages of pest and predator. The experiment continued for one month, or until either pest or predator became extinct in a replicate.

The total numbers of all life stages of *T. urticae* and *N. californicus* (or *P. macropilis*) were log transformed ( $x + 100$  for phytophagous mites and  $x + 50$  for predatory mites) and compared between treatments using a linear mixed effects model (LME, package nlme of R<sup>58</sup>). Treatment was considered an independent fixed factor and numbers of mites and day were considered random factors. Means were separated between treatments with general linear hypothesis testing (function glht of the package lsmeans in R<sup>59</sup>). All analyses were performed using R statistical software.<sup>56</sup>

### **3 RESULTS**

#### **3.1 Suitability of supplementary foods for *N. californicus***

The survival of *N. californicus* differed significantly among diets (Likelihood ratio test = 106, d.f. = 5,  $P < 0.001$ ), but only due to progressive mortality in the starvation treatment, which began on day eight of the experiment and reached 13.3% on day 16 (Fig. 1). Survival in all other diet treatments was similar and close to 100% over the period of the experiment (Likelihood ratio test = 4.1, d.f. = 4,  $P = 0.38$ ). Oviposition was also significantly affected by diet (GLM,  $F = 880.42$ , d.f. = 5,  $P < 0.001$ ), with the highest oviposition rate observed on a mixed diet of *T. urticae* + cattail pollen, and the lowest on bee pollen (Fig. 2). As expected, starved predators did not lay eggs.

### **3.2 Development and reproduction of *N. californicus* on *T. urticae* and cattail pollen**

The developmental period of *N. californicus* (from egg to adult) differed significantly among diets (GLM,  $\chi^2 = 8.49$ , d.f. = 2,  $P = 0.014$ ), being shorter on *T. urticae* and the mixed diet than when predators fed only on cattail pollen (Table 1). The oviposition rate of *N. californicus* fed the mixed diet was not significantly different from those fed solely *T. urticae* during the ten-day evaluation period (GLM,  $\chi^2 = 7.12$ , d.f. = 1,  $P = 0.57$ ). Oviposition peaked between days two and five, when females laid slightly more than three eggs per day (Fig. 3). Females fed only on cattail pollen during both the immature and adult stages did not oviposit, but after receiving *T. urticae* on day ten, their fertility was restored and peaked four days later.

### **3.3 Population dynamics of *T. urticae* and predatory mites on strawberry plants in the greenhouse**

The population of *T. urticae* became significantly different among treatments two weeks after the release of predators (LME,  $\chi^2 = 13.64$ , d.f. = 3,  $P < 0.001$ , Fig. 4) when plants inoculated with *P. macropilis* had the fewest *T. urticae*, and controls without predators, the most. The *P. macropilis* treatment drove the spider mites to extinction first, followed by the *N. californicus* + pollen treatment, whereas spider mites did not go extinct in either of the other treatments.

Numbers of predatory mites peaked 14 days after their release on plants, after which time the numbers of *P. macropilis* decreased more rapidly than did the number of predator life stages in other treatments, which became significantly different on day 28 (LME,  $\chi^2 = 0.56$ ,  $df = 2$ ,  $P < 0.001$ , Fig. 5). The numbers of predator life stages in *N.*

*californicus* in the treatment with pollen declined more slowly than in the treatment without pollen, and at 35 days after release, significantly more *N. californicus* remained alive on plants with pollen than without (LME,  $\chi^2 = 0.02$ , d.f. = 1, P = 0.024).

#### 4 DISCUSSION

The pollen sources we tested sustained the survival of *N. californicus* as well as an exclusive diet of its primary prey species, *T. urticae*. However, these food sources differed in quality when developmental time and oviposition were considered. All pollen sources tested provided nutritional supplementation for *N. californicus*, with cattail pollen the most suitable, and commercial bee pollen the least. Thus, supplementary foods could potentially support the persistence of *N. californicus* in strawberry fields prior to the arrival and establishment of prey. This could improve retention of the predator in fields at low spider mite densities and thus increase the efficiency of biological control. This result illustrates how the ability of generalist predatory mites to exploit supplementary foods can confer to them advantages over specialist species that are entirely dependent upon prey.<sup>25,37,38</sup> In addition, generalists have the potential to control more than one pest species, which could be advantageous to growers in some circumstances.

In some cases, a mixed diet that includes supplementary foods can be more beneficial for the performance of generalist predators than an exclusive prey diet.<sup>28,60</sup> The generalist predator *Euseius scutalis* (Acari: Phytoseiidae) had a higher rate of oviposition on a mixed diet (spider mites + anthers of almond) than it did on a monotypic diet.<sup>61</sup> Here, we observed that predatory mites fed on a mixed diet of *T. urticae* plus cattail pollen had a similar oviposition rate compared to females fed exclusively on *T. urticae*. However, considering the total number of eggs laid over seven days, the oviposition of *N.*

*californicus* was improved by feeding on cattail pollen in the presence of *T. urticae* compared to all other monotypic diets evaluated. Similarly, the oviposition rate of *Amblyseius swirskii* (Acari: Phytoseiidae) was higher when feeding on eriophyid mites in the presence of castor bean pollen, or feeding only on eriophyid mites than when feeding only on pollen.<sup>62</sup>

We observed that, although *N. californicus* successfully completed all developmental stages on a diet of cattail pollen, *T. urticae* or on a mixture of both, development was fastest on *T. urticae* or the mixed diet of *T. urticae* plus cattail pollen. The suitability of a food source for a predator depends on both the nutritional content of the food and the nutritional requirement of the predator. An exclusive pollen diet is known to support the survival, development and reproduction of some phytoseiid species.<sup>2,3,5,6,10,12,14,15,17-23,63,64</sup> It has been already demonstrated that *N. californicus* could survive, develop and oviposit successfully on a diet of almond pollen and maize pollen.<sup>2</sup> Although *N. californicus* could complete its development and could survive feeding only on cattail pollen, individuals that received only cattail pollen during immature and adult stages did not oviposit. Similar results were observed when *N. californicus* fed on pollen of *Urtica urens*, *Lamium amplexicaule*, *Convolvulus arvensis*, *Sonchus oleraceus*, *Galega officinalis*, and strawberry.<sup>13</sup> Thus, in these cases pollen can be considered a supplementary food for *N. californicus*, as it does not support reproduction in this species. However, the fertility of pollen-fed mites was quickly restored once mites were provided *T. urticae* as prey, as observed previously for *N. californicus* fed on an artificial diet containing honey, sucrose, tryptone, yeast extract and egg yolk.<sup>65</sup> The nutritional requirements of larval and adult arthropod life stages can be different, and prey that may be suitable for larval development are not necessarily suitable for adult reproduction or vice versa.<sup>66</sup> Beneficial dietary complementation can occur across life stages when larvae

and adults consume different prey; when larvae of *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) were reared on moth eggs and then switched to greenbugs, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) as adults, both fecundity and fertility were higher compared to individuals that remained on the same diet.<sup>11</sup>

The specialist predator *P. macropilis* suppressed the population of *T. urticae* faster than *N. californicus*, leading to extinction of both pest and predator populations three weeks after predators were released on plants. The high efficiency of *P. macropilis* may explain its frequent use by strawberry growers.<sup>38</sup> However, the generalist *N. californicus* also controlled the *T. urticae* population on strawberry plants and its efficiency improved when cattail pollen was added to plants, suggesting that provision of supplementary food could improve the efficacy of *N. californicus* in biological control of *T. urticae* on strawberry. Although *N. californicus* took longer to suppress the *T. urticae* population compared to *P. macropilis*, it persisted longer on the plants after prey were reduced to low densities, especially in the treatment with cattail pollen. About 35 days after predator release, a considerable number of *N. californicus* mites remained alive, while no *P. macropilis* were observed on plants. Furthermore, on plants with cattail pollen, there were more *N. californicus* remaining than there were *T. urticae*, which suggests a low likelihood of pest resurgence. Similarly, pollen of wild vegetation surrounding the crop can contribute to the persistence of *N. californicus* and help the preventive biological control of *T. urticae*.<sup>13</sup> In contrast, *P. macropilis* is more prey-dependent and tends to start emigrating as soon prey densities decline.<sup>67–69</sup>

Our results indicate that provision of pollen as supplementary food for *N. californicus* will foster a higher population of this predator on strawberry plants and result in a lower population of spider mites. Other studies have supported the idea that supplementary food provisioning can improve the performance of generalist predatory

mites in biological control.<sup>2-5,12-14,18,19,21,22,31,32</sup> The addition of pollen on the diet of the predatory mite *Iphiseius degenerans* (Acari: Phytoseiidae) improved its efficiency in controlling *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae).<sup>7</sup> Similarly, when the predatory mite *Euseius hibisci* (Acari: Phytoseiidae) has access to pollen it is more efficient in suppressing *Oligonychus punicae* (Hirst) (Acari: Tetranychidae).<sup>31</sup> A combination of pollen and honeydew can be used to improve biological control of whiteflies by the predatory mites *E. scutalis* and *Typhlodromips swirskii* (Acari: Phytoseiidae).<sup>60</sup>

In summary, although *T. urticae* was the only food that supported the development, survival and reproduction of *N. californicus* as a monotypic diet, this species has the potential to contribute preventively to the biological control of spider mites. Provisioning of supplementary food such as pollen could serve to retain mites in the strawberry crop at low pest densities, either before their arrival, or after they have been to low numbers by predation. In addition, its generalist feeding habits endow it with the potential to exert biological control of other phytophagous mites that may occur in strawberry, such as the cyclamen mite, *Phytonemus pallidus* (Banks), (Acari: Tarsonemidae). Future studies are warranted to measure its efficacy against *P. pallidus* and other species in the field, and to clarify its other roles within the guild of acariphagous predators in strawberry.

## **ACKNOWLEDGEMENTS**

We are grateful to Students-Postgraduate Agreement Program - PEC-PG, CAPES/CNPq – Brazil, Minas Gerais Research Support Foundation (FAPEMIG), Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council of Science and Technological Development (CNPq) for the scholarships and the financial support. The technical assistance provided by the company ECONTROLE research and consulting, especially to the technical support provided by Igor Gomes, is gratefully acknowledged. We are also thankful to Dr. J.P. Michaud for assisting with English composition of the manuscript. HEVA was supported by the Students-Postgraduate Agreement Program - PEC-PG, CAPES/CNPq – Brazil, and MV, AP by FAPEMIG and CNPq.

## **AUTHOR CONTRIBUTIONS**

HEVA, FC, and AP conceived and designed the experiments. HEVA, FC, PHM, ECF, WSV performed the experiments. HEVA, FC and FL analyzed the data. AP and FC contributed reagents/materials/analysis tools. HEVA, FC, FL, MV, EEO, and AP wrote the paper. All authors read, revised and approved the manuscript.

## **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

## INFORMED CONSENT

All the authors of this manuscript accepted that the paper is submitted for publication in the *Pest Management Science* journal, and report that this paper has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

## REFERENCES

- 1 Sabelis MW and Van Rijn PC, When does alternative food promote biological pest control?, *IOBC WPRS Bull* **29**:195 (2006).
- 2 Khanamani M, Fathipour Y, Talebi AA, and Mehrabadi M, Linking pollen quality and performance of *Neoseiulus californicus* (Acari: Phytoseiidae) in two-spotted spider mite management programmes, *Pest Manag Sci* **73**:452–461 (2016).
- 3 Beltrà A, Calabuig A, Navarro-Campos C, José Ramírez-Soria M, Soto A, Garcia-Marí F, *et al.*, Provisioning of food supplements enhances the conservation of phytoseiid mites in citrus, *Biol Control* **115**:18–22 (2017).
- 4 Khanamani M, Fathipour Y, Asghar Talebi A, and Mehrabadi M, How pollen supplementary diet affect life table and predation capacity of *Neoseiulus californicus* on two-spotted spider mite, *Syst Appl Acarol* **22**:135–147 (2017).
- 5 Soltaniyan A, Kheradmand K, Fathipour Y, and Shirdel D, Suitability of pollen from different plant species as alternative food sources for *Neoseiulus californicus* (Acari: Phytoseiidae) in comparison with a natural prey, *J Econ Entomol* **111**:2046–2052 (2018).
- 6 Van Rijn PC and Tanigoshi LK, Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history, *Exp Appl Acarol* **23**:785–802 (1999).
- 7 Van Rijn PCJ, Van Houten Yvonne M., and Sabelis Maurice W., How plants benefit from providing food to predators even when it is also edible to herbivores, *Ecology* **83**:2664–2679 (2002).
- 8 Van Rijn PC and Sabelis MW, Impact of plant-provided food on herbivore-carnivore dynamics, *Plant-provided food for carnivorous insects: a protective mutualism and its applications*, Cambridge University Press, Cambridge, pp. 223–266 (2005).

- 9 Wäckers FL, van Rijn PCJ, and Bruin J, Plant-provided food for carnivorous insects: a protective mutualism and its applications, Cambridge University Press, Cambridge (2005).
- 10 Delisle JF, Brodeur J, and Shipp L, Evaluation of various types of supplemental food for two species of predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae), *Exp Appl Acarol* **65**:483–494 (2015).
- 11 Michaud J and Jyoti J, Dietary complementation across life stages in the polyphagous lady beetle *Coleomegilla maculata*, *Entomol Exp Appl* **126**:40–45 (2007).
- 12 Goleva I and Zebitz CPW, Suitability of different pollen as alternative food for the predatory mite *Amblyseius swirskii* (Acari, Phytoseiidae), *Exp Appl Acarol* **61**:259–283 (2013).
- 13 Gugole Ottaviano MF, Cédola CV, Sánchez NE, and Greco NM, Conservation biological control in strawberry: effect of different pollen on development, survival, and reproduction of *Neoseiulus californicus* (Acari: Phytoseiidae), *Exp Appl Acarol* **67**:507–521 (2015).
- 14 Kishimoto H, Ohira Y, and Adachi I, Effect of different plant pollens on the development and oviposition of seven native phytoseiid species (Acari: Phytoseiidae) in Japan, *Appl Entomol Zool* **49**:19–25 (2014).
- 15 Messelink GJ, Maanen R van, van Steenpaal SEF, and Janssen A, Biological control of thrips and whiteflies by a shared predator: Two pests are better than one, *Biol Control* **44**:372–379 (2008).
- 16 Delisle JF, Shipp L, and Brodeur J, Apple pollen as a supplemental food source for the control of western flower thrips by two predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae), on potted chrysanthemum, *Exp Appl Acarol* **65**:495–509 (2015).
- 17 Massaro M, Martin JPI, and de Moraes GJ, Factitious food for mass production of predaceous phytoseiid mites (Acari: Phytoseiidae) commonly found in Brazil, *Exp Appl Acarol* **70**:411–420 (2016).
- 18 Nomikou M, Sabelis MW, and Janssen A, Pollen subsidies promote whitefly control through the numerical response of predatory mites, *BioControl* **55**:253–260 (2010).
- 19 Lee MH and Zhang Z-Q, Assessing the augmentation of *Amblydromalus limonicus* with the supplementation of pollen, thread, and substrates to combat greenhouse whitefly populations, *Sci Rep* **8**:12189 (2018).
- 20 de Figueiredo ES, Massaro M, do Carmo S, and de Moraes GJ, Rearing system for the predatory phytoseiid *Euseius concordis* (Acari: Phytoseiidae), *Exp Appl Acarol* **74**:13–23 (2018).

- 21 Ghasemzadeh S, Leman A, and Messelink GJ, Biological control of *Echinothrips americanus* by phytoseiid predatory mites and the effect of pollen as supplemental food, *Exp Appl Acarol* **73**:209–221 (2017).
- 22 Warburg S, Inbar M, Gal S, Salomon M, Palevsky E, and Sadeh A, The effects of a windborne pollen-provisioning cover crop on the phytoseiid community in citrus orchards in Israel, *Pest Manag Sci* **0** (2018).
- 23 Park H-H, Shipp L, Buitenhuis R, and Ahn JJ, Life history parameters of a commercially available *Amblyseius swirskii* (Acari: Phytoseiidae) fed on cattail (*Typha latifolia*) pollen and tomato russet mite (*Aculops lycopersici*), *J Asia-Pac Entomol* **14**:497–501 (2011).
- 24 Van Houten YM and van Stratum P, Control of western flower thrips on sweet pepper in winter with *Amblyseius cucumeris* (Oudemans) and *A. degenerans* Berlese, *Thrips biology and management*, Springer, Boston, MA, pp. 245–248 (1995).
- 25 Muñoz-Cárdenas K, Fuentes LS, Cantor RF, Rodríguez CD, Janssen A, and Sabelis MW, Generalist red velvet mite predator (*Balaustium sp.*) performs better on a mixed diet, *Exp Appl Acarol* **62**:19–32 (2014).
- 26 Pijnakker J, Arijs Y, de Souza A, Cellier M, and Wäckers F, The use of *Typha angustifolia* (cattail) pollen to establish the predatory mites *Amblyseius swirskii*, *Iphiseius degenerans*, *Euseius ovalis* and *Euseius gallicus* in glasshouse crops, *IOBC WPRS Bull* **120**:47–54 (2016).
- 27 Vantornhout I, Minnaert H, Tirry L, and de Clercq P, Effect of pollen, natural prey and factitious prey on the development of *Iphiseius degenerans*, *BioControl* **49**:627–644 (2004).
- 28 Marques RV, Sarmiento RA, Lemos F, Pedro-Neto M, Sabelis MW, Venzon M, *et al.*, Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost, *Funct Ecol* **29**:1317–1324 (2015).
- 29 Tomczyk A and Kropczynska D, Effects on the host plant, Spider mites: their biology, natural enemies and control, Elsevier, Amsterdam, pp. 317–327 (1985).
- 30 Gerson U, Smiley RL, and Ochoa R, *Mites (Acari) for pest control*, Blackwell Science, Oxford, England (2003).
- 31 McMurtry J and Scriven G, The influence of pollen and prey density on the number of prey consumed by *Amblyseius hibisci* (Acarina: Phytoseiidae), *Ann Entomol Soc Am* **59**:147–149 (1966).
- 32 McMurtry JA, Dynamics and potential impact of “generalist” phytoseiids in agroecosystems and possibilities for establishment of exotic species, *Exp Appl Acarol* **14**:371–382 (1992).

