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CÂMPUS DE JABOTICABAL

DISTRIBUTION, BIOECOLOGY AND MANAGEMENT OF THE CITRUS BROWN MITE *Tegolophus brunneus* Flechtmann (ACARI: ERIOPHYIDAE)

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Biologist

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TÍTULO DA TESE: DISTRIBUTION, BIOECOLOGY AND MANAGEMENT OF THE CITRUS BROWN MITE *Tegolophus brunneus* FLECHTMANN (ACARI: ERIOPHYIDAE)

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Matheus Rovere De Morais was born in Tabatinga, São Paulo, on February 21th, 1990. Son of José Caetano de Morais Neto and Aparecida Célia Rovere de Morais, he started studies in Tabatinga, São Paulo, where he finished basic level education in 2004. In 2005, He enrolled at Colégio Técnico Agrícola "José Bonifácio" of Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP/FCAV), in Jaboticabal, São Paulo, where he finished high school and the course of Agricultural Technician in 2007. In 2008, he began studying biological sciences at the same university and graduated in January 2013. In March 2013, he initiated a Masters at the Graduate Program in Sciences (Entomology) in Escola Superior de Agricultura "Luiz de Queiroz", of Universidade de São Paulo (ESALQ/USP), where he was funded by Coordination for the Improvement of Higher Education Personnel (CAPES) and developed his dissertation about integrated pest management, advised by Prof. Dr. Pedro Takao Yamamoto. Matheus obtained his Masters degree in June 2015 and, in August 2015, he started his doctorate in the Graduate Program in Agronomy (Agricultural Entomology) at UNESP/FCAV, advised by Prof. Dr. Daniel Júnior de Andrade, and funded by the São Paulo Research Foundation (FAPESP) and CAPES.

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SUMMARY

Page

DISTRIBUIÇÃO, BIOECOLOGIA E MANEJO DO ÁCARO-MARROM-DOS-CITROS *Tegolophus brunneus* FLECHTMANN (ACARI: ERIOPHYIDAE)

RESUMO - O ácaro-da-ferrugem-dos-citros *Phyllocoptruta oleivor*a é uma das principais pragas dos citros no Brasil. Os problemas atribuídos a *P. oleivora* têm se intensificado e suspeita-se que os danos estejam relacionados a uma nova espécie de eriofiídeo descrita recentemente, o ácaro-marrom-dos-citros *Tegolophus brunneus*. No entanto, não há estudos com essa espécie e informações sobre sua distribuição, características bioecológicas, danos, suscetibilidade a acaricidas e inimigos naturais associados são ausentes. O objetivo principal do projeto foi estudar a distribuição de *T. brunneus* na principal região citrícola brasileira e sobre as principais espécies e variedades cítricas. Além disso, estudou-se a biologia de *T. brunneus* em laboratório, determinou-se a suscetibilidade desse ácaro aos principais acaricidas, caracterizando seus danos em plantas cítricas, bem como o potencial de predação das principais espécies de predadores associadas ao ácaro e seu potencial para o uso no controle biológico. As coletas realizadas em vários municípios do estado São Paulo e Triângulo Mineiro demonstraram que *T. brunneus* infestou apenas lima ácida 'Tahiti', enquanto *P. oleivora* infestou todas as outras espécies e variedades cítricas. O ácaro completa o desenvolvimento em 7 dias, com período de incubação de 3 dias, duração de larva de 2,1 dias e de ninfa 2,8 dias e as fêmeas apresentam fecundidade de 8,5 ovos a 25°C. Os danos da espécie caracterizam-se pelo prateamento dos frutos e formação de manchas escuras na casca, a semelhança dos causados por *P. oleivora*. *Tegolophus brunneus* foi 13 vezes mais tolerante a abamectina que *P. oleivora*, enquanto a toxicidade de ambas as espécie foi a mesma a enxofre. Os predadores coletados nas áreas de produção foram *Iphiseiodes zuluagai*, *Euseius concordis* e *Proprioseiopsis neotropicus*. Os predadores *E. concordis*, *Euseius citrifolius* e *Amblyseius acalyphus* apresentaram resposta funcional tipo II predando sobre *P. oleivora* e *T. brunneus*, enquanto *I. zuluagai* apresentou resposta do tipo III. *Amblyseius acalyphus* predou em média 150 ácaros adultos de ambas as presas, enquanto *I. zuluagai* and *E. concordis* predaram em média 100 e 87 ácaros adultos de *P. oleivora* e 115 e 72 ácaros adultos de *T. brunneus*, respecticamente. *Euseius citrifolius* apresentou uma predação menor de aproximadamente 20 ácaros adultos de ambas as espécies. Os resultados obtidos na pesquisa permitem classificar *T. brunneus* como uma espécie de grande importância para lima ácida Tahiti devido aos danos ocasionados e ao rápido desenvolvimento da espécie evidenciado pelo estudo do desenvolvimento biológico do ácaro. Ainda, deve-se ter atenção ao manejo químico da espécie utilizando abamectina, pelos maiores níveis de tolerância dessa espécie em relação a *P. oleivora*. Os ácaros predadores mais comumente encontrados em áreas de produção apresentaram elevado consumo de *T. brunneus* e *P. oleivora*, com destaque para *A. acalyphus* que apresentou a maior predação, sendo necessários maiores estudos para o uso dessa espécie nos pomares cítricos.

Palavras chave: Manejo integrado de pragas, controle biológico, especificidade hospedeira, controle químico.

DISTRIBUTION, BIOECOLOGY AND MANAGEMENT OF CITRUS BROWN MITE *Tegolophus brunneus* (ACARI: ERIOPHYIDAE)

ABSTRACT - The citrus rust mite *Phyllocoptruta oleivora* is one of the key pests of citrus in Brazil. The problems attributed to *P. oleivora* have been increasing recently and it is suspected that the damages are caused due to a new species of rust mite recently described, the citrus brown mite *Tegolophus brunneus*. However, this species has not been studied so far, and its distribution, bioecological characteristics, potential damage, susceptibility to acaricides and associated natural enemies are unknown. Thus, the objective of the work was to study *T. brunneus* distribution in the main citrus production region of Brazil and on the main citrus varieties. Furthermore, *T. brunneus* biological development was studied, as well as its susceptibility to the main acaricides, damages caused to the plants, as well the main predatory mites in citrus orchards and its potential of predation to *T. brunneus* and *P. oleivora*. The survey conducted in many municipalities in São Paulo and Minas Gerais States showed that *T. brunneus* infests only ‘Tahiti’ acid lime, while *P. oleivora* infests all citrus species and varieties. *Tegolophus brunneus* completes its life cycle in 7 days, with an incubation period of 3 days, larvae duration of 2.1 days and nymh duration of 2.8 days and female fecundity of 8.5 eggs at 25°C. The damages caused by this species are characterized by the silvering and formation of dark spots in the fruit skin, similar to the caused by *P. oleivora*. *Tegolophus brunneus* was 13 times more tolerant to abamectin than *P. oleivora*, while the toxicity of sulfur was equal in both species. The predatory mites collected in citrus production areas were *Iphiseiodes zuluagai*, *Euseius concordis* and *Proprioseiopsis neotropicus*. The predators *E. concordis*, *Euseius citrifolius* and *Amblyseius acalyphus* showed a type II functional response preying on adults of *P. oleivora* and *T. brunneus*, while *I. zuluagai* showed a type III functional response. *Amblyseius acalyphus* preyed on average 150 adult mites of both mite prey species, while *I. zuluagai* and *E. concordis* preyed on average of 100 and 87 adult mites of *P. oleivora* and 115 and 72 adult mites of *T. brunneus*, respectively. *Euseius citrifolius* demonstrated a lower predation than the other predators, and consumed on average 20 adult mites of both prey species. The results obtained permit classification of *T. brunneus* as an important pest of the ‘Tahiti’ acid lime due to the damages caused and the fast development of this mite in the laboratory. Furthermore, it is important to be aware of the higher tolerance levels of this species to abamectin compared to *P. oleivora*. The predatory mites commonly found in citrus orchards showed high feeding rates on *T. brunneus* and *P. oleivora*, especially *A. acalyphus*, which showed the highest predation, being necessary to further study this species to use in biological control against citrus pests.