- 33 Broufas GD and Koveos DS, Effect of different pollens on development, survivorship and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae), *Environ Entomol* **29**:743–749 (2000).
- 34 Simmonds SP, The possible control of *Steneotarsonemus pallidus* on strawberries by *Phytoseiulus persimilis*, *Plant Pathol* **19**:106–107 (1970).
- 35 Easterbrook MA, Fitzgerald JD, and Solomon MG, Biological control of strawberry tarsonemid mite *Phytonemus pallidus* and two-spotted spider mite *Tetranychus urticae* on strawberry in the UK using species of *Neoseiulus* (*Amblyseius*) (Acari: Phytoseiidae), *Exp Appl Acarol* **25**:25–36 (2001).
- 36 Rhodes EM and Liburd OE, Evaluation of predatory mites and acramite for control of twospotted spider mites in strawberries in North Central Florida, *J Econ Entomol* **99**:1291–1298 (2006).
- 37 Oliveira H, Janssen A, Pallini A, Venzon M, Fadini M, and Duarte V, A phytoseiid predator from the tropics as potential biological control agent for the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), *Biol Control* **42**:105–109 (2007).
- 38 Oliveira H, Fadini MAM, Venzon M, Rezende D, Rezende F, and Pallini A, Evaluation of the predatory mite *Phytoseiulus macropilis* (Acari: Phytoseiidae) as a biological control agent of the two-spotted spider mite on strawberry plants under greenhouse conditions, *Exp Appl Acarol* **47**:275–283 (2009).
- 39 McMurtry JA and Croft BA, Life-styles of phytoseiid mites and their roles in biological control, *Annu Rev Entomol* **42**:291–321 (1997).
- 40 Mcmurtry JA, Moraes GJD, and Sourassou NF, Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies, *Syst Appl Acarol* **18**:297–320 (2013).
- 41 Chiavegato L and Mischán M, Efeito do acaro *Tetranychus* (T.) *urticae* (Koch, 1836) Boudreaux & Dosse, 1963 (Acari, Tetranychidae) na produção do morangueiro (*Fragaria* spp.) cv. 'Campinas', *Científica* **9**:257–266 (1981).
- 42 Edge VE and James DG, Organo-tin Resistance in *Tetranychus urticae* (Acari: Tetranychidae) in Australia, *J Econ Entomol* **79**:1477–1483 (1986).
- 43 Greco NM, Sánchez NE, and Liljesthröm GG, *Neoseiulus californicus* (Acari: Phytoseiidae) as a potential control agent of *Tetranychus urticae* (Acari: Tetranychidae): effect of pest/predator ratio on pest abundance on strawberry, *Exp Appl Acarol* **37**:57–66 (2005).
- 44 Fraulo AB and Liburd OE, Biological control of twospotted spider mite, *Tetranychus urticae*, with predatory mite, *Neoseiulus californicus*, in strawberries, *Exp Appl Acarol* **43**:109 (2007).

- 45 Ahn JJ, Kim KW, and Lee JH, Functional response of *Neoseiulus californicus* (Acari: Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae) on strawberry leaves, *J Appl Entomol* **134**:98–104 (2010).
- 46 García-Marí F and Enrique González-Zamora J, Biological control of *Tetranychus urticae* (Acari: Tetranychidae) with naturally occurring predators in strawberry plantings in Valencia, Spain, *Exp Appl Acarol* **23**:487–495 (1999).
- 47 Ferla NJ, Marchetti MM, and Gonçalves D, Ácaros predadores (Acari) associados à cultura do morango (*Fragaria sp.*, Rosaceae) e plantas próximas no Estado do Rio Grande do Sul, *Biota Neotropica* **7**:1–8 (2007).
- 48 Sato ME, Da Silva MZ, De Souza Filho MF, Matioli AL, and Raga A, Management of *Tetranychus urticae* (Acari: Tetranychidae) in strawberry fields with *Neoseiulus californicus* (Acari: Phytoseiidae) and acaricides, *Exp Appl Acarol* **42**:107–120 (2007).
- 49 Reis PR and Alves EB, Criação do ácaro predador *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae) em laboratório, *Soc Entomol Bras* **26**:565–568 (1997).
- 50 Lofego A and Moraes G, Taxa de oviposição dos predadores *Amblyseius acalyphus* e *Amblyseius neochiapensis* (Acari: Phytoseiidae) com diferentes tipos de alimento, *Arq Inst Biol* **72**:379–382 (2005).
- 51 Yamamoto PT and Gravena S, Influência da temperatura e fontes de alimento no desenvolvimento e oviposição de *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae), *Soc Entomol Bras* **25**:109–115 (1996).
- 52 Rodríguez-Cruz FA, Venzon M, and Pinto CMF, Performance of *Amblyseius herbicolus* on broad mites and on castor bean and sunnhemp pollen, *Exp Appl Acarol* **60**:497–507 (2013).
- 53 Messelink G, Ramakers P, Cortez J, and Janssen A, How to enhance pest control by generalist predatory mites in greenhouse crops, presented at the Proceedings of the Third International Symposium on Biological Control of Arthropods, Christchurch, New Zealand, 8-13 February, 2009, 2009, 309–318.
- 54 Bellini MR, de Araujo RV, Silva ES, de Moraes GJ, and Berti Filho E, Ciclo de vida de *Proprioseiopsis cannaensis* (Muma) (Acari: Phytoseiidae) com diferentes tipos de alimentos, *Neotrop Entomol* **39**:360–364 (2010).
- 55 Sabelis MW, How to analyse prey preference when prey density varies? A new method to discriminate between effects of gut fullness and prey type composition, *Oecologia* **82**:289–298 (1990).
- 56 R-Development-Core-Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2014).
- 57 Crawley MJ, *The R book*, John Wiley & Sons (2012).

- 58 Pinheiro J, Bates D, DebRoy S, Sarkar D, and R Core Team, nlme: Linear and Nonlinear Mixed Effects Models, R Package Version 3:96 (2009).
- 59 Lenth RV, Least-squares means: the R package lsmeans, J Stat Softw 69:1–33 (2016).
- 60 Nomikou M, Janssen A, and Sabelis MW, Phytoseiid predators of whiteflies feed and reproduce on non-prey food sources, Exp Appl Acarol 31:15–26 (2003).
- 61 Swirski E, Amitai S, and Dorzia N, Laboratory studies on the feeding, development and reproduction of the predaceous mites *Amblyseius rubini* Swirski and Amitai and *Amblyseius swirski* Athias (Acarina: Phytoseiidae) on various kinds of food substances., Isr J Agric Res 17:101–119 (1967).
- 62 Ragusa S and Swirski E, Feeding habits, post-embryonic and adult survival, mating, virility and fecundity of the predacious mite *Amblyseius swirskii* [Acarina: Phytoseiidae] on some coccids and mealybugs, Entomophaga 22:383–392 (1977).
- 63 Dicke M and Sabelis MW, How plants obtain predatory mites as bodyguards, Neth J Zool 38:148–165 (1987).
- 64 Gerson U, Pest control by mites (Acari): present and future, Acarologia 54:371–394 (2014).
- 65 Ogawa Y and Osakabe M, Development, long-term survival, and the maintenance of fertility in *Neoseiulus californicus* (Acari: Phytoseiidae) reared on an artificial diet, Exp Appl Acarol 45:123–136 (2008).
- 66 Michaud J, On the assessment of prey suitability in aphidophagous Coccinellidae, Eur J Entomol 102:385 (2005).
- 67 Takafuji A, The effect of the rate of successful dispersal of a Phytoseiid mite, *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae) on the persistence in the interactive system between the predator and its prey, Res Popul Ecol 18:210–222 (1976).
- 68 Bernstein C, Prey and predator emigration responses in the acarine system *Tetranychus urticae*-*Phytoseiulus persimilis*, Oecologia 61:134–142 (1984).
- 69 Pels B and Sabelis MW, Local dynamics, overexploitation and predator dispersal in an acarine predator-prey system, Oikos 86:573–583 (1999).

## FIGURE LEGENDS

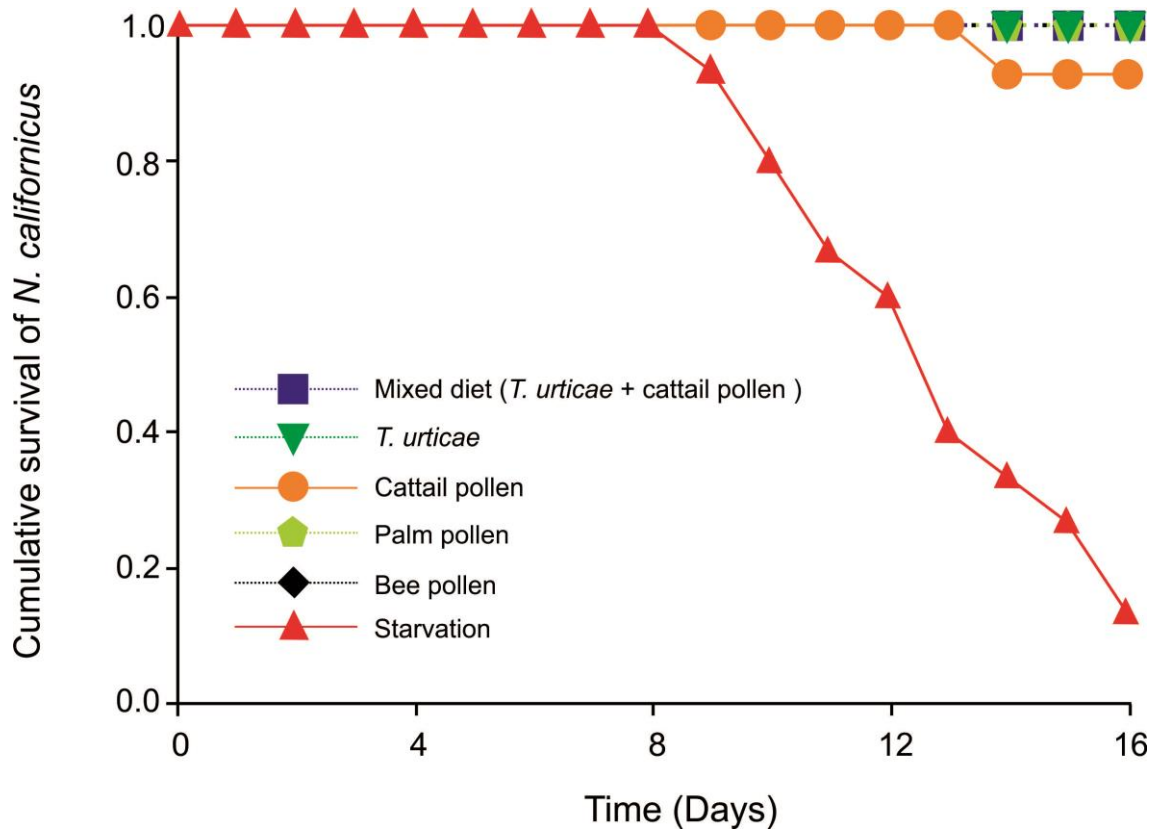
**Fig. 1** Survival of *Neoseiulus californicus* fed on mixed diet (*T. urticae* + cattail pollen) (blue squares), *T. urticae* (inverted green triangles), cattail pollen (orange circles), palm pollen (light green pentagons), bee pollen (close rhombus) and without food (red triangles) at laboratory, ( $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH and 12:12 h).

**Fig. 2** Number (mean  $\pm$  SE) of eggs of *Neoseiulus californicus* during 7 days with mixed diet (*Tetranychus urticae* + cattail pollen), *T. urticae*, cattail pollen, palm pollen, with bee pollen and without food at the laboratory ( $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH and 12:12 h).

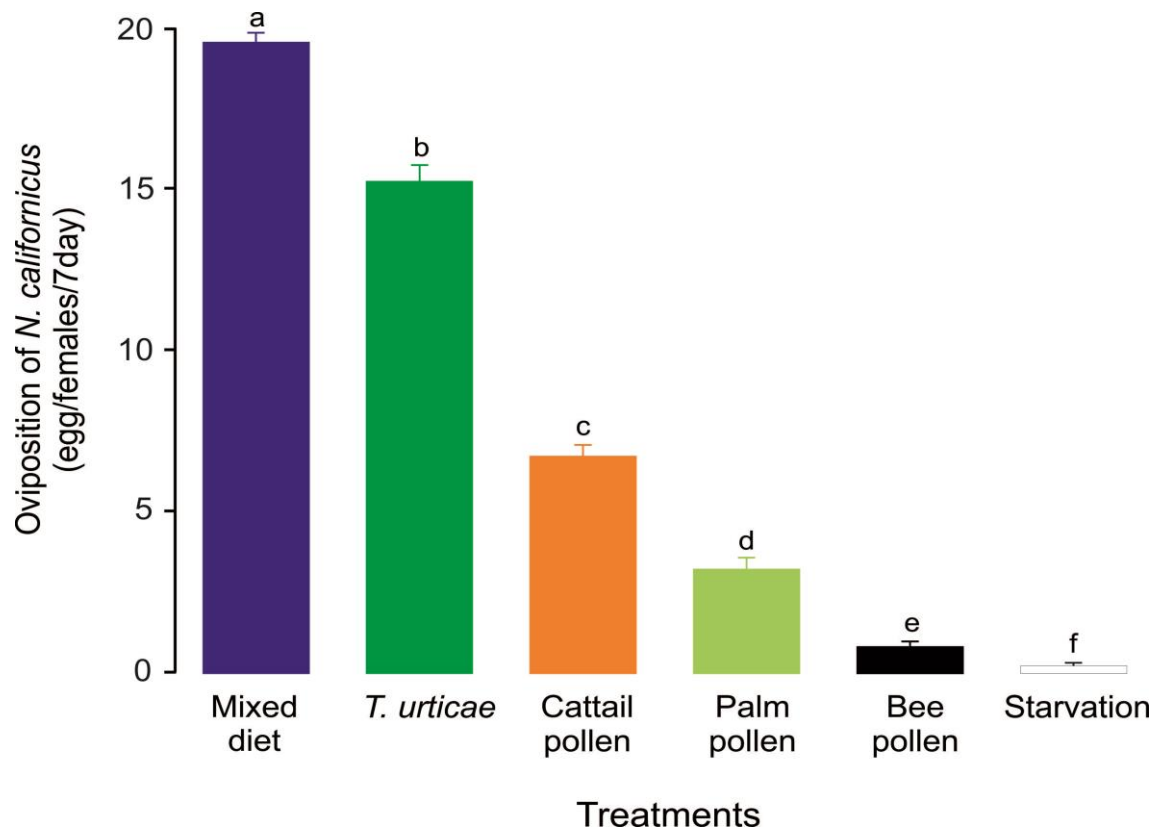
**Fig. 3** Numbers (mean  $\pm$  SE) of *Neoseiulus californicus* eggs as a function of time on strawberry leaf disc with mixed diet (*Tetranychus urticae* + cattail pollen) (blue squares), *T. urticae* (inverted green triangles), and cattail pollen (orange circles) at laboratory, ( $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH and 12:12 h). Prey *T. urticae* was offered to females that had only pollen for twenty days after the release of *N. californicus* (indicated by an arrow in the figure).

**Fig. 4** Numbers (mean  $\pm$  SE) of mobile and immobile stages of *Tetranychus urticae* as a function of time on strawberry plants with *Neoseiulus californicus* (gray circles), with *N. californicus* + cattail pollen (blue squares), with *Phytoseiulus macropilis* (orange circles) and without predator (inverted green triangles) in the greenhouse ( $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH and 12:12 h). Predators were released on plants seven days after infestation with spider mites (indicated by an arrow in the figure).

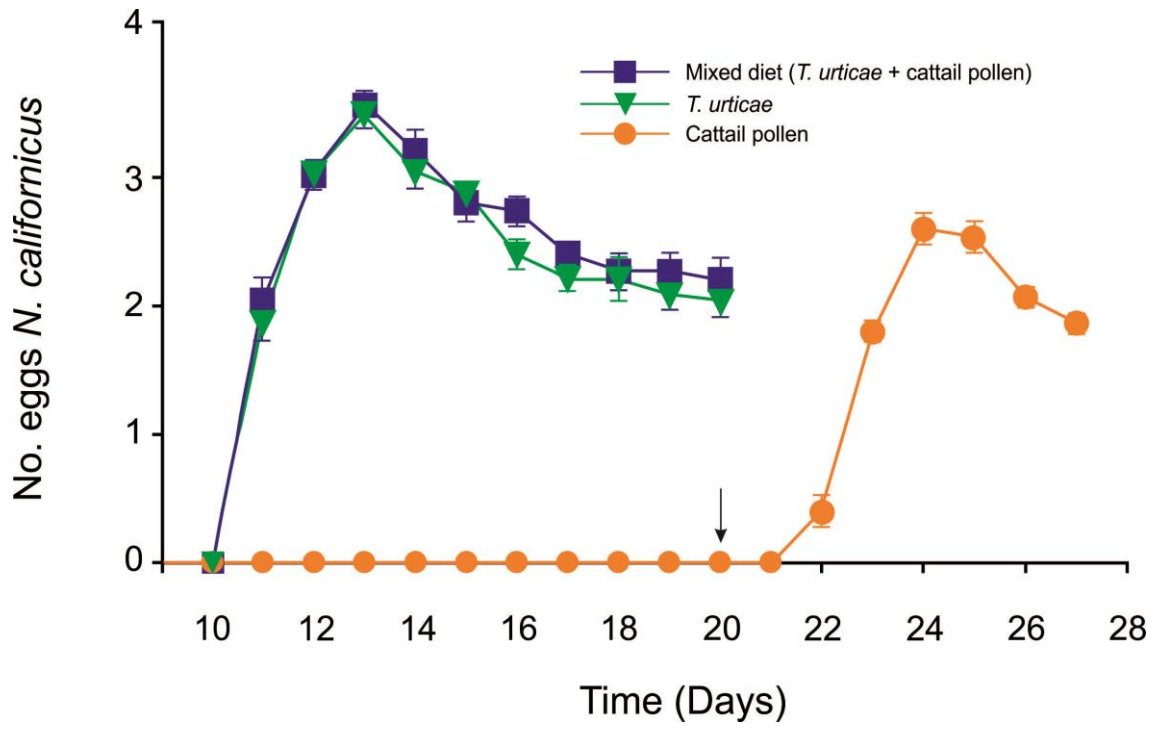
**Fig. 5** Numbers (mean  $\pm$  SE) of predatory mites (all stages) as a function of time on strawberry plants after infestation with *Tetranychus urticae*. Plants with *Neoseiulus californicus* (grey circles), with *N. californicus* + cattail pollen (blue squares) and with *Phytoseiulus macropilis* (orange circles) under green-house ( $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH and 12:12 h).