Keywords: Integrated pest management, biological control, host specificity, chemical control

Chapter 1 – General considerations

1. Introduction

Brazil is the largest orange producer and exporter of concentrated orange juice in the world. This crop is an important resource and generates jobs to the country (Neves and Trombini, 2017). One of the main challenges to citrus production is damage caused by pests and diseases. Among pests, the mites should be highlighted, as some species are key pests of citrus, requering investments in pest control every year to prevent losses of production (Rodrigues and Oliveira, 2005; Vacante, 2010).

The citrus sector has faced phytosanitary challenges over the years, but has been able to keep up with production due to the use and development of management tactics, although these actions have had short durations and high economic and environmental costs (Bastianel et al., 2006; Bové 2006; Picazo-Tadeo and Reig-Martínez, 2006; Belasque Jr. et al., 2010; Grogan and Goodhue, 2012). Citriculture exiges nowadays high investments to produce, mainly due to the management of citrus psyllidae *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), transmissor of Huanglongbing (HLB), and for mite control, mainly the citrus leprosis mite *Brevipalpus yothersi* Baker and *Brevipalpus papayensis* Baker (Acari: Tenuipalpidae) and the citrus rust mite *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) (Van Leeuwen et al., 2010, Neves at al., 2010, Nunes et al, 2018).

The citrus rust mite *P. oleivora* is considered less harmful than *D. citri* and *B. yothersi*, however, it is surprising growers and technicians in the main citrus growing region of Brazil, i.e., São Paulo State and West-Southwest Minas Gerais belt (Rodrigues and Oliveira, 2005; Fundecitrus, 2018), due to increasing of its crop damage and the failure to control this mite. Over the past decades, the management of *P. oleivora* in Brazil was based on the use of two acaricides: sulfur and abamectin, which can have selected mite populations resistant to these products (Omoto and Alves, 2004).

A new species that causes russeting in citrus was described in São Paulo State: the citrus brown mite *Tegolophus brunneus* Flechtmann (Acari: Eriophyidae) (Flechtmann, 1999), and some growers suspect that this species is responsible for the increase of rust damages in citrus and the difficulty to control pests, despite no scientific proof having been presented so far.

Knowledge about *T. brunneus* is needed, since one of the tenets of Integrated pest management (IPM) is the precise identification of the organisms that infest a crop and its damage potential, as well as the main natural mortality agents of this species, allowing the development of management strategies suitable to keep the population levels of this organism below economic threshold levels (Kogan, 1998; Hoy, 2016). However, studies have not been performed with *T. brunneus* involving host specificity, damage potential, biological development, natural factors of mortality and susceptibility to main acaricides

The lack of knowledge about *T. brunneus* pest status and the current challenges to control rust mites in citrus could lead to management decisions with little to no scientific basis, which goes against the urgent necessity of rationalize the pesticide use in citrus, through the integration of management strategies based on IPM precepts.

2. Literature Review

2.1 Citrus economic importance

The citriculture has a high economic importance to Brazilian economy. The country is responsible for 34% of orange fruits and 76% of orange juice produced in the world, being the greatest producer of that commodity (USDA, 2018). The orange production in Brazil is concentrated in São Paulo State and the West-Southwest Minas Gerais belt, with 191.69 million trees in the region, producing 10 million tons of oranges in the 2016/2017 season. The citrus sector is fundamental to the economy of at least 350 orange-producing municipalities in this region, generating $6.5 billion annually in all chain of production. Besides that, citrus industry plays an important social role, as the crop employs a large amount of hand labor, especially during harvesting, generating around 200 thousand direct and indirect jobs in the region (Neves and Trombin, 2017; Fundecitrus, 2018).