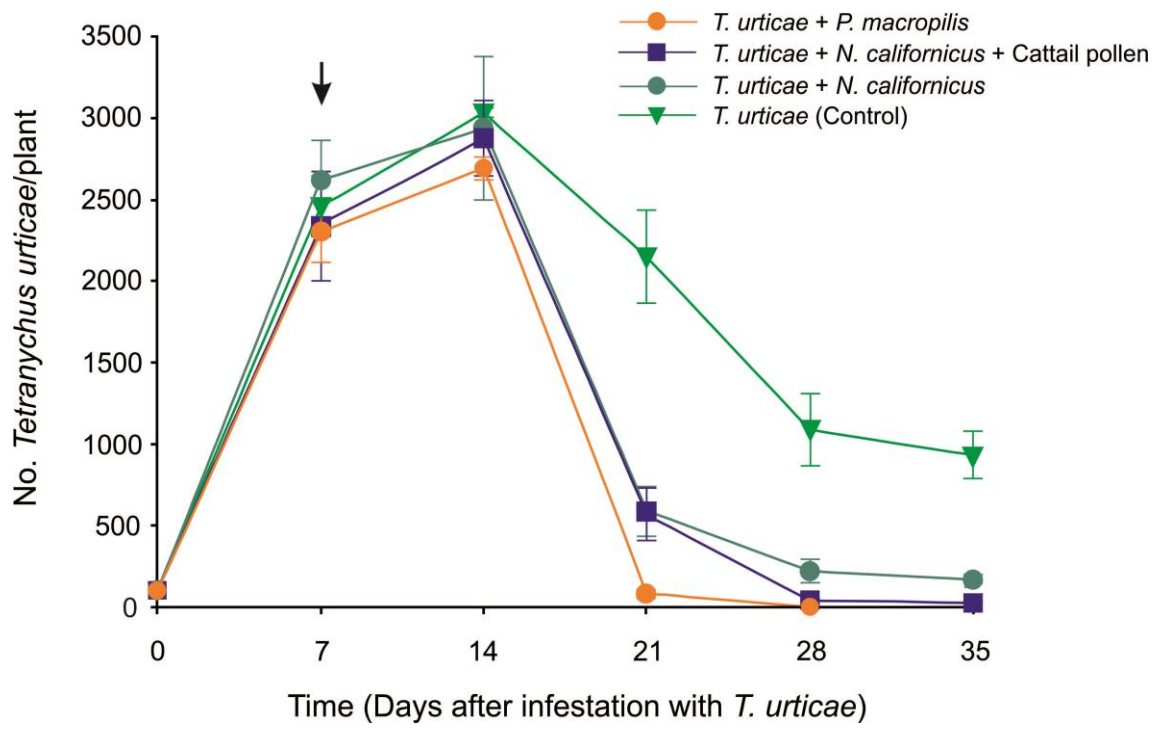
**Figure 1.**



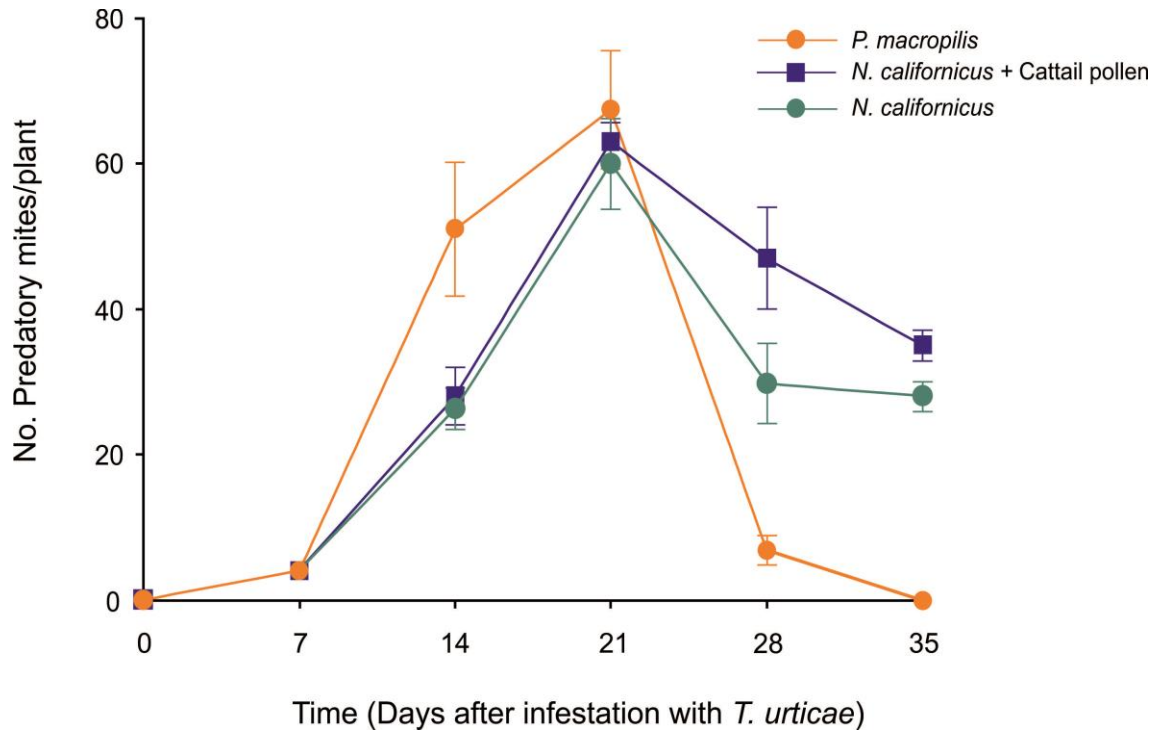
**Figure 2.**



**Figure 3.**



**Figure 4.**



**Figure 5.**

**Table 1.** Mean ( $\pm$  SE) duration of immature stages of *Neoseiulus californicus* feeding on an either mixed diet (*T. urticae* + cattail pollen), *T. urticae*, or cattail pollen under laboratory conditions of  $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH, and 12:12 (L/D) photoperiod.

Treatment	Duration (days)			
	Larva	Protonymph	Deutonymph	Total
<i>(T. urticae</i> + cattail pollen)	$0.95 \pm 0.05$ a	$1.67 \pm 0.06$ b	$1.50 \pm 0.06$ a	$6.86 \pm 0.11$ a
<i>T. urticae</i>	$0.96 \pm 0.04$ a	$1.49 \pm 0.09$ a	$1.59 \pm 0.09$ a	$6.73 \pm 0.13$ a
Cattail pollen	$0.87 \pm 0.06$ a	$1.77 \pm 0.13$ b	$2.06 \pm 0.14$ b	$7.29 \pm 0.15$ b

## CHAPTER 3.

### **Flipping advantages to adversity: lime sulfur side effects on predatory mites of natural occurrence in strawberry plants**

Henry E. Vacacela Ajila<sup>1</sup>, Eugênio E. Oliveira<sup>1</sup>, Felipe Lemos<sup>2</sup>, Khalid Haddi<sup>1</sup>, Felipe Colares<sup>2</sup>, Pedro H. Marques Gonçalves<sup>1</sup>, Madelaine Venzon<sup>3</sup>, Angelo Pallini<sup>1</sup>

*<sup>1</sup>Department of Entomology, Federal University of Viçosa, 36570-900, Viçosa-Minas Gerais, Brazil*

*<sup>2</sup>Econtrole Research & Consulting Ltda., Technological Center of Regional Development of Viçosa (CenTev), Federal University of Viçosa, 36570-900, Viçosa-Minas Gerais, Brazil*

*<sup>3</sup>Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), Vila Gianetti 46, 36570-000, Viçosa-Minas Gerais, Brazil*

\*Corresponding author:  
Henry E. Vacacela Ajila  
Tel. (55)(31) 3899-2472  
E-mail: henryeduar2@hotmail.com

## Abstract

The application of lime sulfur is a common practice used to control arthropod pests in organic production of fruits. However, the unintended effects of this insecticide preparation on non-targeted organisms have not received the adequate attention. Here, we evaluated the lime sulfur toxicity on the phytophagous mites *Tetranychus urticae* Koch (Acari: Tetranychidae) and on two predatory mite species (i.e., *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) of natural occurrence in strawberry plants. We also assessed the repellent activities and potential effects on the oviposition rates and the egg viability of mites that were exposed to field used lime sulfur concentrations (i.e., 2 %). The lime sulfur exhibited higher toxicity to the predators *N. californicus* (LC<sub>50</sub> = 5.4 [4.5-6.8 %]) and *P. macropilis* (LC<sub>50</sub> = 5.0 [4.0-6.5 %]) than to the herbivore *T. urticae* (LC<sub>50</sub> = 13.2 [7.3-28.5 %]). However, the exposure to field-applied concentrations resulted in higher reductions on the oviposition rate of *T. urticae* (36 %) than on the predatory mites (*N. californicus* = 18 %, *P. macropilis* = 19 %). Interestingly, although the egg viability of *P. macropilis* was less affected (i.e., reduction of 50 %) by such lime sulfur exposures, these mites were unable to avoid lime sulfur-treated areas and the egg viability of *N. californicus* was only 18.6 %. Thus, by demonstrating undesirable effects to naturally occurring predatory mites, our findings indicate that applications of lime sulfur could compromise the biological control of herbivore mites in organic production of strawberries. However, studies are needed to estimate the overall impact on the mite populations in the field.

**Keywords:** *T. urticae*, *P. macropilis*, *N. californicus*, lime sulfur, strawberry, organic crops

## Key message

- Lime sulfur is commonly used to control pests in organic production, but the side effects on non-target organisms are still not well understood.
- Lime sulfur exhibited higher toxicity to the predators *Neoseiulus californicus* and *Phytoseiulus macropilis* than to *Tetranychus urticae*.
- *Tetranychus urticae* exhibited higher reductions on the oviposition rate than the predatory mites *N. californicus* and *P. macropilis*.
- Egg viability of *P. macropilis* was less affected by lime sulfur exposures, but these mites were unable to avoid lime sulfur-treated areas.

## 1 Introduction

In conventional strawberry crops, pest control is often carried out using synthetic chemical pesticides (Branzanti et al. 1989; Sances and Ingham 1997; García-Marí and Enrique González-Zamora 1999; Sato et al. 2007; Urbaneja et al. 2008). However, the use such synthetic chemicals has limitations once adequate products for different types of pests may be not always available (Easterbrook et al. 2001). Additionally, the selection of pest populations resistant to different pesticides makes pest management more complicated (Carbonaro et al. 1986; Easterbrook et al. 2001; Sato et al. 2007). So, to reduce dependence on the use of synthetic agrochemicals, ecological strategies of pest control appears as an alternative for sustainable production (Fadini et al. 2004). This alternative prioritises the use of non-conventional phytosanitary methods to regulate the populations of pests without causing adverse impacts on populations of natural enemies (Fadini et al. 2004; Madail et al. 2007).

The lime sulfur is an alternative product used in crops for control of pest and diseases (Russell 2005; Venzon et al. 2013; Tuelher et al. 2014). Its effect on insects occurs when air passes through the solution of calcium carbonate and sulfur, thus releasing gases such as sulfur dioxide and hydrogen sulfide. The main insecticidal effect is generally attributed to the latter (Abbott 1945). In many crops, the lime sulfur is efficient in controlling phytophagous mites without causing lethal effects on natural enemies, when suitable concentration are used (Soto 2009; Tuelher et al. 2014). For instance, this is the case in the control of mites such as *Oligonychus ilicis* (McGregor) on coffee (Tuelher et al. 2014), *Tetranychus evansi* (Baker & Pritchard) on tomato (Soto et al. 2010) and *Polyphagotarsonemus latus* (Banks) on chili pepper (Venzon et al. 2006).

*Phytoseiulus macropilis* (Banks) and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) are predatory mites that often occur in strawberry crops (Garcia and Chiavegato 1997; Marchetti and Ferla 2004; Ferla et al. 2007). They have different feeding habits; they may be classified as a specialist (carnivorous) or a generalist (omnivore) respectively (McMurtry and Croft 1997; Mcmurtry et al. 2013). However, despite differences in dietary habits, the two predatory species have been successfully used to control a key strawberry pest, the two-spotted spider mite *Tetranychus urticae* (Greco et al. 2005; Oliveira et al. 2007, 2009; Fraulo and Liburd 2007; Ahn et al. 2010).

It has been shown that the lime sulfur causes a more significant impact in the pest when compared to the natural enemies (Venzon et al. 2006, 2013; Soto 2009; Efrom et al. 2011; Tuelher et al. 2014). Some studies observed adverse effects of lime sulfur on natural enemies of pest, but it depends on the concentration used (Daniel et al. 2001; Beers et al. 2009; Venzon et al. 2013; Armas et al. 2018). In order to better understand the lethal and sublethal effects of lime sulfur and its indirect effects on natural enemies, further studies are needed. This information could be used to guide practices based on the compatibility of this product with biological control of pest in organic crops.

Thus, the objective of the present study was to evaluate the toxicity of the lime sulfur to the phytophagous mite *T. urticae* and its natural enemies *P. macropilis* and *N. californicus*. We also evaluated the effect of sublethal concentrations of lime sulfur on the oviposition, egg viability and foraging behaviour of the three mite species.

## 2 Materials and methods

### 2.2 Production of plants

We cultivated commercial strawberry seedlings (*Fragaria x ananassa* 'Portola') and jack bean (*Canavalia ensiformis*) in a greenhouse ( $25 \pm 7^\circ\text{C}$ ,  $70\% \pm 20\%$  RH) for use on experiments and rearing of mite species. We transplanted these plants in plastic pots (3L) filled with commercial soil substrate (Bioplant®, a mixture of vermiculite and organic fertiliser). Five new pots of jack bean were sowed weekly using five seeds per pot. The plants were watered twice a day (250 mL/day). The strawberry seedlings were fertilized with a solution of NPK (5-15-5, 6.2 gr/plant) every thirty days plus ammonium sulphate (21%; 0.83 gr/plant) and potassium chloride (60%; 0.42 gr/plant) every fifteen days.

### 2.2 Mite rearing

We started colonies of the phytophagous mite *T. urticae* and the predatory mite *P. macropilis* from individuals obtained from a rearing kept in the Laboratory of Acarology at the Federal University of Viçosa. The phytoseiid mite *N. californicus* was collected in the year of 2016 from fruits and leaves of strawberry plants attacked by phytophagous mites in a pesticide-free crop at the municipality of Ervália in the State of Minas Gerais, Brazil ( $20.842778^\circ\text{S}$  -  $42.675306^\circ\text{W}$ ).

We kept the colonies of spider mites (*T. urticae*) in a climatic chamber at the temperature of  $25 \pm 1^\circ\text{C}$ , relative humidity of  $70 \pm 10\%$  and a photoperiod of 12 hours. We reared the spider mites on jack bean plants of 21 days old and with only cotyledonary leaves. We isolated the plants to prevent possible contamination of the infested plants by

other arthropods or escaping of the spider mites. The plant pots were arranged on plastic trays (44 x 30 x 8 cm) and placed over larger trays (60 x 40 x 10 cm). We then filled the space between the trays with water plus detergent to create a barrier. Approximately one week after being infested, the leaves of jack bean showed evident symptoms of spider mite attack. At that moment, we collected the leaves with mites and used them for different purposes (for instance, to feed the cultures of predatory mites, to collect spider mites for experiments, or to replicate the colonies).

The colonies of predatory mites (*N. californicus* and *P. macropilis*) were kept in a climatic chamber as mentioned before. Each species of predatory mite was kept in a rearing unit composed of two plastic trays. A smaller tray (44 x 30 x 8 cm) was kept inside a larger one (60 x 40 x 10 cm), and the space between them was filled with water plus detergent to avoid contaminations and evasion of the predatory mites. Both species of predatory mites were fed with *T. urticae* three times per week. To this, we dropped on each rearing unit of predatory mite three leaves of jack beans infested with spider mites.

We carried out bioassays with 15-days-old females of each mite species from their cohorts. The population of *T. urticae* was kept in jack bean plants, but the experiments we done in strawberry leaves. Thus, for the cohort of *T. urticae*, we arranged strawberry leaflets over a layer of wet cotton wool laid inside a plastic tray. To each leaflet, we transferred 50 adult females from the lab colonies kept on jack bean plants. After 24 hours feeding and laying eggs, these females were removed and discarded, remaining on strawberry leaflets only the eggs of *T. urticae*. The arenas with the egg waves of *T. urticae* were kept in a climatic chamber. The strawberry leaflets were replaced by new ones when necessary (i.e. when they began to show symptoms of deterioration). We removed the old leaflet and placed a new one in the arena. The old leaflet was then cut into small sections

and placed on the top of the new one. The pieces of old leaflet were removed after 3-4 days when the spider mites had moved away to the new leaflet.

Arenas from leaves of jack bean previously infested with *T. urticae* were prepared to obtain the cohort of the predators *N. californicus* and *P. macropilis*. First, we put a water soaked foam (11 x 18 x 2.5 cm) inside a plastic tray (15.5 x 22 x 4 cm). Then, we covered the foam with a black plastic sheet surface (11 x 19 cm). A full-developed leaf of jack bean was then laid over the plastic sheet and divided into two sections (7 x 8 cm) by strips of wet paper tissue (Kleenex®). We also wrapped the edges of the jack bean leaf with wet strips of paper tissue to maintain the turgidity of the leaf and prevent escaping of the mites. We released on the arenas 60 pregnant females (30 at each section) of each predatory mite species. Approximately 24 hours later, these adult females were removed from the arenas, leaving only the eggs laid. The cohorts were kept in a climatic room at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and with a photoperiod of 12 hours. After hatching and throughout development, individuals from predatory mites were fed three times a week with a mixture of all stages of *T. urticae* offered on stripes cut from infested jack bean leaves. Mites of all species remained in the arenas until reaching adulthood, when they were used in the experiments.

### **2.3 Lime sulfur**

We evaluated the lethal and sublethal effect of lime sulfur on the phytophagous mite *T. urticae* and on the predatory mites *N. californicus* and *P. macropilis*. For the preparation of the lime sulfur, we proceeded according to Venzon et al (2011). We made a liter of lime sulfur by boiling 250 gr of sulfur and 125 gr of virgin lime with water. Lime sulfur was used for a maximum period of one month. After that, a new preparation was made.

The density of the stock solution of lime sulfur was 30-31°B, from which we took aliquots to prepare dilutions for the experiments. The dilutions tested were prepared to reach suitable concentrations as previously described (Soto et al. 2010; Venzon et al. 2011, 2013).

To determine the concentrations of lime sulfur to be used in the bioassays, we performed preliminary tests with broad concentration range. So, we determine the minimal concentrations of lime sulfur that did not cause mortality on mites and the maximal concentrations that caused high mortality (100% of mortality) on each mite species.

#### **2.4 Concentration-response bioassays**

Aiming to select a suitable working dose of the lime sulfur that causes a negative effect on the phytophagous mite *T. urticae* but with no or small impact over the natural enemies *N. californicus* and *P. macropilis*, we assessed the mortality of mites when exposed to different doses as described below. For *T. urticae*, strawberry leaf discs (2.8 cm Ø) were sprayed with different concentrations of lime sulfur and distilled water (control) using a Potter Tower (Bukard, Rickmansworth, UK). At each spray, a 2.5 mL aliquot of lime sulfur was applied at 0.34 bar pressure, according to the recommendations of the Organization for Biological Control of Noxious Animals and Plants/West Palearctic Regional Section (IOBC/WPRS) (Hassan 1989).

After being sprayed, the leaf discs were fixed to Petri dishes with an agar-water solution (1 g agar in 10 mL of water). Approximately 25 mL of this solution was transferred to Petri dishes (60 mm x 15 mm). As the solution cooled down, but before the solidification, the leaf discs were arranged over the gelled agar. Approximately one hour

later, we removed the solidified agar that was in the contour of the leaf disk of each arena. The hollow (between the agar and the border of the Petri dish) was filled with water up to the edge of the leaf disk to avoid mites from escaping. Finally, ten adult females of *T. urticae* were released per arena (15 days old). We tested eight concentrations for the spider mites (40, 30, 20, 10, 5, 2, 1, 0.1 % and control as distilled water) and each concentration was repeated four times for each species.

We used the same methodology described above for *T. urticae* for the predatory mites. But one hour before proceeding with the release of predators, the arenas were infested with *T. urticae* (approximately 90 individuals per arena). The previous infestation intended to provide enough food for predators. After that, we transferred to leaf discs ten pregnant females (15 days old) of *P. macropilis* or *N. californicus*. Each concentration (10, 8, 6, 5, 4, 2, 1, 0,1 %) plus the control (distilled water) were repeated four times for each species. Mortality was assessed 24 hours after the release of the mites on the sprayed leaf discs. We considered as dead the individuals that were unable to walk the length of their body when disturbed with a fine brush.

## **2.5 Sublethal effects on oviposition and egg viability**

We used concentrations of lime sulfur commonly applied in the field by strawberry farmers (1 and 2%) to evaluate the effect of the lime sulfur on oviposition and viability of the mite eggs from *T. urticae*, *P. macropilis* and *N. californicus*.

For oviposition, we used the same methodology described above (item 2.5). Thus, after spraying the strawberry leaf discs, ten females of *T. urticae* or five females of *P. macropilis* or *N. californicus* (15 days old) were released on each disc. The leaf discs that received predatory mites were previously infested with *T. urticae* (20 adult females, 48

hours before receiving predatory mites). These spider mites and their eggs served as food for the predatory mites. We counted the number of eggs laid for five consecutive days in 24 hours time intervals. Each treatment (1%, 2% and control) were replicated ten times for each mite species.

To study the effect of the lime sulfur on the viability of mite eggs, we submitted the eggs to a topic spray of lime sulfur. First, we prepared arenas with strawberry leaf disc placed directly on a wet cotton layer that was inside a plastic tray (60 x 40 x 10 cm). Thus, 48 hours before the beginning of the experiment, the leaf discs assigned for the treatments with predatory mites were infested with 20 adult females of *T. urticae*. These spider mites and their eggs served as food for the predatory mites. Two days later, ten mated 15 days-old females from the cohorts of *P. macropilis*, *N. californicus* and *T. urticae* were released on each leaf disc. On the treatment with *T. urticae*, the mated females were released on a clean leaf disc. After 24 hours, all adult females were removed from all leaf discs and the number of eggs laid was recorded. Thus, leaf discs with eggs were sprayed with lime sulfur using the Potter tower with the respective doses of 1% and 2% or with distilled water (control). Ten replicates were made for each treatment and evaluations were performed every 24 hours. We recorded the number of emerged larvae on each treatment until no more hatching could be observed on the leaf discs. After being recorded, the emerged larvae were removed from the arenas and discarded. Eggs showing no emergence were considered unviable.

## **2.6 Repellent effect of the lime sulfur**

We evaluated the repellency effect of lime sulfur over the three mite species. For this, we used a methodology according to Marques et al. (2015) with some modifications. So, we

observed the distribution of mites between patches sprayed with lime sulfur or water (control). We performed the trials on arenas consisting of two strawberry leaf discs (2.8 cm Ø) connected by a bridge made from black plastic sheet (8.0 x 0.5 cm). The leaf discs and bridges were arranged on wet cotton in plastic trays (8 arenas/plastic tray). The position of leaf discs (left or right) assigned to treatments was reversed on half of the replicates to avoid unforeseen bias. The plastic bridge connecting the leaf discs was placed in a way that the edges of the bridge overlap the border of the leaf discs, to allow the mites walking freely without drowning in the water. Forty-eight hours before trials, the leaf discs were infested individually with ten mated females of *T. urticae*. Forty-eight hours later, the spider mites were removed and the number of eggs was recorded and standardised by removing the eggs in excess using a fine brush and leaving 70 eggs per leaf disc. The tests with *T. urticae* were performed on arenas made of clean (not previously infested) strawberry leaf discs.

All strawberry leaf discs were sprayed in Potter tower as described above. In a first experiment, we sprayed one of the leaf discs of each arena with lime sulfur at 2 % (from now on it will be referred as "treated") and the other one with distilled water (from now on referred to as "untreated"). In a second experiment, we used the LC<sub>50</sub>s of each species in order to observe whether the increase of the concentration causes a change in the repellency behavior. So, after being sprayed, the leaf discs were left drying out for one hour at the laboratory. Finally, one 15 days-old mated female was released in the centre of the bridge of each arena and observations were made every hour during 5 hours. Hence, after 24 hours the chosen position was recorded. We replaced only the mites showing no movement after 5 minutes. Preliminary experiments done with leaf discs sprayed only with distilled water showed no preference of the mites for choosing sides of the arena.

## 2.7 Statistical analyses

Probit analysis estimated Concentration-response curves in SAS statistical software package (SAS Institute 2008). The total number of eggs laid per female was compared between treatments using ANOVA and for the comparison of means, the Holm-Sidak method was used for *T. urticae* and *P. macropilis*. Dunn's Method was used for *N. californicus*. All analysis and comparison of means were performed using Sigma Plot 12.5 software. The percentage of viable eggs was compared between treatments using ANOVA and for the comparison of means was used Tukey's test through Sigma Plot 12.5 software. The differences between the number of mites that chose one side over the other was analyzed between treatments with the non-parametric Proc FREQ (SAS Institute 2008) followed by  $\chi^2$  test ( $\alpha = 0.05$ ).

## 3 Results

### 3.1 Concentration-response bioassays

The results showed that the Probit model suited the mortality of mites in response to increasing concentrations of lime sulfur solution. Among the three mite species studied, the herbivore *T. urticae* was less susceptible than the two predatory mites to lime sulfur. Indeed, the two-spotted spider mite *T. urticae* showed an  $LC_{50}$  of 13.22% (7.33 - 28.54 % C.I.) higher than  $LC_{50}$ s of the predatory mites *N. californicus* [ $LC_{50} = 5.43\%$  (4.46 - 6.76 C.I.)] and *P. macropilis* [ $LC_{50} = 5.04\%$  (4.00 - 6.54 % C.I.)]. (Fig. 1).

Noteworthy is that the  $LC_{50}$ s found for all the three mites are higher than the recommended concentrations applied in strawberry fields ( e.g. 1-2 %).

### 3.2 Sublethal effects on oviposition and egg viability

Sublethal concentrations of lime sulfur caused a negative effect on the oviposition of the three mite species studied (Fig. 2). We observed significant differences ( $F_{2,24} = 33.640$ ,  $P < 0.001$ ) in the oviposition of *T. urticae* in function of the concentrations of lime sulfur used. The lowest oviposition rate was observed from females of *T. urticae* on the leaf discs sprayed with lime sulfur at 2%. These females laid on average  $4.56 \pm 0.28$  eggs/day, which represents a reduction of 36% on their oviposition rate when compared to the oviposition rate of *T. urticae* females on the leaf discs sprayed with water.

Moreover, there were significant differences in oviposition rate of the predatory mite *P. macropilis* ( $F_{2,27} = 5.271$ ,  $P = 0.012$ ), which was reduced on leaf discs sprayed with different sublethal concentrations of lime sulfur. However, no significant difference was observed between females from the treatments that received the concentrations 2% and 1% of lime sulfur ( $2.41 \pm 0.15$  and  $2.59 \pm 0.15$  eggs/day, respectively) (Fig. 2). In addition, we also observed significant differences in the oviposition of *N. californicus* ( $H = 9.69$ ,  $df = 2$ ,  $P = 0.008$ ). This phytoseiid mite showed an average oviposition rate of  $1.79 \pm 0.14$  and  $1.83 \pm 0.10$  eggs per day at lime sulfur concentrations of 2 and 1%, respectively. The oviposition rates of *N. californicus* on leaf discs sprayed with lime sulfur were not significantly different among them, but significantly inferior to the oviposition rate observed for females on control leaf discs sprayed with water ( $2.19 \pm 0.04$  eggs/day) (Fig. 2).

Regarding the viability of the mite eggs, there were significant differences in hatching rates following the spraying of sublethal concentrations of lime sulfur (Fig 3). The eggs of the predatory mite *P. macropilis* ( $H = 19.35$ ,  $df = 2$ ,  $P < 0.001$ ) were the least affected, presenting means of viable eggs of 50.7 and 68.7% corresponding to the 2% and 1% concentrations of the lime sulfur, respectively. In the same context, it was observed that *T. urticae* ( $H = 22.34$ ,  $df = 2$ ,  $P < 0.001$ ) and *N. californicus* ( $H = 19.90$ ,  $df = 2$ ,  $P < 0.001$ ) were the most affected. Thus, *T. urticae* presented 12.7 and 28.1% of viable eggs, whereas that *N. californicus* presented percentages of 18.6% and 20.9% of hatching when the eggs were sprayed with lime sulfur at 2% and 1%, respectively (Fig. 3).

### **3.2.1 Repellent effect of the lime sulfur**

The lime sulfur solution at the concentration of 2% affected the choice in two of the three mite species tested. *Tetranychus urticae* selected the untreated patches ( $P < 0.05$ ) in all observation times (Fig. 4C). Similarly, *N. californicus* chose the untreated patches in the first five hours ( $P < 0.05$ ). However, at 24 hours we did not find significant difference ( $P > 0.05$ ) (Fig 4A). The predator *P. macropilis* showed no significant difference ( $P > 0.05$ ) as 60.13 % of the individuals went to the untreated patch and 39.87% for the treated patch (Fig. 4B).

Similar patterns of mite behaviour were found when we tested the effects of the lime sulfur using the experimentally determined  $LC_{50s}$  of each species. In this way, *T. urticae* was selected the untreated area from the treated one, presenting significant difference ( $P < 0.05$ ) (Fig. 4F). We also observed that *N. californicus* in the first hour and after 24 hours was not able to discriminate the untreated area from the treated one ( $P > 0.05$ ) whereas it was able to avoid the treated areas in the period between the second

and fifth hour ( $P < 0.05$ ) (Fig. 4D). In the same way as when using 2% of the sulfur solution, *P. macropilis* did not show significant difference between treated and untreated patches ( $P > 0.05$ ) (Fig. 4E).

#### 4. Discussion

Lime sulfur is widely accepted and used in organic crops for pest and disease control although many of its direct and indirect effects on non-target organisms are not yet well known (Venzon et al. 2006, 2013). Lime sulfur presents lethal activity and sublethal negative effects on biological traits of *T. urticae*. Such negative effects of lime sulfur over the main pest of strawberry crops are essential in the context of integrated pest management. Our results showed also deleterious effects including mortality and reduction of oviposition and eggs viability of the main natural enemies of *T. urticae*, namely the predatory mites *N. californicus* and *P. macropilis*. These results indicate that lime sulfur could be more harmful to the natural enemies than to the pest itself. Moreover, the application of the lime sulfur resulted contrasting choice behaviors in the three mite species suggesting that a deeper reflection on the real benefits and effects of this alternative product is still needed.

The application of the inorganic preparations called ‘lime sulfur’ has been recommended since the 19<sup>th</sup> century, representing one of the oldest effective pest control methods (Russell 2005). This application, through sprays on leaves or fruits, continues to be commonly recommended in integrated pest management (IPM) programs. Its ecological impacts have always been considered low and it is compatible with organic management practices of phytophagous mites in high value crops such as strawberry (Soto 2009). Our results showed lethal activities of the lime sulfur on *T. urticae* although

the lethal concentrations needed to control this mite were found to be far higher than the actual recommended field rates of 2% (Penteado 2000). The toxicity against *T. urticae* is in line with the common practice recommending the application of this preparation as an alternative product capable of controlling multiple pests in the organic production of strawberries particularly the two-spotted spider mite *T. urticae* (Solomon et al. 2001; Klingen and Westrum 2007; Moraes and Flechtmann 2008; Bernardi et al. 2015) and even more recently, the spotted-wing drosophila, *Drosophila suzukii* (Marques-Francovig et al. 2014; Andrezza et al. 2017). The toxic effects of the lime sulfur on mites can be attributed to the elemental sulfur and hydrogen sulfide (H<sub>2</sub>S) released by the degradation of the lime sulfur after application (Dahlawi and Siddiqui 2017). The H<sub>2</sub>S inhibits the cytochrome oxidase, whereas the elemental sulfur oxidizes the cytochrome B to C, releasing more H<sub>2</sub>S inside the cell (Smith et al. 1977; Dorman et al. 2002).

Nevertheless, lethal effects were found also for the two species of predator *N. californicus* and *P. macropilis*. Moreover, the LC<sub>50</sub>s observed for these predatory mite species were inferior to the two-spotted spider mite *T. urticae*. This indicates that the predatory mites tested here are more susceptible to the lime sulfur than the phytophagous mite. Similar negative side effects on natural enemies have been previously reported by Venzon et al. (2013b), who showed that the lime sulfur despite having a toxic effect on the pest *Polyphagotarsonemus latus* (Acari: Tarsonemidae), can also cause negative effects on non-target organisms as the predatory mite *Amblyseius herbicolus* (Acari: Phytoseiidae) and predatory larva of *Chrysoperla externa* (Neuroptera: Chrysopidae).

Beyond the lethal effect, sublethal effects of the lime sulfur were found on the oviposition and egg viability not only for the two-spotted spider mite but also for its two predators *N. californicus* and *P. macropilis*. These results confirm that the lime sulfur applications, even at sublethal concentrations, may decrease the oviposition capacity and

egg viability of mites. This contrasts with previous hypothesis claiming that the reduction in oviposition of phytoseiid mites is more related to the decrease in the rate of predation due to the effect of the applied products (Hassan 1982; Overmeer 1985). However, the reduction in the oviposition rate and egg viability of *T. urticae* by lime sulfur application will contribute to maintaining low levels of the pest in the crop and this is positive from a pest management perspective. The effect on reproductive parameters of such applications need to be seriously considered to better estimate the effects of these products on natural populations of predators and on non-target organisms in general.

We have also shown here that a concentration of the lime sulfur commonly used in field sprays may produce changes in the mites' behaviour. We observed repellency of this product for the herbivore *T. urticae* and the predator *N. californicus*. In the meantime, our results also revealed that the phytoseiid mite *P. macropilis* is affected by the lime sulfur concentration used as it was not able to choose between treated area and untreated one. The observed repellency effect may be the result of the released gases from the lime sulfur on the sensory perception abilities that these mite species possess. Mite behaviour can be affected by chemicals as acaricides (McMurtry et al. 1970; Mansour et al. 1997; Lima et al. 2013, 2017) and their ability of foraging can be negatively affected due to interference of such chemicals with their odour perception (Lima et al. 2017) resulting in repellency and irritability (Mansour et al. 1997; Lima et al. (2013). However, the behaviour observed here in the species *P. macropilis* challenges these findings especially because when a higher dose ( $LC_{50}$ ) was tested the selection behaviour of the mites was not different from what was observed when the 2% dose was used.

In summary, although the lethal effect of the lime sulfur was more significant for natural enemies than for the pest, the doses necessary to cause mortality of fifty per cent of the population are very high and therefore not applicable since they could cause

problems like phytotoxicity. Furthermore, the effect on the reduction of the oviposition and the ovicidal effect observed in *T. urticae* together indicate that this alternative product could be still an alternative for the control of this pest since the population would be affected to a great extent. Additionally, from the three species studied, *P. macropilis* was the only one that did not show repellency to the lime sulfur, which can be positive due to the possible compatibility between these two control methods. Finally, to recommend the use of the lime sulfur in organic strawberry crops, it would be of primary importance firstly to know the phytosanitary status of the crop and the biodiversity of beneficial organisms to avoid causing harm to the populations of natural enemies in the agroecosystem.

### **Author's contributions**

HEVA, EEO, FL, KH and AP conceived and designed the experiments. HEVA, FC and PHM performed the experiments. HEVA, EEO, FL, KH, and AP analyzed the data. AP contributed reagents/materials/analysis tools. HEVA, EEO, FL, KH, FC, MV and AP wrote the paper. All authors read, revised and approved the manuscript.

### **Acknowledgements**

We are grateful to Students-Postgraduate Agreement Program - PEC-PG, CAPES/CNPq – Brazil, Minas Gerais Research Support Foundation (FAPEMIG), Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council of Science and Technological Development (CNPq) for the scholarships and the financial support. The technical assistance provided by the companies ECONTROLE research & consulting

and Mineração Lapa Vermelha. Author HEVA was supported by the Students-Postgraduate Agreement Program - PEC-PG, CAPES/CNPq – Brazil, and author AP by FAPEMIG and CNPq and MV by CNPq.

### **Compliance with ethical standards**

### **Conflict of interest**

The authors have declared that no conflict of interest exists.

### **Informed consent**

Informed consent was obtained from all individual participants included in the study.