Other important areas of citrus production are the production of lemons, limes and mandarins. Brazil produces nearly 1.3 million tons of ‘Tahiti’ acid lime *Citrus latifolia* Tanaka, and São Paulo State produces 978 thousand tons, being the main producing state. Brazil is the second largest producer of this fruit in the world and exports only 10% of production (IBGE, 2019).

Brazil also produces 965 thousand tons of mandarins, including 370 just in São Paulo State. It is important to note that these fruits are produced mainly by small farmers, creating important resources and jobs to the municipalities close to fruit production areas (IBGE, 2019). Citrus also generates important sub products, such as citrus pulp, used to feed animals like cattle, and oils and essences used in the cosmetic industry (Rezzadori and Benedetti, 2009).

The citrus sector also invests in research and training in institutions such as the Fund for Citrus Protection (Fundecitrus) in Brazil, as well as in partnerships with many universities and research centers. This investment in the formation of human capital and knowledge produces a great return to the society that goes beyond citriculture, as the results produced impact other agrobusiness sectors (Moricochi et al., 1981; Figueiredo et al., 2012).

2.2. Bioecological aspects of Eriophyidae

Eriophyidae is the mite family with the highest number of species described so far, with more than four thousand species, many of which still need to be investigated more in depth. The great diversity of these mites is dependent on the strong relationship and specificity that they have with their hosts (Lillo and Scoracka, 2010).

The eriophyids present important peculiarities, such the presence of two legs during all development stages and the reduced size compared to other mites, being some of the tiniest known arthropods. These mites present egg, larvae, nymph and adult stages (Lillo and Skoracka, 2010). There is some controversy in these terms and some authors prefer to denominate egg, protonymph, deutonymph and adult or egg, nymph I, nymph II and adult stages (Sabelis and Bruin, 1996).

These mites undergo both sexual and asexual reproduction, and copulation is absent. The males release a structure in the substrate called a spermatophore which contains the spermatozoids. The female then absorbs the spermatophore through the genital opening (Michalska et al., 2010). The asexual reproduction happens through arrhenotokous parthenogenesis, in which non-fertilized eggs will originate only males (Helle and Wysoki, 1983).

Eriophyidae also presents deuteroginy, which consists of a resistant form to cope with adverse climatic periods. In the species that this behavior is observed, the females present a protogyne form when the environmental conditions are suitable to mite development and a deutoginy form in adverse climatic conditions. In the majority of species a constriction of opistossomal annuli occurs to reduce water loss and resist extreme periods. The morphological changes in deutoginy females caused equivocated description of species, which consisted in the deutoginy forms of described species (Sabelis and Bruin, 1996).

The eriophyid mites are important pests in many crops, causing significant damages and huge economic losses, being considered the second most important mite family in agricultural production, preceded only by Tetranychidae (Van Leeuwen et al., 2010). Other important aspects of these Eriophyidae refer to its role as biological control agents of invasive plants, due to its high specificity, fast populational growth and damages caused to the plants (Smith et al., 2010).

Due to reduced size and for living hidden in plant structures, these mites have a great quarantine importance, since they can be easily transported from place to place in vegetative structures or even by human beings. Thus, approaches are necessary to detect these mites before they invade countries where they were not reported (Navia et al., 2010).

The hidden lifestyle of eriophyids makes mite management difficult, as the natural enemies and acaricides applied do not reach these mites easily (Van Leeuwen et al., 2010). It is not the same for eriophyid species that do not live in hidden structures, and present a vagrant life style, walking on vegetal substrates throughout their development, being exposed to natural control agents. Sabelis and Bruin (1996) discussed about these different lifestyles and suggested that there is a tradeoff between the higher protection conferred to the mites which live hidden in vegetal structures and the higher food resources that the vagrant mites can exploit. Also, competition is higher for mites which live concealed in vegetal structures, as usually many mites of these species live together.

The damages caused by Eriophyidae to the plants are diverse, causing galls, blisters, erinium, russeting, bronzing and silvering of vegetative structures (Royalt and Perring, 1996). These damages develop due to physiological alteration of the host. It is also suspected that in some cases the damages are the result of association with microorganisms and algae (Mccoy and Albrigo, 1975; Sharma, 1991).

The dispersal potential by walking for eriophyid mites is limited and the main ways of dispersion is by the wind or phoresy. Phoresy dispersion happens when these mites adher to other organisms to be carried to a new host (Michalska et al., 2010). Wind dispersion is riskier to these organisms, as due to the high host specificity of these mites, there is little probability of reaching a suitable host. In contrast, when associated with a pollinator of the host plant, the chances of getting to a suitable host increases significantly, as occurs for *Aceria litchii* (Keifer) (Acari: Eriophyidae), which associates with bees that pollinate litchi trees (Waite, 1999) and *Aceria pallida* Keifer (Acari: Eriophyidae), which is associated to psyllidae *Bactericera gobica* (Loginova) (Hemiptera: Triozidae) to disperse to its host, being an obligatory association to dispersion (Liu et al., 2016). However, the dispersion behaviour in Eriophyidae mites is poorly studied (Mickalska et al., 2010).

Eriophyidae present high economic importance also by transmission of phytopathogens, especially viruses (Oldfield, 1970). The transmission of the *Wheat Streak Mosaic Virus* (WSMV) and *Wheat Mosaic Virus* (WMoV), by the mite *Aceria tosichella* Keifer (Acari: Eriophyidae) to wheat plants is a good example (Navia et al., 2010). One of the main management tools against these diseases is the use of resistant varieties to the pathogens and/or to the mites (Ali et al., 2016, Chuang et al., 2017).

In general, however, the main management strategy used for eriophyid control is chemical control, even though this control method presents several disadvantages, such as the difficulty to reach mites that live hidden in vegetative structures (Childers et al, 1996; Van Leeuwen et al., 2010), and selection of mite populations resistant to the main acaricides molecules (Marcic, 2012; Van Leeuwen and Dermauw, 2016).

Unfortunately, biological control is underused for management of these mites so far, even though the entomopathogens fungi are one of the most promising alternatives, mainly the species *Hirsutella thompsonii* (Fischer), *Beauveria bassiana* Balsam and *Lecanicillium lecanii* (Zimmermann), which demonstrate important mortality levels for eriophyids in the field (McCoy, 1996). Predatory mites have also been studied to the control of these mites, however the results were less promising (Palevsky et al., 2003; Maoz et al., 2014)

2.3. Citrus rust mites in Brazil

The citrus rust mite *P. oleivora* is one of the most important species in the family Eriophyidae, causing high citrus damages worldwide (Van Leeuwen et al., 2010). The mite infests all citrus species and varieties and damages can reach 70% (Gerson, 2003; Vacante, 2010).

The biological development of *P. oleivora* was studied by Allen et al. (1995) in fruits of sweet orange *Citrus sinensis* L. Osbeck ‘Valencia’ and the species took seven days to complete the development at 25 °C. The egg incubation period was 3.5 days, larval duration 2.1 days, nymphal development 1.6 days and fecundity of 10.5 eggs per female.