### **Research involving human participants and/or animals**

This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

### **References**

- Abbott CE (1945) The toxic gases of lime-sulfur. *J Econ Entomol* 38:618–620
- Ahn JJ, Kim KW, Lee JH (2010) Functional response of *Neoseiulus californicus* (Acari: Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae) on strawberry leaves. *J Appl Entomol* 134:98–104
- Andreazza F, Vacacela Ajila HE, Haddi K, et al (2017) Toxicity to and egg-laying avoidance of *Drosophila suzukii* (Diptera: Drosophilidae) caused by an old alternative inorganic insecticide preparation. *Pest Manag Sci* 74:861–867

- Armas FS, Rakes M, Pasini RA, et al (2018) Toxicidade de diferentes concentrações de calda sulfocálcica utilizada em cultivos orgânicos de soja sobre o parasitoide *Telenomus podisi*. In: 14<sup>a</sup> Jornada de Pós-Graduação e Pesquisa. pp 2747–2761
- Beers EH, Martinez-Rocha L, Talley RR, Dunley JE (2009) Lethal, sublethal, and behavioral effects of sulfur-containing products in bioassays of three species of orchard mites. *J Econ Entomol* 102:324–335
- Bernardi D, Botton M, Nava DE, Zawadneak MAC (2015) Guia para a identificação e monitoramento de pragas e seus inimigos naturais em morangueiro. Embrapa 46 p
- Branzanti EC, José A, Echevarría PH (1989) La fresa. Mundi-prensa, Madrid, Spain
- Carbonaro MA, Moreland DE, Edge VE, et al (1986) Studies on the mechanism of cyhexatin resistance in the twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *J Econ Entomol* 79:576–579
- Dahlawi SM, Siddiqui S (2017) Calcium polysulphide, its applications and emerging risk of environmental pollution—a review article. *Environ Sci Pollut Res* 24:92–102
- Daniel C, Haeseli A, Weibel F (2001) The side effects of lime sulphur on predaceous arthropods, ie *Typhlodromus pyri*, and other leaf occupying arthropods. Plant protection: pest and beneficials, Frick, Switzerland, Research Institute of Organic Agriculture (FiBL), 8p. Disponível em: <<http://orgprints.org/2932/01/daniel-et-al-2001-lime-sulphur.pdf>>. Accessed in 10 Oct. 2017.
- Dorman DC, Moulin FJ-M, McManus BE, et al (2002) Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium. *Toxicol Sci* 65:18–25
- Easterbrook MA, Fitzgerald JD, Solomon MG (2001) Biological control of strawberry tarsonemid mite *Phytonemus pallidus* and two-spotted spider mite *Tetranychus urticae* on strawberry in the UK using species of *Neoseiulus* (*Amblyseius*) (Acari: Phytoseiidae). *Exp Appl Acarol* 25:25–36
- Efrom CF, Redaelli L, Meirelles RN, Ourique C (2011) Selectivity of phytosanitary products used in organic farming on adult of *Cryptolaemus montrouzieri* (Coleoptera, Coccinellidae) under laboratory conditions. *Semina Ciênc Agrár* 32:1429–1438
- Fadini MAM, Pallini A, Venzon M (2004) Controle de ácaros em sistema de produção integrada de morango. *Ciênc Rural* 34:1271–1277
- Ferla NJ, Marchetti MM, Gonçalves D (2007) Ácaros predadores (Acari) associados à cultura do morango (*Fragaria sp.*, Rosaceae) e plantas próximas no Estado do Rio Grande do Sul. *Biota Neotropica* 7:1–8
- Fraulo AB, Liburd OE (2007) Biological control of twospotted spider mite, *Tetranychus urticae*, with predatory mite, *Neoseiulus californicus*, in strawberries. *Exp Appl Acarol* 43:109

- Garcia I, Chiavegato L (1997) Resposta funcional e reprodutiva de *Phytoseiulus macropilis* (Banks, 1905) (Acari: Phytoseiidae) a diferentes densidades de ovos de *Tetranychus urticae* (Koch, 1836)(Acari: Tetranychidae). *Científica* 25:35–43
- García-Marí F, Enrique González-Zamora J (1999) Biological control of *Tetranychus urticae* (Acari: Tetranychidae) with naturally occurring predators in strawberry plantings in Valencia, Spain. *Exp Appl Acarol* 23:487–495
- Greco NM, Sánchez NE, Liljeström GG (2005) *Neoseiulus californicus* (Acari: Phytoseiidae) as a potential control agent of *Tetranychus urticae* (Acari: Tetranychidae): effect of pest/predator ratio on pest abundance on strawberry. *Exp Appl Acarol* 37:57–66
- Hassan S (1989) Testing methodology and the concept of the IOBC/WPRS working group. In: Pesticides and non-target invertebrates/editor: Paul C. Jepson, Intercept Ltd. Wimborne & Dorset, pp 1–18
- Hassan SA (1982) Relative tolerance of three different strains of the predatory mite *Phytoseiulus persimilis* A.-H. (Acari, Phytoseiidae) to 11 pesticides used on glasshouse crops1. *Z Für Angew Entomol* 93:55–63
- Klingen I, Westrum K (2007) The effect of pesticides used in strawberries on the phytophagous mite *Tetranychus urticae* (Acari: Tetranychidae) and its fungal natural enemy *Neozygites floridana* (Zygomycetes: Entomophthorales). *Biol Control* 43:222–230. doi: 10.1016/j.biocontrol.2007.07.013
- Lima DB, Melo JWS, Guedes RNC, et al (2013) Survival and behavioural response to acaricides of the coconut mite predator *Neoseiulus baraki*. *Exp Appl Acarol* 60:381–393
- Lima DB, Oliveira HKV, Melo JWS, et al (2017) Acaricides impair prey location in a predatory phytoseiid mite. *J Appl Entomol* 141:141–149
- Madail JCM, Antunes LE, Belarmino LC, et al (2007) Avaliação econômica dos sistemas de produção de morango: convencional, integrado e orgânico. Pelotas: Embrapa Clima Temperado (Comunicado técnico, 181) 4 p
- Mansour FA, Ascher KRS, Abo-Moch F (1997) Effects of neemgard on phytophagous and predacious mites and on spiders. *Phytoparasitica* 25:333
- Marchetti M, Ferla N (2004) Flutuação populacional de ácaros (Acari) na cultura do morango no Estado do Rio Grande do Sul. *Sociedade Brasileira de Zoologia*, Brasília, p 15
- Marques RV, Sarmiento RA, Lemos F, et al (2015) Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost. *Funct Ecol* 29:1317–1324
- Marques-Francovig C, Mikami A, Dutra V, et al (2014) Fertilização orgânica e inseticidas botânicos para controle do ácaro rajado em morango. *Ciênc Rural* 44:7

- McMurtry J, Huffaker C, van de Vrie M (1970) Ecology of tetranychid mites and their natural enemies: A review: I. Tetranychid enemies: Their biological characters and the impact of spray practices. *Hilgardia* 40:331–390
- McMurtry JA, Croft BA (1997) Life-styles of phytoseiid mites and their roles in biological control. *Annu Rev Entomol* 42:291–321
- McMurtry JA, Moraes GJD, Sourassou NF (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. *Syst Appl Acarol* 18:297–320
- Moraes GJ, Flechtmann CHW (2008) Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil. Holos
- Oliveira H, Fadini MAM, Venzon M, et al (2009) Evaluation of the predatory mite *Phytoseiulus macropilis* (Acari: Phytoseiidae) as a biological control agent of the two-spotted spider mite on strawberry plants under greenhouse conditions. *Exp Appl Acarol* 47:275–283
- Oliveira H, Janssen A, Pallini A, et al (2007) A phytoseiid predator from the tropics as potential biological control agent for the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Biol Control* 42:105–109
- Overmeer W (1985) Toxicological methods. In: Helle & Sabelis, Spider mites their biology, natural enemies and control. Elsevier, Amsterdam, New York, pp 183–189
- Penteado SR (2000) Controle alternativo de pragas e doenças com as caldas bordalesa, sulfocálcica e Viçosa, 3rd edn. Via Orgânica - Fraga Penteado & Cia Ltda, Valinhos SP
- Russell PE (2005) A century of fungicide evolution. *J Agric Sci* 143:11–25
- Sances FV, Ingham ER (1997) Conventional and Organic Alternatives To Methyl Bromide on California Strawberries. *Compost Sci Util* 5:23–37
- SAS Institute (2008) Statistical analysis system: Getting started with the sas learning, SAS Institute. SAS Institute, Carry NC
- Sato ME, Da Silva MZ, De Souza Filho MF, et al (2007) Management of *Tetranychus urticae* (Acari: Tetranychidae) in strawberry fields with *Neoseiulus californicus* (Acari: Phytoseiidae) and acaricides. *Exp Appl Acarol* 42:107–120
- Smith L, Kruszyna H, Smith RP (1977) The effect of methemoglobin on the inhibition of cytochrome c oxidase by cyanide, sulfide or azide. *Biochem Pharmacol* 26:2247–2250
- Solomon MG, Jay CN, Innocenzi PJ, et al (2001) Review: Natural enemies and biocontrol of pests of strawberry in Northern and Central Europe. *Biocontrol Sci Technol* 11:165–216

- Soto A (2009) Manejo alternativo de ácaros em morango e tomate. Universidade Federal de Viçosa
- Soto A, Venzon M, Oliveira RM, et al (2010) Alternative control of *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on tomato plants grown in greenhouses. *Neotrop Entomol* 39:638–644
- Tuelher ES, Venzon M, Guedes RNC, Pallini A (2014) Toxicity of organic-coffee-approved products to the southern red mite *Oligonychus ilicis* and to its predator *Iphiseiodes zuluagai*. *Crop Prot* 55:28–34
- Urbaneja A, Pascual-Ruiz S, Pina T, et al (2008) Efficacy of five selected acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side effects on relevant natural enemies occurring in citrus orchards. *Pest Manag Sci* 64:834–842
- Venzon M, Amaral D, Perez A, et al (2011) Identificação e manejo ecológico de pragas da cultura de pimenta. Epamig, Belo Horizonte MG, Brazil
- Venzon M, Oliveira RM, Perez AL, et al (2013) Lime sulfur toxicity to broad mite, to its host plants and to natural enemies. *Pest Manag Sci* 69:738–743
- Venzon M, Rosado M da C, Pinto CMF, et al (2006) Potencial de defensivos alternativos para o controle do ácaro-branco em pimenta “Malagueta” *Hortic Bras* 24:224–227

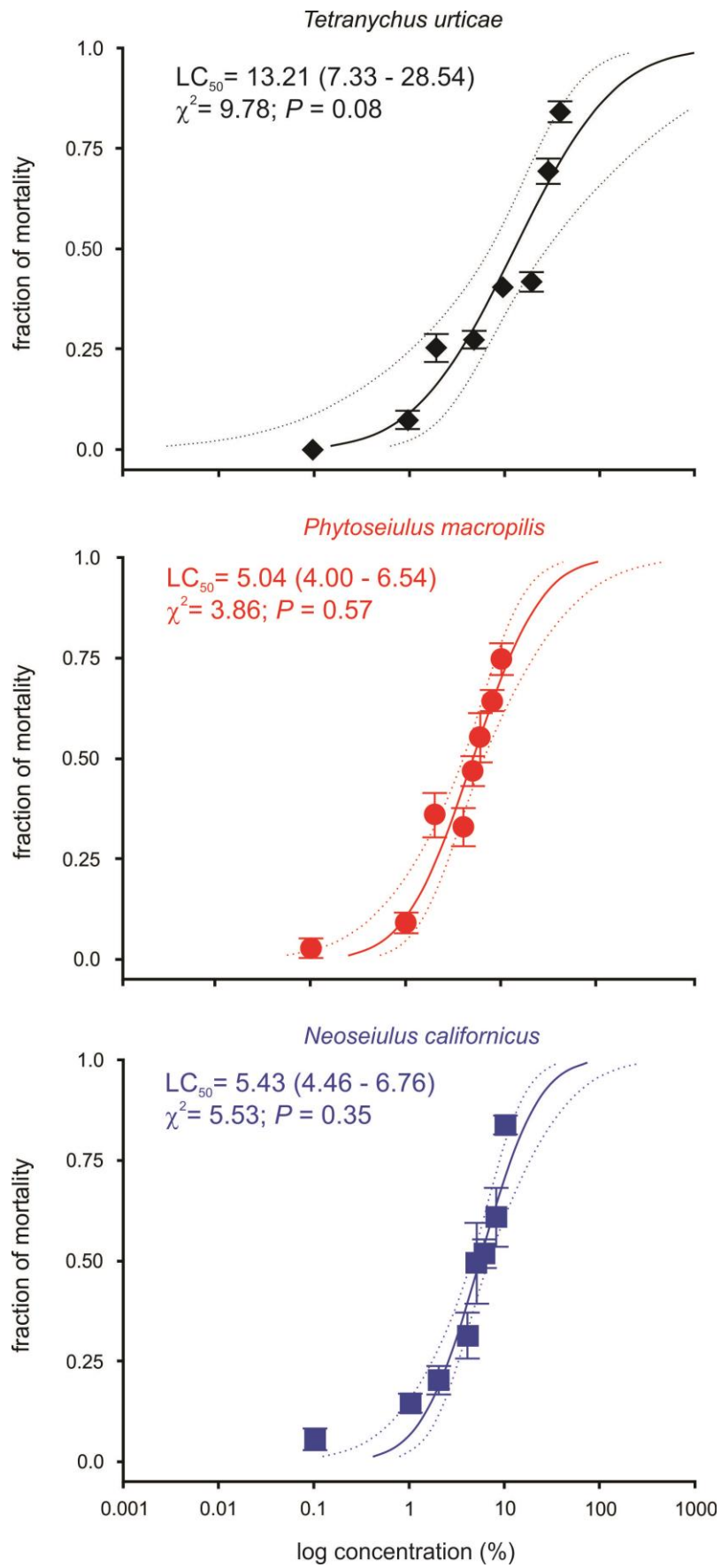
## Figure legends

**Fig. 1.** Toxicity of the lime sulfur to adult females of *T. urticae* (black triangles), *N. californicus* (blue squares) and *P. macropilis* (red circles).

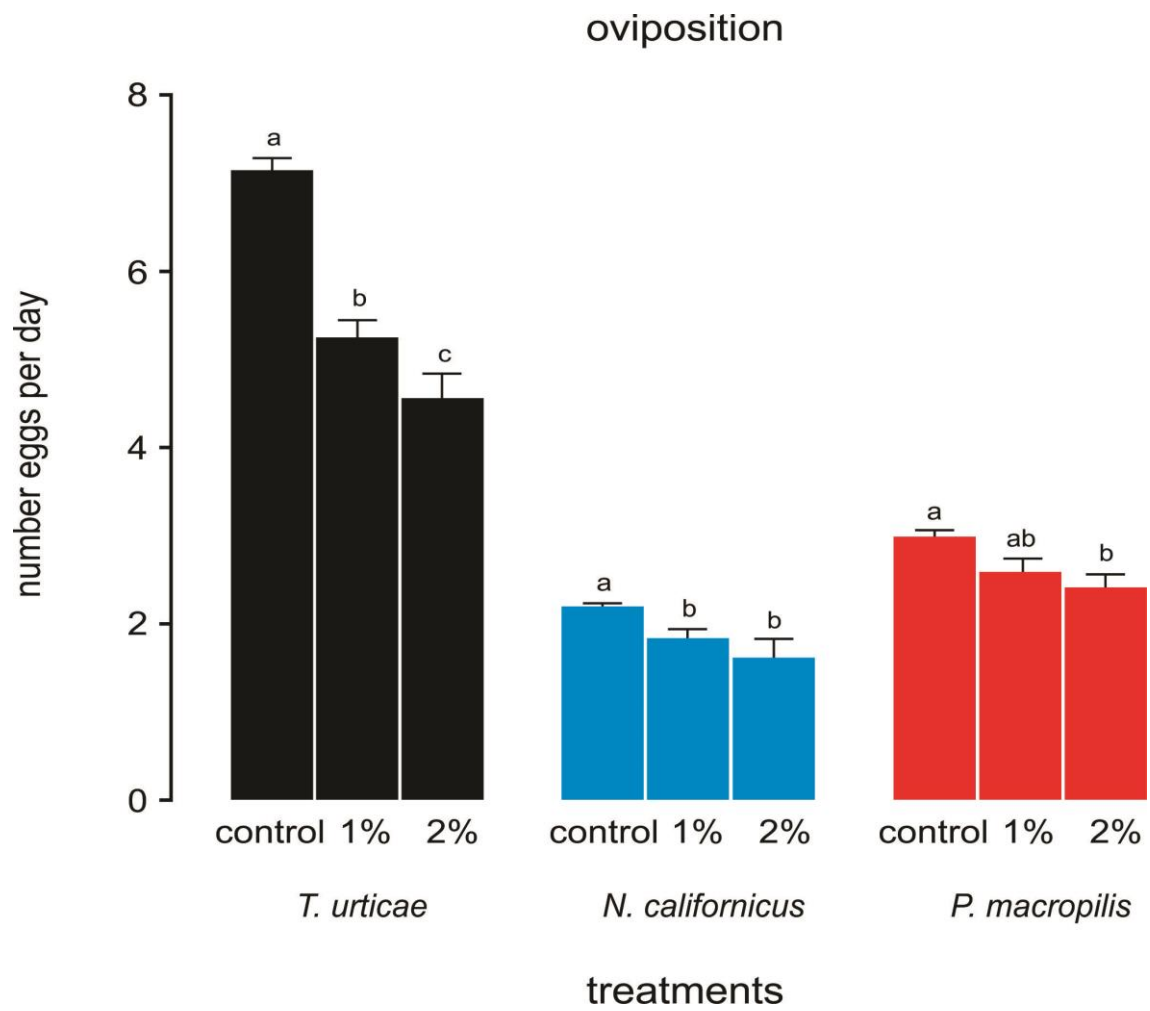
**Fig. 2.** Number of eggs per day (mean  $\pm$  SE) laid by adult females of *T. urticae* (black bars), *N. californicus* (blue bars) and *P. macropilis* (red bars), as a function of concentrations of lime sulfur. Letters above each group of bars indicate differences within each treatment.

**Fig. 3.** Fraction of viable eggs (mean  $\pm$  SE) of *T. urticae* (black bars), *N. californicus* (blue bars) and *P. macropilis* (red bars), as a function of concentrations of the lime sulfur. The letters above each bar indicate the differences within each treatment.

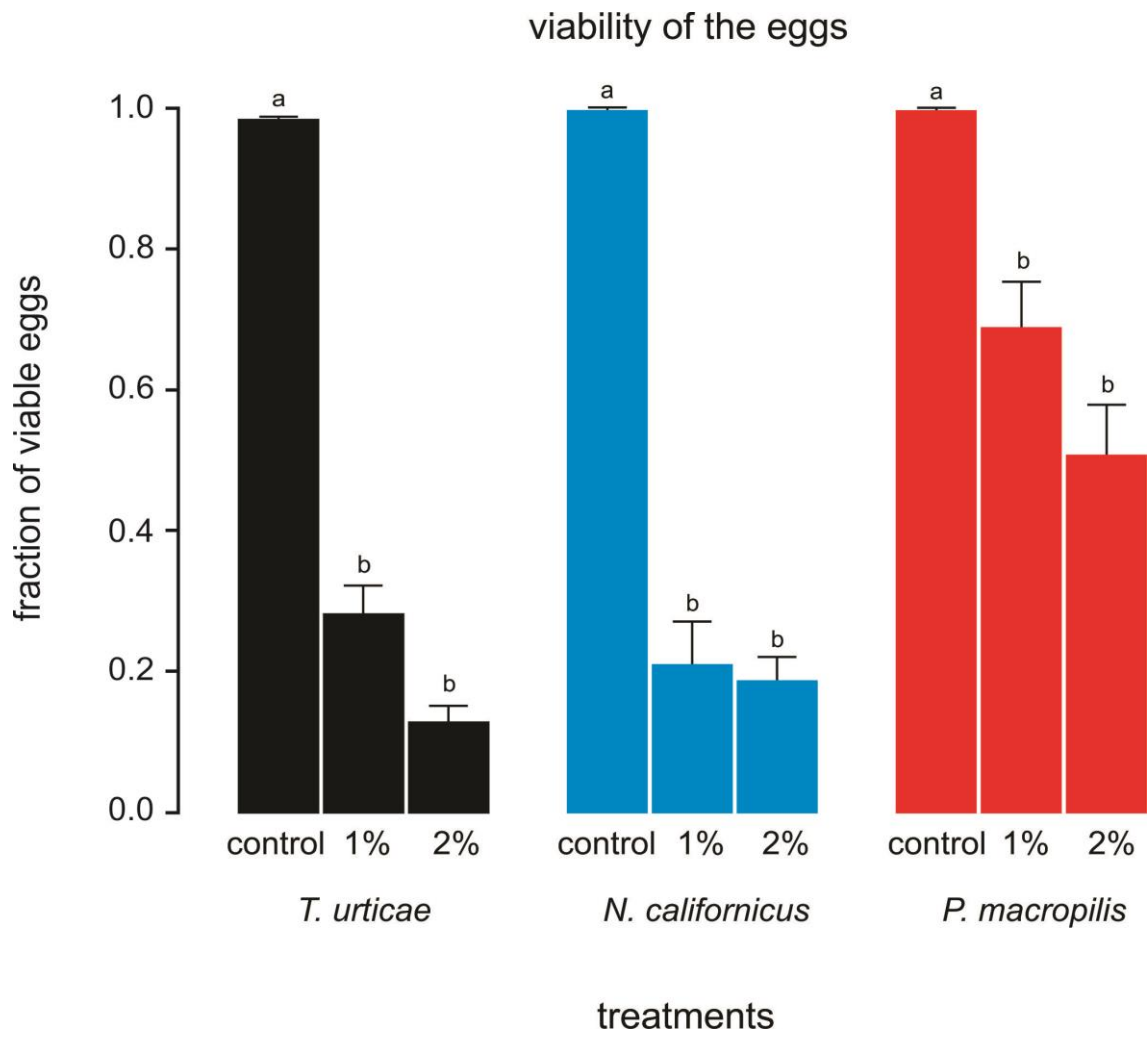
**Fig. 4.** Repellency of lime sulfur at 2% and LC<sub>50</sub> to the mites *T. urticae* (**A, D**), *N. californicus* (**B, E**) and *P. macropilis* (**C, F**). Asterisks indicate significant differences ( $P < 0.05$ ).



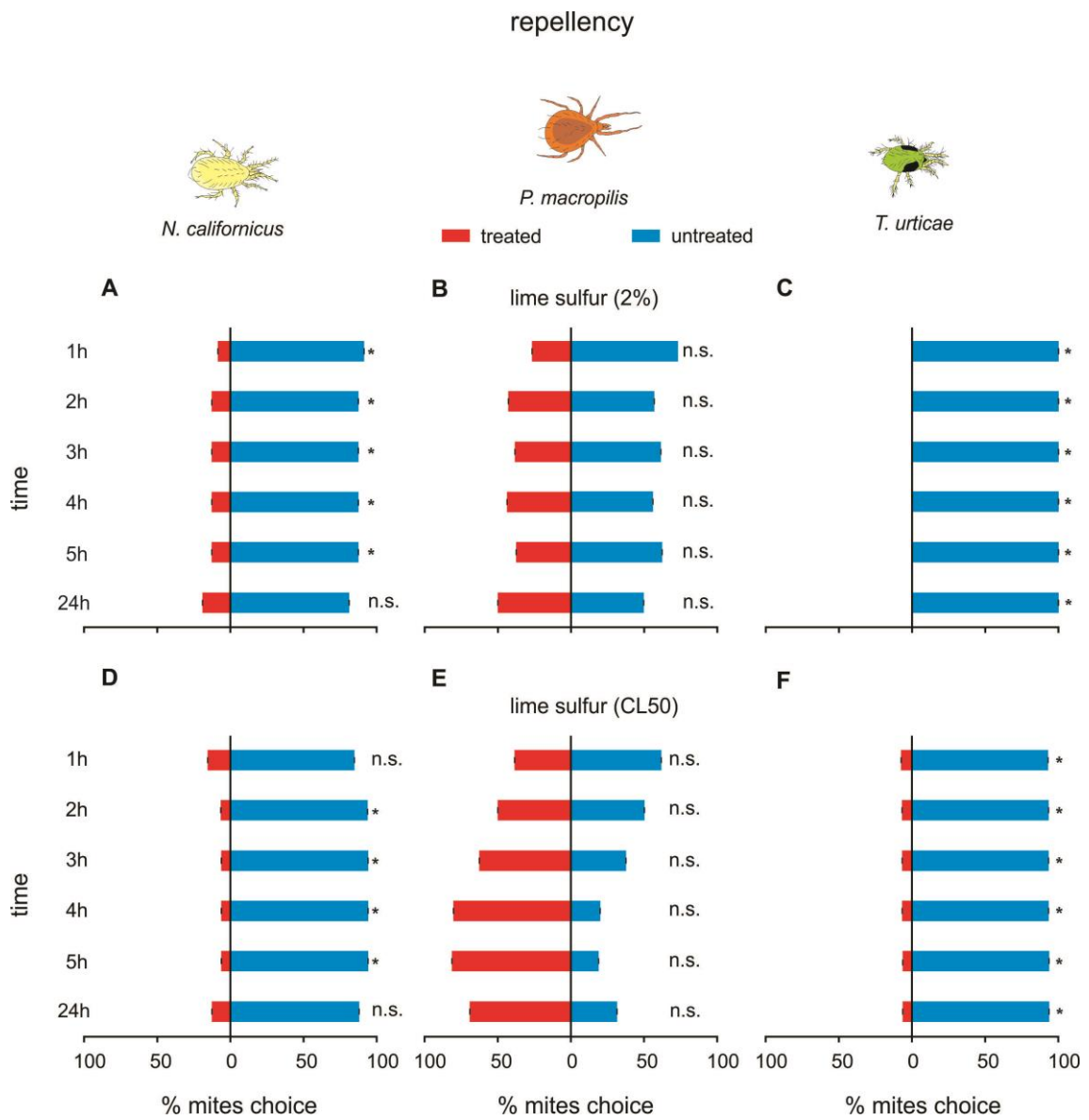
**Figure 1.**



**Figure 2.**



**Figure 3.**



**Figure 4.**

## CHAPTER 4.

# **Ultraviolet (UV) irradiation causes sterility in *Tetranychus urticae* (Koch): a technique to improve the release and commercialization of predatory mites**

Henry E. Vacacela Ajila<sup>1</sup>, André L. Perez<sup>2</sup>, Pedro H. Marques Gonçalves<sup>1</sup>, Angelo Pallini<sup>1</sup>

<sup>1</sup>*Department of Entomology, Section Acarology, Federal University of Viçosa, 36570-900, Viçosa-Minas Gerais, Brazil*

<sup>2</sup>*Econtrole Research & Consulting Ltda., Technological Center of Regional Development of Viçosa (CenTev), Federal University of Viçosa, 36570-900, Viçosa-Minas Gerais, Brazil*

\*Corresponding author:  
Henry E. Vacacela Ajila  
Tel. (55)(31) 3899-2472  
E-mail: henryeduar2@hotmail.com

## ABSTRACT

UV irradiation causes various negative effects on crop pests and diseases. The effect can be lethal depending on the dose especially in tiny organisms such as mites. We estimated an adequate time for UV irradiation that causes mortality on adult females and sterility on the eggs of *Tetranychus urticae* Koch (Acari: Tetranychidae). We also evaluated the relative suitability of inactive mites (females and eggs of *T. urticae*) on the biological parameters of the predatory mites *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae). UV irradiation caused the sterility of the eggs (5 min) and the mortality of the females (40 min) on the phytophagous mite *T. urticae*. The biological parameters (oviposition and survival) of both predatory mites *N. californicus* and *P. macropilis* were not affected by feeding on females and sterile eggs of *T. urticae*. UV irradiation is an efficient technology that causes the sterility of *T. urticae* and the sterile material (females and eggs) can be used as a suitable resource for the rearing and commercialization of predatory mites.

**Keywords:** negative effects, inactive mites, *P. macropilis*, *N. californicus*, biological parameters

## INTRODUCTION

The herbivorous mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) also known as two-spotted spider mite (TSSM) is considered one of the most important pests in many agricultural crops (Simmonds 1970; Tomczyk and Kropczynska 1985; Easterbrook et al. 2001; Rhodes and Liburd 2006; Oliveira et al. 2009; Ohtsuka and Osakabe 2009). This herbivorous mite has a high reproductive capacity, which can compromise the production (Tomczyk and Kropczynska 1985; Sato et al. 2007) or cause the total loss in crops if control measures are not taken (Chiavegato and Mischan 1981). Some control measures can be used to the management of this pest; however, chemical control is the most widely used by farmers, especially in conventional crops (Sato et al. 2007). The inappropriate and excessive use of chemical control has led to selection of resistant populations to various acaricides, thus becoming a challenging situation for managing them (Easterbrook et al. 2001; Sato et al. 2007).

There are some non-chemical alternatives that can be used in the management of *T. urticae*. One of them is the use of biological control agents, specifically predatory mites (Edge and James 1986; Easterbrook et al. 2001; Greco et al. 2005; Rhodes and Liburd 2006; Oliveira et al. 2007b, 2009; Fraulo and Liburd 2007). The species that are mostly used for biological control are within the Phytoseiidae family (Chant 1985; McMurtry and Croft 1997; Gerson et al. 2003; Mcmurtry et al. 2013). Some species such as *Phytoseiulus macropilis* (Banks) (Oliveira et al. 2007b, 2009), *Phytoseiulus persimilis* (Athias-Henriot) (Simmonds 1970; Rhodes and Liburd 2006), *Neoseiulus californicus* (McGregor) (Easterbrook et al. 2001; Rhodes and Liburd 2006), *Neoseiulus cucumeris* (Oudemans) (Easterbrook et al. 2001) stand out for the effective control of *T. urticae*.

Currently, the commerce of biological control agents for pest control is a reality on the world stage. However, the quality of predatory mites may be compromised due to long periods of starvation during transport from biofactories to the crops. Companies use many strategies to deal with this limitation; in some cases, they send a certain amount of prey together with the predators, this in order to guarantee the supply of food during the transportation. In other cases, predatory mites are deposited on substrates such as rice husk (Bellini 2008) or vermiculite.

However, there are risks associated with this strategy. Sending large numbers of prey (phytophagous mites) together with predatory mites can lead to unexpected infestations in crops. On the other hand, placing a smaller amount of prey may not be sufficient and promote several problems as mortality, cannibalism, loss of efficiency. Oliveira et al. (2007a) showed that starvation (*T. urticae*) can cause negative effects on *P. macropilis* females. Mortality, reduction in oviposition and even starvation can lead to offspring with less sexual ratio, that is, the production of a low number of female descendants than male.

It is known that UV irradiation in certain doses can promote the mortality (Ohtsuka and Osakabe 2009), escape (Barcelo and Calkins 1980; Barcelo 1981; Ohtsuka and Osakabe 2009; Tachi and Osakabe 2012) and inhibition of oviposition (Barcelo 1981; Ohtsuka and Osakabe 2009; Sakai et al. 2012; Suzuki et al. 2014) of *T. urticae* (Suzuki et al. 2009). Suzuki et al. (2009) suggests that populations of *T. urticae* can be effectively reduced with the use of UV irradiation. Specifically, UV irradiation directly damages DNA (Suzuki et al. 2009) and other important components such as lipids, lipid membranes, nucleic acids and proteins (Jurkiewicz and Buettner 1994; Shindo et al. 1994).

Thus, pre-exposure of prey to UV irradiation can ensure that predatory mites are sent to the destination together with food (eggs and mobile forms) and at the same time eliminates the risk of possible unexpected infestations. So, the objective of this study was to determine the appropriate UV irradiation time to cause female mortality and egg sterility of *T. urticae*. Additionally, we investigated whether UV irradiated mites (females and eggs of *T. urticae*) may have negative effects on the biological parameters of the predatory mites *N. californicus* and *P. macropilis*.

## **MATERIALS AND METHODS**

### **Jack bean plants**

We sowed four seeds of jack bean (*Canavalia ensiformis*) every week in four plastic pots (3L). We used the commercial soil substrate (Bioplant®) to fill all the pots. The plants were watered twice a day (250 ml). Jack bean plants were pesticide free and kept in a greenhouse ( $25 \pm 7^\circ\text{C}$ ,  $70\% \pm 20\%$  RH).

### **Mite rearing**

The predatory mite *P. macropilis* was obtained from colonies kept in the Laboratory of Acarology at the Federal University of Viçosa. *Neoseiulus californicus* was collected from pesticide-free strawberry plantations set in the municipality of Ervália at the State of Minas Gerais, Brazil ( $20^\circ 50' 34.0''\text{S}$  -  $42^\circ 40' 31.1''\text{W}$ ). The predatory mites found were placed on plastic trays (44 x 30 x 8 cm), which were put inside larger ones (60 x 40 x 10 cm). The larger tray was filled with a water layer to prevent the mites from escaping. A

portion of cotton was put underneath the smaller tray in order to fix it and avoid that it moved and touched the walls of the larger one. Leaves of jack bean infested with *T. urticae* were added in the tray as food source for the predatory mites. The leaves of jack bean infested with *T. urticae* were provided by the company Econtrôle Research & Consulting Ltda. Both colonies of predatory mites were fed three times per week, offering three or more leaves (depending of the degree of infestation) of jack bean infested with *T. urticae*. We kept the colonies in a climatic room at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and with a photoperiod of 12 hours.

### **Cohorts**

The spider mite cohort was obtained collecting adult females of *T. urticae* and transferring them to plastic trays (15.5 x 22 cm). Previously, on the bottom of this tray we had placed a layer of cotton and we added water until the cotton was totally soaked. Two leaves of jack bean (depending on the size) were placed over the soaked cotton and 200 females of *T. urticae* were transferred to the leaves. All females were removed 24h later and only the eggs laid were kept on the leaves. The eggs were incubated in a climatic room at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and with a photoperiod of 12 hours until the phytophagous mites reach adulthood. The jack bean leaves were replaced when necessary, as soon as they had the first symptoms of deterioration.

A similar methodology was used for both cohorts of the predators *P. macropilis* and *N. californicus*. We collected 60 adult females of each predator species from the colonies and transferred them to rectangular arenas (7 x 8 cm) cut from jack bean leaves infested with *T. urticae* (eggs, juveniles and adults). The arenas were accommodated over a plastic sheet (11 x 19 cm), which had previously been placed over a sponge soaked in

water inside a plastic tray (15.5 x 22 cm). The edges of the arenas were covered with wet paper tissues to prevent the mites from escaping. After 24 h the females were removed from the arenas, leaving only the eggs laid. The arenas with the egg waves of the predatory mites were incubated in a climatic room until the predatory mites reach adulthood.

### **Ultraviolet exposure**

For our experiments, we built a wooden box (length: 100 cm, width: 50 cm, height: 25 cm) with a UV lamp installed inside it (Fig. 1). A warning lamp was installed on the top of the box to indicate when the UV light was turned on. On the left side of the box two switches were installed to turn on or off the UV and the fluorescent lamps inside the box (Fig. 2). The germicidal lamp used was a fluorescent ultraviolet (UV) lamp “Moran Light”, which reaches a peak of wavelength of 254 nm, 15 w power, bulb T8, size 437mm x 26mm, base G13 and angle of beam 320°.

### **Experimental procedures**

#### **Ultraviolet (UV) exposure and its effects on egg and females of *T. urticae***

Aiming to select an adequate time to achieve the sterility of eggs and death of females of *T. urticae*, we exposed them to UV in different times recording hatching of eggs and reproduction and mortality of females. First, to test the effect on the eggs, we put a cotton layer in the bottom of the plastic tray (15.5 x 22 cm) and added water until the cotton was totally soaked. On each tray, we placed 20 leaf disc of jack bean (2.8 cm Ø) separated equidistantly from one another. In these discs, we placed ten females of *T. urticae* coming

from the rearing during 48 hours to oviposit. After that, the females were removed and the number of egg was standardized, leaving 40 eggs on the leaf discs. Subsequently, the eggs were exposed to UV according to the following treatments: 60 min, 40 min, 20 min, 15 min, 10 min, 5 min, 1 min and control (without exposure). The total number of the eggs hatched was recording during 7 days when no more hatching was observed. The total number of eggs hatched was compared between treatments and analyzed by Kruskal-Wallis ranks. For contrast of means between treatments (times), we used Dunn's method or Tukey test. All analyses were performed using SigmaPlot 12.5 (SigmaPlot 2011).

Also, we conducted experiments to evaluate the UV effect on females of *T. urticae*. We used a similar methodology of the previous experiment. So, once we had the plastic tray with the jack leaf discs, we released 10 females of the cohort (12 days old since egg) in each experimental unit. Thus, we exposed the *T. urticae* females to UV according the treatments: 60 min, 40 min, 20 min, 15 min, 10 min, 5 min, 1 min and control (without exposure). We recorded the mortality, oviposition, hatching and percentage of unviable eggs of the *T. urticae* females exposed to UV. Mortality and oviposition was evaluated for 3 days and hatching of the eggs until it was no more observed. Unviable eggs were recorded at the end of the experiment comparing the number of laid eggs vs hatching eggs. The total number of *T. urticae* dead or alive, laid eggs, hatched eggs and the percentage of inviable eggs were compared between treatments and analyzed by Kruskal-Wallis ranks. Tukey test was used for the contrast of means between treatments. Statistical analyses we performed using SigmaPlot 12.5 (SigmaPlot 2011).

## Ultraviolet (UV) exposure and its indirect effects on predatory mites

We carried-out experiments to evaluate whether egg and females of *T. urticae* after UV exposure continued to be a suitable food source for the predatory mites *P. macropilis* and *N. californicus*, recording predation, oviposition and hatching predatory eggs. First, we tested if the time after exposure could affect the eggs acceptance or rejection of *T. urticae* exposed to UV by the predatory mites. A similar methodology was used for both phytoseiid mites and we prepared arenas made from agar/water solution (1 g agar in 10 mL of water). In a glass beaker (1 L), we added water and we took into the microwave for warming-up. After, we gradually added the agar until dissolved it completely. Then, we put the hot solution in each petri dish (approximately 25 ml). As the solution cooled, we added the leaf jack bean discs in the center of the petri dish. After the solution solidified, we removed the agar around the leaf disc and filled the space with distilled water to avoid the mites escaping. Each arena was infested with 15 females of *T. urticae* from the rearing for 48 hours. Subsequently, the females were removed and the number the eggs were recorder and standardized; we left 80 eggs per arena. Four treatments were tested: a) eggs of *T. urticae* exposed to UV (40 min) after 3 days, b) 2 days, c) 1 day and d) the control treatment without UV exposure. On each day, we were exposing the arenas according to the treatments and after the arenas were kept in a climatic room until the day when the experiment was set. Previously to the predatory mites release, we put three sections (1 cm) of thread in each arena in order to offer a refuge for them (*Canavalia ensiformis* does not have trichomes). Finally, we released one mated female of each predatory mite (10 days old from egg) on each arena. The number of eggs predated and eggs laid by the predator was recorded during 3 days. The hatching of the predatory eggs was evaluated until it was no more observed. The total number of *T. urticae* eggs predated

by *P. macropilis* and *N. californicus* as well the oviposition of them were analyzed by one-way Anova with the treatment as the independent variable using SigmaPlot 12.5 (SigmaPlot 2011). We did not analyze the hatching of predatory mites because in all the treatments it was recording very close to 100%.