Phyllocoptruta oleivora feeds on leaves, fruits and twigs, causing russeting in these structures. Due to the reduced stylets of *P. oleivora* (7 µm), the damages are limited superficially to the epidermic cells of plants. The damages in fruits vary depending on development or citrus species, causing silvering in lemon, limes and madarines in the beginning of development, progressing to russeting in more developed fruits. In oranges, when infestation occurs in young fruits, the occurrence of cracking of epidermic cells is common, producing a symptom known as ‘shark skin’. In more developed fruits russeting and bronzing of the fruit skin occurs. Damages in leaves are less severe, causing dark spots similar to grease mainly on the border of these organs. *Phyllocoptruta oleivora* prefers to infest the regions less exposed to solar radiation (Mccoy and Albrigo, 1975).

As the result of feeding of *P. oleivora* in the plants, ethylene is produced, causing premature fruit ripening and alteration of soluble solids levels, with higher concentration of acetaldeids and ethanol in the damaged fruits when compared to non-infested fruits (McCoy et al., 1976). Furthermore, lignin accumulates in the fruit skin due to mite feeding, making the fruits to present corticated aspect. The economic threshold level was estimated in 70-80 mites/cm^2 of fruit (McCoy and Albrigo, 1975).

The feeding process of these mites damage the guard cells of stomates and increase transpiration rate, making the plants severely attacked to be more susceptible to dry periods. High infestation also causes reduction in the fruit diameter and fruit abnormal drop (Allen, 1978; Yang et al., 1994) and high defoliation (McCoy, 1976).

The main management tactic used against these mites is spraying chemical pesticides, although many researchers have shown the potential of entomopathogenic fungi to control *P. oleivora* (McCoy, 1996). Studies with predatory mites were also performed, with less promising results (Argov et al., 2002; Palevsky et al., 2003; Maoz et al., 2014).

More than 81 acaricides are registered to manage *P. oleivora* in citrus (Agrofit, 2018). However, pest control has been performed mainly with the acaricides sulfur and abamectin. This is very concerning, as acaricide resistance in populations of *P. oleivora* have already been reported in other countries (Omoto et al., 1994, Bergh et al., 1999). These studies have not been performed yet in Brazil (Omoto and Alves, 2004).

The management of *P. oleivora* in Brazilian orchards is causing concern, as the damages are increasing in the country, especially on the main citrus production region of São Paulo State and West-Western region of Minas Gerais. Some farmers and technicians have attributed these damages to a mite species described in 1999 and which also causes russeting, the citrus brown mite *Tegolophus brunneus* Flechtmann (Acari: Eriophyidae).

This mite is similar to the species *Tegolophus australis* Keifer (Acari: Tetranychidae), a key pest of citrus in Australia (Smith et al., 1997). However, there are no studies evaluating the damage potential of this pest to the main citrus species and varieties planted. *Tegolophus brunneus* was not reported in other countries, being a quarantine important pest, as Brazil exports citrus fruits to countries as Argentina and Uruguay (Embrapa, 2007).

The only information known about *T. brunneus* is that this mite has been found in some mite surveys in citrus. The mite was reported in sweet orange ‘Pera’ in the municipality of Jaguariúna-SP (Albuquerque, 2006), in Amazonia State (Bobot et al., 2011), and in Rio Grande do Sul State infesting sweet orange 'Pera' and tangor 'Murcott' (Horn et al., 2011; Bressan and Ott, 2017).

The toxicity of the main acaricides to this species is unknown, as well as the main mortality factors in the field, although there are speculations without scientific basis. Studies with *T. brunneus* are necessary to allow for more adequate management decisions to be performed in citrus orchards.

Aculops pelekassi (Keifer) (Acari: Eriophyidae), an important citrus rust species in many citrus producing countries, has not been reported in Brazil (Vacante, 2010).