We also performed experiments to evaluate the effect of *T. urticae* females exposed to UV on the predation and oviposition of *P. macropilis* and *N. californicus*. The methodology used in these experiments was similar to the above. So, once the jack bean leaf disc was fixed in the center of the dish, we placed 20 females of *T. urticae* from the rearing on each arena. Then, according to the treatment we exposed the arenas (40 min) and kept them in a climatic room until the day when the experiment was set. We tested four treatments as previously described. One mated female of each predatory mite (10 days old from egg) was released on each arena according to the treatments. The total number of predated females and eggs laid was analyzed by one-way Anova or Kruskal-Wallis ranks when assumptions of normality were not satisfied. Dunn's method was used for the contrast of means between treatments. All analyzes was performed using SigmaPlot 12.5 (SigmaPlot 2011).

## **RESULTS**

### **Ultraviolet (UV) exposure and its effects on egg and females of *T. urticae***

The exposure to UV caused negative effects on the egg hatching and mortality of *T. urticae* females. We observed significant difference (*Kruskal-Wallis rank sum test*,  $F = 127.412$ ,  $df = 7$ ,  $P < 0.001$ ) on the egg hatching of *T. urticae* according to different times tested. All UV exposure times tested caused almost 100% of sterility on *T. urticae* eggs

(Fig 3 A). As expected, the egg hatching of *T. urticae* on the control was significantly higher than the in the treatments exposed to UV light (Fig. 3A).

The survival of *T. urticae* females after UV exposure was significantly different among the different times tested (*Kruskal-Wallis rank sum test*,  $H = 119.461$ ,  $df = 7$ ,  $P = <0.001$ ). When females of *T. urticae* were exposed to UV during 40 and 60 min, it was recorded 100% of mortality at the end of the third day of evaluation (Fig. 3B). As the UV exposure time was reduced, it was possible to observe alive individuals in all treatments being the control with the largest number of alive individuals  $6.9 \pm 0.19$  (Fig 3B). The oviposition of *T. urticae* females after UV exposure was also significantly different and varied among the different times of UV exposure (*Kruskal-Wallis rank sum test*,  $H = 136.911$ ,  $df = 7$ ,  $P <0.001$ ). The highest oviposition was observed in the control ( $106.90 \pm 6.22$  eggs/3 days) and in the treatment with one minute of UV exposure ( $88.35 \pm 6.35$  eggs/3 days) (Fig. 3C). The lowest oviposition was observed when females were exposed during 40 and 60 minutes to UV with an average of  $1.75 \pm 0.39$  eggs/3 days and  $2.85 \pm 0.46$  eggs/3 days, respectively (Fig. 3C). Furthermore, the percentage of unviable eggs laid by females of *T. urticae* exposed to UV was higher when females were exposed to 40 and 60 min to UV ( $53.90 \pm 10.37$  and  $70.78 \pm 6.37$ , respectively) compared to the control ( $13.05 \pm 1.43$ ) (*Kruskal-Wallis rank sum test*,  $H = 61.094$ ,  $df = 7$ ,  $P <0.001$ ) (Fig. 3D).

### **Ultraviolet (UV) exposure and its indirect effects on predatory mites**

We observed significant differences in the predation of *T. urticae* eggs by *N. californicus* after UV exposure among treatments (one-way ANOVA,  $df = 3$ ,  $P = <0,001$ ). The highest predation rate of *N. californicus* occurred when the predatory mite fed on sterile eggs of

*T. urticae* from one day after UV exposure ( $55.57 \pm 3.48$  eggs/3days). On the other treatments (3 days, 2 days and control) predation rate was very similar with  $35.28 \pm 2.16$ ,  $38.83 \pm 2.35$  and  $42.44 \pm 2.55$ , respectively (Fig. 4A). Similarly, the oviposition of *N. californicus* was also significantly different (one-way ANOVA,  $df = 3$ ,  $P = 0.023$ ). The higher rate of oviposition occurred when *N. californicus* fed on sterile eggs of *T. urticae* from 3 days old ( $5.11 \pm 0.44$ ), 2 days ( $4.72 \pm 0.37$ ) and without exposure (control) ( $6.22 \pm 0.49$ ). Females of *N. californicus* laid fewer eggs when fed on *T. urticae* sterile eggs of one-day old (Fig. 4B). However, *N. californicus* was able to feed on sterilize females of *T. urticae*. We observed statistical difference on the predation among treatments (Kruskal-Wallis rank sum test,  $H = 41.450$ ,  $df = 3$ ,  $P = <0.001$ ). The higher predation rate of sterilized *T. urticae* females by *N. californicus* was observed in three treatments 1, 2 and 3 days after UV females exposure ( $9 \pm 0.94$ ;  $8.93 \pm 1.18$ ;  $9.80 \pm 0.14$  females/3days) (Fig. 4C). On the control the predation rate was lower ( $0.30 \pm 0.14$  females/3days) (Fig. 4C). The oviposition of *N. californicus* fed on sterilized females of *T. urticae* also was statistically different among the different times tested (Kruskal-Wallis rank sum test,  $H = 41.450$ ,  $df = 3$ ,  $P = <0.001$ ). In the control, we observed the highest oviposition rate of *N. californicus* ( $5.95 \pm 0.35$  eggs/3days). Furthermore, we observed a smaller and similar amount of eggs in all other treatments (3 days:  $3.65 \pm 0.38$ ; 2 days:  $4.13 \pm 0.48$ ; and 1 day:  $4.07 \pm 0.47$  eggs/3days) (Fig. 4D).

On the other predatory species, we observed statistical differences on the predation of the sterile eggs of *T. urticae* (Kruskal-Wallis rank sum test,  $H = 20.074$ ,  $df = 3$ ,  $P = <0.001$ ). The higher rate of predation occurred when the eggs of *T. urticae* had one day old after UV exposure  $71.88 \pm 1.34$  egg/3 days. On the treatment with 3 days after UV exposure and control the predation was lower as  $59.47 \pm 3.08$  and  $50.89 \pm 3.54$ , respectively (Fig. 5A). We also observed that the oviposition of *P. macropilis* when fed

with sterile eggs of *T. urticae* was very similar in all treatments, approximately 6.54 egg/3days (one-way ANOVA,  $df = 3$ ;  $P = 0.055$ ) without showing significant difference (Fig 5B). Additionally, the predation of *T. urticae* females after UV exposure by *P. macropilis* was significantly different among treatments (*Kruskal-Wallis rank sum test*,  $H = 39.556$ ,  $df = 3$ ,  $P = <0.001$ ). The predation rate of *P. macropilis* was higher and similar between the treatments on 1, 2 or 3 days ( $12.87 \pm 0.74$ ;  $11.71 \pm 0.64$ ;  $11.87 \pm 0.78$ ) (Fig. 5C). However, in the control the predation was lower ( $1.16 \pm 0.46$  females/3days). Additionally, *P. macropilis* did not show significant differences in the oviposition when they ate sterilized females of *T. urticae* (one-way ANOVA,  $df = 3$ ;  $P = 0,208$ ). This predator laid an average of 6.54 egg/3 days in all treatments (Fig. 5D).

## DISCUSSION

Our results show that UV irradiation causes egg sterility and mortality of *T. urticae* females. So, in the present study we firstly showed that almost all times of exposure tested caused the total sterility of the eggs of *T. urticae*. The exposure of 5 minutes was the ideal time to reach total sterility. On the other hand, total mortality of *T. urticae* females occurred when they received 40-60 min of UV irradiation. Secondly, our results showed that the natural enemies of frequent occurrence in strawberry *N. californicus* and *P. macropilis* do not suffered harmful effects when fed on “sterile” eggs and females of *T. urticae*. Results indicate that these two predators can take advantage of this resource without compromising their biological parameters.

UV irradiation can cause negative effects in various organisms such as pests (Antignus et al. 1996, 2001; Doukas and Payne 2007) and diseases of crops (Aylor and

Sanogo 1997; Fourtouni et al. 1998; Raviv and Antignus 2007). On many insect pests UV irradiation can alter the orientation, navigation and communication (Raviv and Antignus 2007). Similarly, in pathogenic fungi UV irradiation can interrupt the life cycle. This is mainly caused due to the negative effects on the reproductive structures (germination and mortality of spores) (Raviv and Antignus 2007). On organisms such as mites, exposure to UV irradiation can cause lethal effects, which is mainly due to their small size (Suzuki et al. 2009). UV irradiation can promote mortality (Ohtsuka and Osakabe 2009), alter behavior (escape) (Barcelo and Calkins 1980; Barcelo 1981; Ohtsuka and Osakabe 2009; Tachi and Osakabe 2012) and inhibit oviposition (Barcelo 1981; Ohtsuka and Osakabe 2009; Sakai et al. 2012) of *T. urticae* (Suzuki et al. 2009).

The lethal effect of UV irradiation on mites can be attributed to the direct damage it causes on DNA (Suzuki et al. 2009) and other important components such as lipids, lipid membranes, nucleic acids and proteins (Jurkiewicz and Buettner 1994; Shindo et al. 1994).

When natural enemies such as predatory mites are commercialized, the maintenance diet is composed of live mites, especially *T. urticae* on its various phases. However, this type of methodology can be unsuitable for biological control because of possible crop risks. Our results showed that the UV irradiation caused negative effects in *T. urticae* and no effects in the natural enemies (*N. californicus* and *P. macropilis*) fed on sterilized eggs and females. This is very important for the applications and implications on biological control practices and specially when transporting predators to the target crops. So, the sterility of both females and eggs of *T. urticae* with 40 minutes of UV irradiation guarantees that these phytophagous mites can be used to commercialize predatory mites without risks to crops. Thus, those sterile mites can help maintain the

quality of the product to be marketed (predatory mites) and because it would help to expand the periods for the commercialization of these biological agents.

UV irradiation is a technology that has been used for several years for different purposes with very satisfactory results. Some studies that had a great highlight was the use of UV irradiation to cause the sterilization of moth eggs *Ephestia kuehniella* and *Anagasta kuehniella* (Lepidoptera: Pyralidae) (Azizoglu et al. 2011; Parra et al. 2014). Sterilized eggs of those moths are usually sold at a high value. The use of them is diverse, however they are mainly used for research in study centers and for the mass rearing of various natural enemies such as parasitoids, ladybugs, predatory bugs.

In summary, the UV irradiation caused a negative effect on the phytophagous mite *T. urticae*, the sterility of the eggs and the mortality of the females. To commercialize predatory mites it is necessary to sterilize the prey with UV for 40 minutes. This time is necessary to cause the sterility of the eggs and mortality of *T. urticae* females. Additionally, the predatory mites *N. californicus* and *P. macropilis* can feed on both sterile eggs and females of *T. urticae* without affecting their biological traits (as oviposition and survival). This technology guarantees the release of predatory mites maintaining the efficiency of them without risks in the target crop and could even extend marketing periods of store and transportation.

## **AUTHORS CONTRIBUTIONS**

HEVA, ALP and AP conceived and designed the experiments. HEVA, ALP and PHMG performed the experiments. HEVA, ALP and AP analyzed the data. AP, ALP contributed reagents/materials/analysis tools. HEVA, ALP and AP wrote the paper. All authors read, revised and approved the manuscript.

## ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. We are grateful to Students-Postgraduate Agreement Program - PEC-PG, CAPES/CNPq – Brazil, Minas Gerais Research Support Foundation (FAPEMIG), and National Council of Science and Technological Development (CNPq) for the scholarships and the financial support. The technical assistance provided by the company ECONTROLE research & consulting. Author HEVA was supported by the Students-Postgraduate Agreement Program - PEC-PG, CAPES/CNPq – Brazil, and author AP by FAPEMIG and CNPq.

## REFERENCES

- Antignus Y, Lapidot M, Cohen S (2001) Interference with ultraviolet vision of insects to impede insect pests and insect-borne plant viruses. In: Harris KF, Smith OP, Duffus JE (eds) *Virus-Insect-Plant Interactions*. Academic Press, San Diego, pp 331–350
- Antignus Y, Mor N, Ben Joseph R, et al (1996) Ultraviolet-absorbing plastic sheets protect crops from insect pests and from virus diseases vectored by insects. *Environ Entomol* 25:919–924
- Aylor DE, Sanogo S (1997) Germinability of *Venturia inaequalis* conidia exposed to sunlight. *Phytopathology* 87:628–633
- Azizoglu U, Yılmaz S, Karaborklu S, Ayvaz A (2011) Ovicidal activity of microwave and UV radiations on Mediterranean flour moth *Ephesia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae). *Tükr Entomol Derg* 35:437–446
- Barcelo JA (1981) Photoeffects of visible and ultraviolet radiation on the two-spotted spider mite, *Tetranychus urticae*. *Photochem Photobiol* 33:703–706
- Barcelo JA, Calkins J (1980) The kinetics of avoidance of simulated solar UV radiation by two arthropods. *Biophys J* 32:921–929
- Bellini MR (2008) Manejo de *Tetranychus urticae* Koch (Acari: Tetranychidae) em plantas ornamentais. Tese de Doutorado em Ecologia de Agroecossistemas, Universidade de São Paulo

- Chant DA (1985) The Phytoseiidae. In: Spider mites their biology, natural enemies and control. Elsevier, Amsterdam, pp 3–32
- Chiavegato L, Mischan M (1981) Efeito do acaro *Tetranychus* (T.) *urticae* (Koch, 1836) Boudreaux & Dosse, 1963 (Acari, Tetranychidae) na produção do morangueiro (*Fragaria* spp.) cv. 'Campinas'. Científica 9:257–266
- Doukas D, Payne CC (2007) Greenhouse whitefly (Homoptera: Aleyrodidae) dispersal under different UV-light environments. J Econ Entomol 100:389–397
- Easterbrook MA, Fitzgerald JD, Solomon MG (2001) Biological control of strawberry tarsonemid mite *Phytonemus pallidus* and two-spotted spider mite *Tetranychus urticae* on strawberry in the UK using species of *Neoseiulus* (*Amblyseius*) (Acari: Phytoseiidae). Exp Appl Acarol 25:25–36
- Edge VE, James DG (1986) Organo-tin Resistance in *Tetranychus urticae* (Acari: Tetranychidae) in Australia. J Econ Entomol 79:1477–1483
- Fourtouni A, Manetas Y, Christias C (1998) Effects of UV-B radiation on growth, pigmentation, and spore production in the phytopathogenic fungus *Alternaria solani*. Can J Bot 76:2093–2099
- Fraulo AB, Liburd OE (2007) Biological control of twospotted spider mite, *Tetranychus urticae*, with predatory mite, *Neoseiulus californicus*, in strawberries. Exp Appl Acarol 43:109
- Gerson U, Smiley RL, Ochoa R (2003) Mites (Acari) for pest control. Blackwell Science, Oxford, England
- Greco NM, Sánchez NE, Liljesthröm GG (2005) *Neoseiulus californicus* (Acari: Phytoseiidae) as a potential control agent of *Tetranychus urticae* (Acari: Tetranychidae): effect of pest/predator ratio on pest abundance on strawberry. Exp Appl Acarol 37:57–66
- Jurkiewicz BA, Buettner GR (1994) Ultraviolet light-induced free radical formation in skin: an electron paramagnetic resonance study. Photochem Photobiol 59:1–4
- McMurtry JA, Croft BA (1997) Life-styles of phytoseiid mites and their roles in biological control. Annu Rev Entomol 42:291–321
- McMurtry JA, Moraes GJD, Sourassou NF (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Syst Appl Acarol 18:297–320
- Ohtsuka K, Osakabe M (2009) Deleterious effects of UV-B radiation on herbivorous spider mites: they can avoid it by remaining on lower leaf surfaces. Environ Entomol 38:920–929
- Oliveira H, Duarte V, Rezende D, et al (2007a) Períodos de ausência de presas e estabilidade do controle biológico do ácaro-rajado. Pesqui Agropecuária Bras 42:1207–1209

- Oliveira H, Fadini MAM, Venzon M, et al (2009) Evaluation of the predatory mite *Phytoseiulus macropilis* (Acari: Phytoseiidae) as a biological control agent of the two-spotted spider mite on strawberry plants under greenhouse conditions. *Exp Appl Acarol* 47:275–283
- Oliveira H, Janssen A, Pallini A, et al (2007b) A phytoseiid predator from the tropics as potential biological control agent for the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Biol Control* 42:105–109
- Parra J, Coelho Jr A, Geremias L, et al (2014) Criação de *Anagasta kuehniella*, em pequena escala, para produção de *Trichogramma*. Piracicaba Ocasio 32
- Raviv M, Antignus Y (2007) UV radiation effects on pathogens and insect pests of greenhouse-grown crops. *Photochem Photobiol* 79:219–226
- Rhodes EM, Liburd OE (2006) Evaluation of predatory mites and acaricide for control of twospotted spider mites in strawberries in North Central Florida. *J Econ Entomol* 99:1291–1298
- Sakai Y, Sudo M, Osakabe M (2012) Seasonal changes in the deleterious effects of solar ultraviolet-B radiation on eggs of the twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *Appl Entomol Zool* 47:67–73
- Sato ME, Da Silva MZ, De Souza Filho MF, et al (2007) Management of *Tetranychus urticae* (Acari: Tetranychidae) in strawberry fields with *Neoseiulus californicus* (Acari: Phytoseiidae) and acaricides. *Exp Appl Acarol* 42:107–120
- Shindo Y, Witt E, Han D, Packer L (1994) Dose-Response effects of acute ultraviolet irradiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. *J Invest Dermatol* 102:470–475
- SigmaPlot (2011) SigmaPlot for windows, version 12.5. Systat Software, Inc, San Jose, CA
- Simmonds SP (1970) The possible control of *Steneotarsonemus pallidus* on strawberries by *Phytoseiulus persimilis*. *Plant Pathol* 19:106–107
- Suzuki T, Watanabe M, Takeda M (2009) UV tolerance in the two-spotted spider mite, *Tetranychus urticae*. *J Insect Physiol* 55:649–654
- Suzuki T, Yoshioka Y, Tsarsitalidou O, et al (2014) An LED-based UV-B irradiation system for tiny organisms: System description and demonstration experiment to determine the hatchability of eggs from four *Tetranychus* spider mite species from Okinawa. *J Insect Physiol* 62:1–10
- Tachi F, Osakabe M (2012) Vulnerability and behavioral response to ultraviolet radiation in the components of a foliar mite prey–predator system. *Naturwissenschaften* 99:1031–1038
- Tomczyk A, Kropczynska D (1985) Effects on the host plant. In: Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam, pp 317–327