2.4. Predatory mites in citrus in Brazil

The principal predatory mite species in the main citrus region of Brazil belongs to the Phyoseiidae family, specifically the species *Iphiseiodes zuluagai* Denmark and Muma and *Euseius concordis* (Chant) (Acari: Phytoseiidae) (Sato et al., 1994; Reis et al., 2000; Silva et al., 2012). Other predatory mites commonly collected, although at lower population levels, are those from the Stigmaeidae family. Other mite families, such as Blattisociidae, Ascidae, Bdellidae, Cunaxidae and Cheyletidae are less commonly collected (Silva et al., 2012).

The main Phytoseiidae species observed in orchards in Rio Grande do Sul State were *Euseius ho* (DeLeon) (Acari: Phytoseiidae), *Neoseiulus tunus* (DeLeon) (Acari: Phytoseiidae), *I. zuluagai* and *Typhlodromips cananeiensis* Gondim Jr. and Moraes (Acari: Phytoseiidae) (Horn et al., 2011). Bobot et al. (2011) found ten species of mites of the Phytoseiidae family in citrus in Amazonas State, one species of Ascidae, one mite of the Bdellidae family and one of Cunaxidae, which were not identified to species level. The highest number of species belonged to the genus *Amblyseius*.

Sato et al. (1994) observed in orchards of Presidente Prudente-SP that the predominant species of predatory mites were *I. zuluagai*, *Euseius citrifolius* Denmark and Muma (Acari: Phytoseiidae) and *E. concordis*, representing, respectively, 47.3; 26.5 and 25.7% of mites collected. These authors also collected the species

Amblyseius chiapensis DeLeon (Acari: Phytoseiidae) (0.4%), *Euseius alatus* DeLeon (Acari: Phytoseiidae)(0.1%) and *Typhlodromina camelliae* (Chant and Yoshida Shaul) (Acari: Phytoseiidae) (0.1%).

Reis et al. (2000) during samplings in sweet orange ‘Valencia’ in Lavras, Minas Gerais, observed that the predominant species were *I. zuluagai* and *E. alatus*, representing 66.2 and 29.8% of predatory mites collected. Besides these species, *Amblyseius compositus* Denmark and Muma (Acari: Phytoseiidae), *Amblyseius herbicolus* (Chant) (Acari: Phytoseiidae) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) were also collected.

Moreira (1993) observed high predominance of *I. zuluagai* (84.5%) in a citrus orchard in Jaboticabal-SP, followed by *E. citrifolius* (8.1%). Ferreira et al. (2018) in a citrus orchard in Amazonia State, found that the dominant species were *Amblyseius aeralis* (Muma) (Acari: Phytoseiidae), followed by *I. zuluagai*, *Iphiseiodes kamahorae* DeLeon (Acari: Phytoseiidae) and *Proprioseiopsis neotropicus* (Ehara) (Acari: Phytoseiidae). The species *E. citrifolius*, *Iphiseiodes quadripilis* Banks (Acari: Phytoseiidae) and *Typhlodromalus aripo* DeLeon (Acari: Phytoseiidae) were also collected in lower numbers.

Mites of the family Stigmaeidae are considered the second most important group of predatory mites to citrus. Matioli et al. (2002) observed in surveys in the main production regions of São Paulo State that the main species of this family were *Agistemus brasiliensis* Matioli, Ueckermann and Oliveira (Acari: Stigmaeidae), *Agistemus floridanus* Gonzales (Acari: Stigmaeidae) and *Zetzellia malvinae* Matioli, Ueckermann and Oliveira (Acari: Stigmaeidae), and *A. brasiliensis* represented 85% of mites collected on this family. Horn et al. (2011) collected *A. floridanus* during surveys in orchards in Rio Grande do Sul in sweet Orange ‘Valencia’, and Ferreira et al. (2018) also collected this mite in orchards in Amazonia.

3. References

Agrofit. Ministério da Agricultura, Pecuária e Abastecimento. 2018. Available in: <http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Acessed in 20 jan. of 2018.