## FIGURE LEGENDS

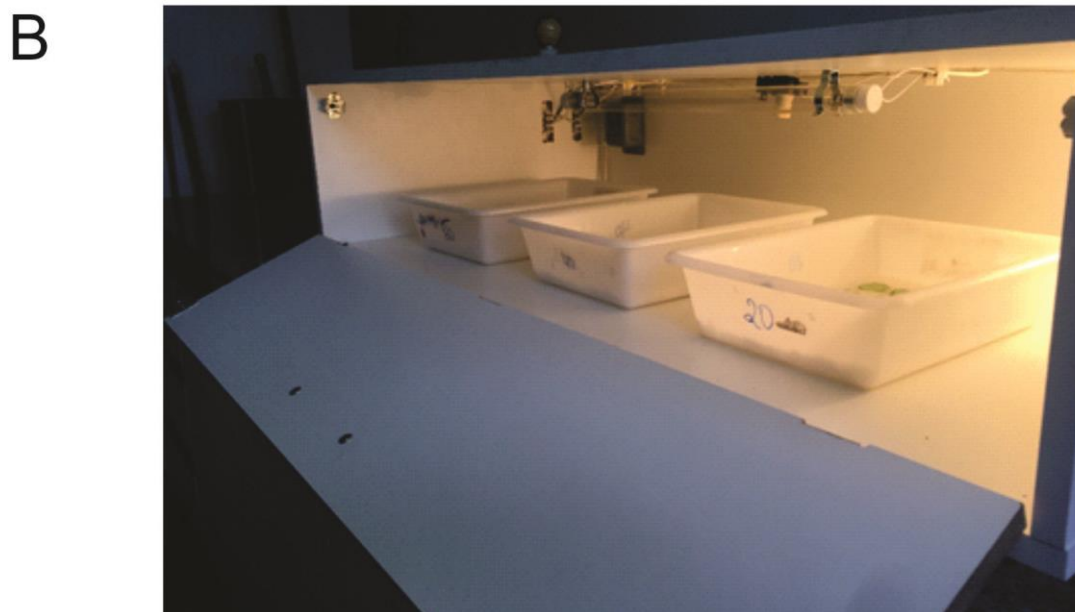
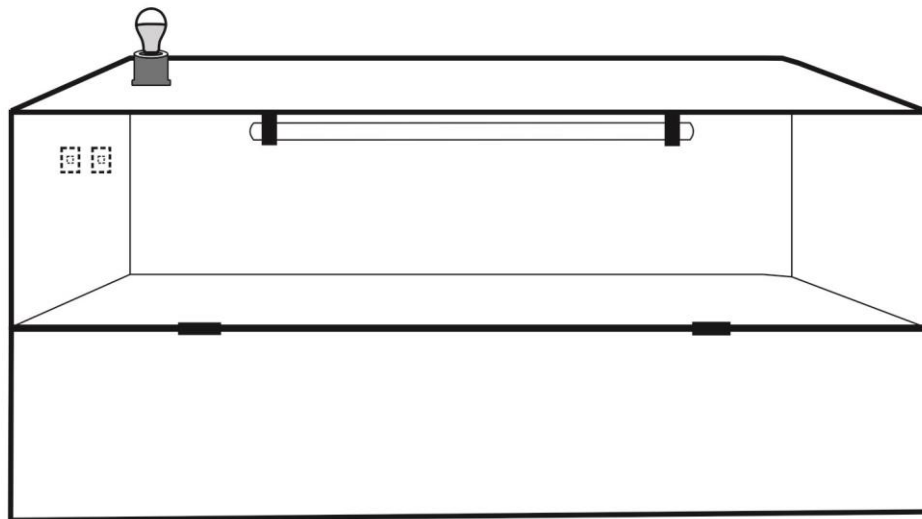
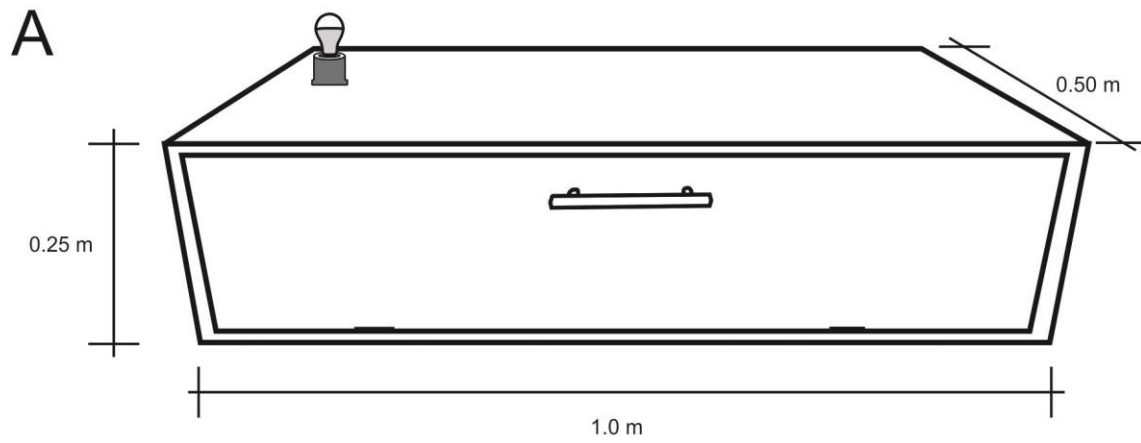
**Fig. 1** A) Design of the wood structure used for UV irradiation. Inside the structure were installed two lamps on the top: a germicidal UV lamp and an incandescent. B) Structure used for UV irradiation. In the interior, it were placed the plastic trays with the material to be sterilized.

**Fig. 2** Ultraviolet (UV) exposure and its effects on eggs and females of *Tetranychus urticae*. A) Number of hatching eggs ( $\pm$  EP) of *T. urticae* after UV exposure. B) Number of individuals ( $\pm$  EP) (alive/dead) of *T. urticae* after UV exposure. C) Number of eggs ( $\pm$  SE) of *T. urticae* females after UV exposure. D) Percentage ( $\pm$  SE) of non-viable eggs after exposure of females to UV. The letters above each bar indicate the differences between treatments.

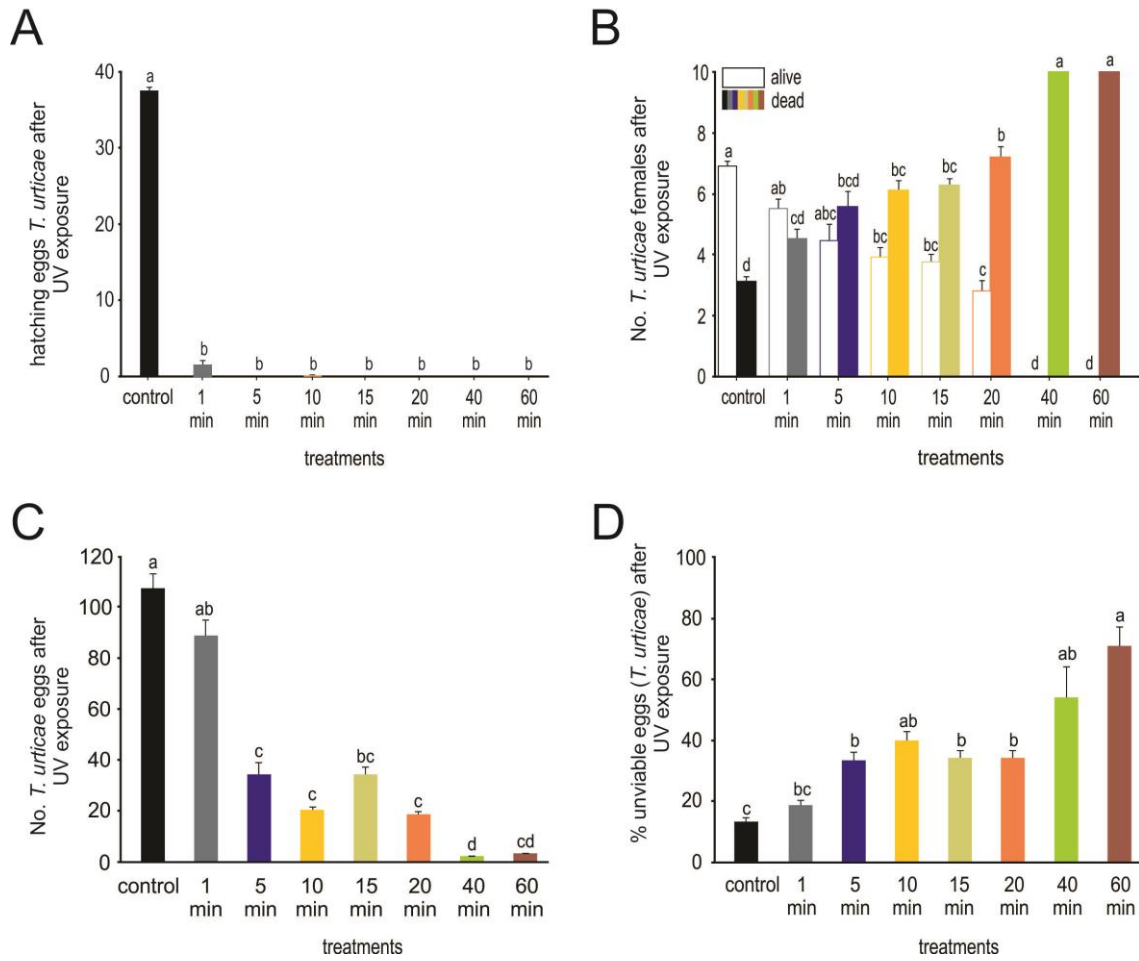
**Fig. 3** Ultraviolet (UV) exposure of eggs and females of *Tetranychus urticae* and their effects on the predatory mite *Neoseiulus californicus*. A) Number of preyed eggs ( $\pm$  SE) of *T. urticae* after UV exposure by *N. californicus*. B) Number of *N. californicus* ( $\pm$  EP) eggs fed with *T. urticae* eggs exposed to UV. C) Number of preyed females of *T. urticae* exposed to UV ( $\pm$  EP) by *N. californicus*. D) Number of *N. californicus* ( $\pm$  EP) eggs fed with UV-exposed *T. urticae* females. The letters above each bar indicate the differences between treatments.

**Fig. 4** Ultraviolet (UV) exposure of eggs and females of *Tetranychus urticae* and their effects on the predatory mite *Phytoseiulus macropilis*. A) Number of preyed eggs ( $\pm$  EP) of *T. urticae* after UV exposure by *P. macropilis*. B) Number of eggs of *P. macropilis* ( $\pm$

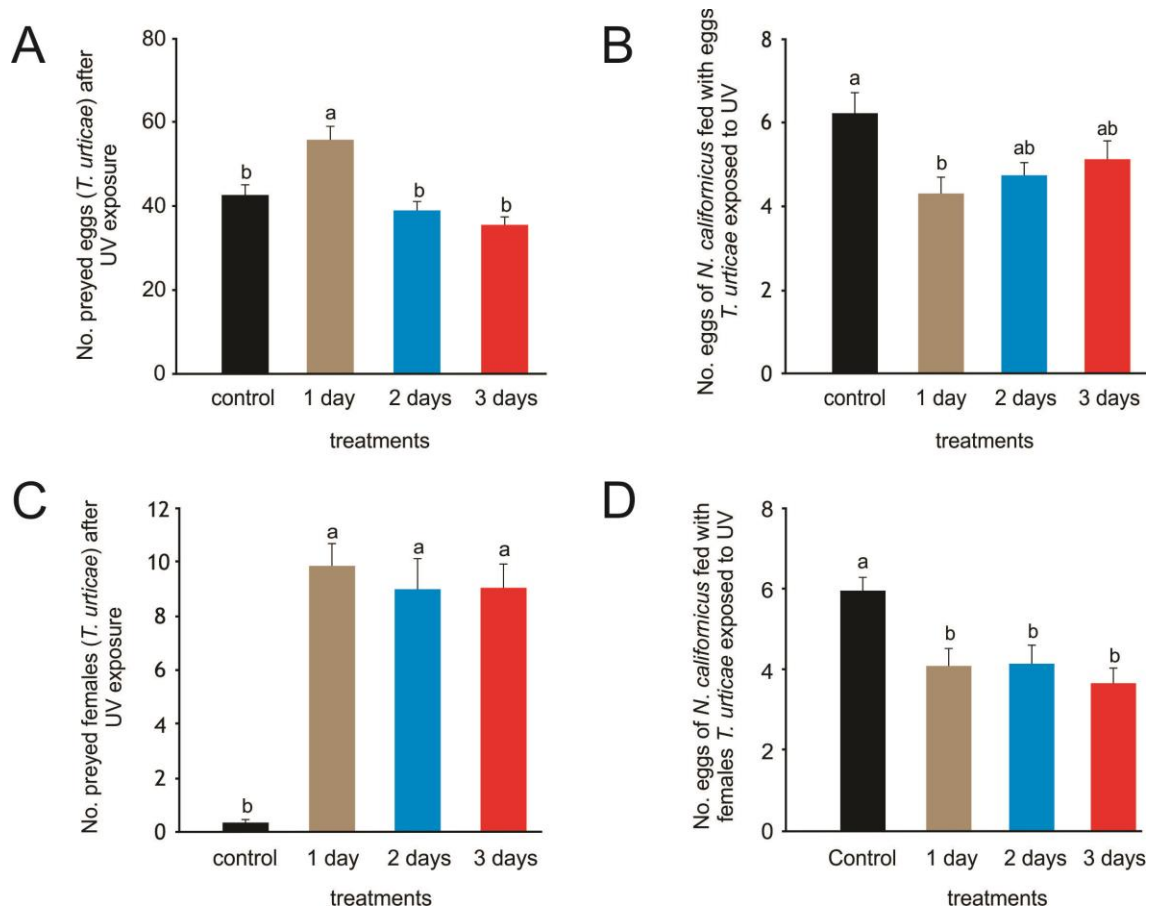
EP) fed with *T. urticae* eggs exposed to UV. C) Number of preyed females of *T. urticae* exposed to UV ( $\pm$  EP) by *P. macropilis*. D) Number of eggs of *P. macropilis* ( $\pm$  EP) fed with *T. urticae* females exposed to UV. The letters above each bar indicate the differences between treatments.



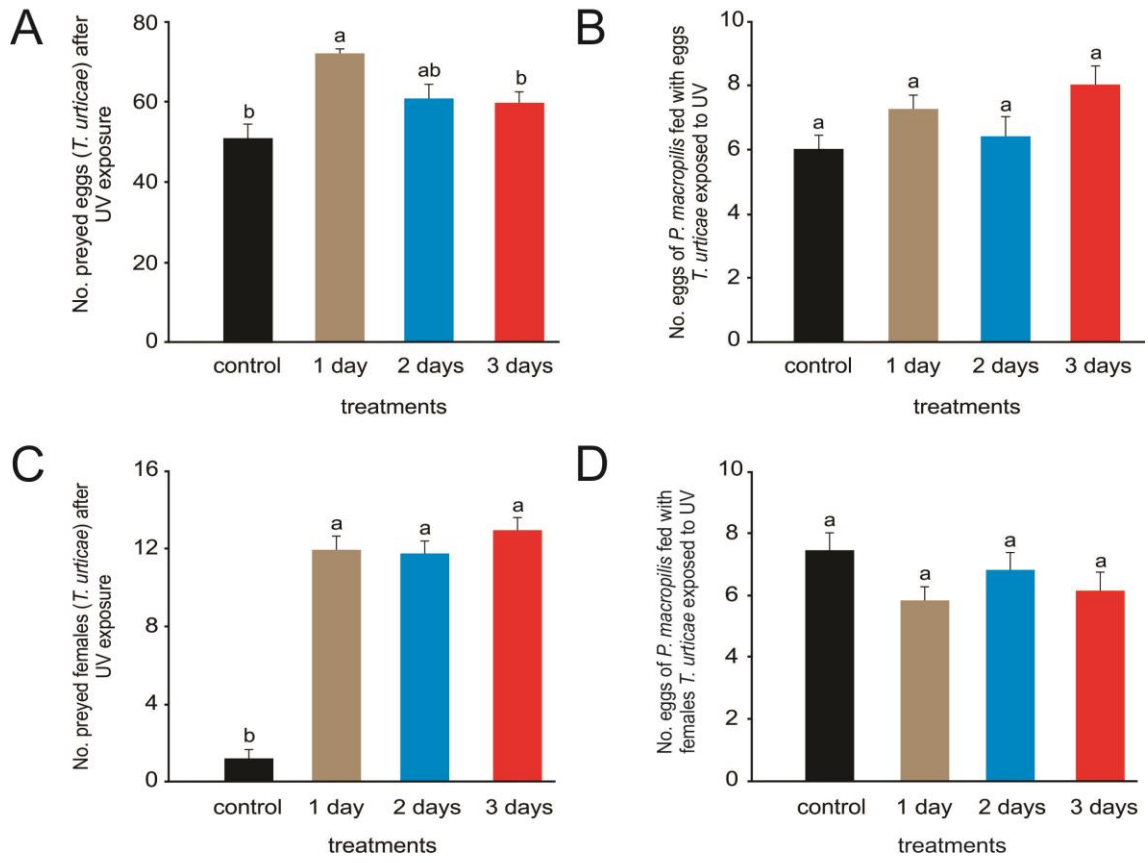
**Figure 1.**



**Figure 2**



**Figure 3**



**Figure 4**

## GENERAL CONCLUSIONS

While managing crops, the herbivore *Phytonemus pallidus* was found in organic strawberry infesting and causing severe losses in two municipalities at the state of Minas Gerais-Brazil.

Our attempt to select better natural enemies to control pest mites in the crop found that *Tetranychus urticae* was the only food that supported the development, survival and reproduction of *N. californicus* as a monotypic diet. This species has the potential to contribute preventively to the biological control of the spider mite on the crop. Provisioning of supplementary food such as pollen could serve to retain mites in the strawberry crops at low pest densities, either before their arrival, or after they have been turned into low numbers due to predation.

We also assessed the effectiveness of lime sulfur in the studied system and came with the results that the lethal effect of the lime sulfur was higher for natural enemies than for the pest. The doses necessary to cause mortality of fifty per cent of the mite populations are very high and therefore not applicable since they could cause problems like phytotoxicity. Furthermore, the effect on the reduction of the oviposition and the ovicidal effect observed in *T. urticae* together indicates that this alternative product could be still an alternative for the control of this pest since the population would be affected to a great extent. Furthermore, out the three species studied, *P. macropilis* was the only one that did not show repellency to the lime sulfur, which can be positive due to the possible compatibility of managing strategies used in the field.

Finally, we innovated and presented results showing that UV irradiation caused a negative effect on the phytophagous mite *T. urticae* as the sterility of the eggs and the mortality of the females. Thus, 5 minutes was the time necessary to cause the sterility of the eggs and 40 minutes to cause the mortality of the females of *T. urticae*. Additionally,

the predatory mites *N. californicus* and *P. macropilis* can feed on both sterile eggs and females of *T. urticae* without affecting their biological parameters (oviposition and survival). So, this new approach and technology provide a formal assurance that the release of predatory mites will maintain the efficiency of them without risks of infestation of the herbivores used as diet in the target crop. And, it could even extend marketing periods of store and transportation of natural enemies for the purpose of biological control.