Albuquerque FA (2006) **Diversidade de ácaros em cultivo orgânico de citros e na vegetação natural circundante, e perspectivas para a criação massal de *Iphiseiodes zuluagai*** (Acari: Phytoseiidae). 95f. Tese (Doutorado em Agronomia) - Unesp de Jaboticabal.

Ali N, Heslop-Harrison JP, Ahmad H, Graybosch RA, Hein GL, Schwarzacher T (2016) Introgression of chromosome segments from multiple alien species in wheat breeding lines with wheat streak mosaic virus resistance. **Heredity** 117:114-123.

Allen JC (1978) The effect of citrus rust mite damage on citrus fruit drop. **Journal of Economic Entomology** 71:746–750.

Allen JC, Yang Y, Knapp JL (1995) Temperature effects on development and fecundity of the citrus rust mite (Acari: Eriophyidae). **Environmental Entomology** 24:996–1004.

Argov Y, Amitai S, Beattie GAC, Gerson U (2002) Rearing, release and establishment of imported predatory mites to control citrus rust mite in Israel. **BioControl** 47:399-409.

Bastianel M, Freitas-Astúa J, Kitajima EW, Machado MA (2006) The citrus leprosis pathosystem. **Summa Phytopathologica** 32:211-220.

Belasque Jr J, Bassanezi RB, Yamamoto PT, Ayres AJ, Tachibana A, Violante AR, Dragone J (2010) Lessons from huanglongbing management in São Paulo State, Brazil. **Journal of Plant Pathology** 92:285-302.

Bergh J, Rugg D, Jansson, RK, McCoy CW, Robertson JL (1999) Monitoring the susceptibility of citrus rust mite (Acari: Eriophyidae) populations to abamectin. **Journal of Economic Entomology** 92:781-787.

Bobot TE, Chilson EF, Navia D, Gasnier TRJ, Lofego AC, Oliveira BM (2011) Mites (Arachnida, Acari) on *Citrus sinensis* L. Osbeck orange trees in the state of Amazonas, Northern Brazil. **Acta Amazonica** 41:557-566.

Bové JM (2006). Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. **Journal of Plant Pathology** 88:7-37.

Bressan LR, Ott AP (2017) Distribuição intra-planta e flutuação populacional de *Tegolophus brunneus* (Acari: Eriophyidae: Phyllocoptinae) em tangerineira. **Caderno de Pesquisa** 29:23-29.

Childers CC, Easterbrook MA, Solomon MG. Chemical control of eriophyoid mites. In: Lindquist EE, Sabelis MW, Bruin J. **World crop pests**. Elsevier,1996. p. 695-726.

Chuang WP, Rojas LMA, Khalaf LK, Zhang G, Fritz AK, Whitfield AE, Smith CM (2017) Wheat genotypes with combined resistance to wheat curl mite, wheat streak mosaic virus, wheat mosaic virus, and Triticum mosaic virus. **Journal of Economic Entomology** 110:711-718.

Embrapa (2017) **Destinos das exportações brasileiras de limão em 2017**. Cruz das Almas: Embrapa: Mandioca e Fruticultura, 4p.

Ferreira CT, Krug C, Garcia MVB, Moraes GD (2018) Leprosis mite and other mite species (Acari) associated to orange groves in Brazilian Central Amazon. **Systematic and Applied Acarology** 23:449-462.

Figueiredo MGD, Barros ALMD, Conceição JCPRD (2012) Retorno econômico dos investimentos em P&D na citricultura paulista. **Revista de Economia e Sociologia Rural** 50:493-502.

Flechtmann CH (1999) *Tegolophus brunneus* n. sp., a new citrus rust mite from Brazil (Acari: Eriophyidae). **International Journal of Acarology** 25:265-267.

Fundecitrus - Fundo de Defesa da Citricultura (2018) Inventário de Árvores e estimativa da safra de laranja 2017/2018 do cinturão citrícola de São Paulo e triângulo/sudoeste mineiro 2017/18. Disponível em: https://www.fundecitrus.com.br/pdf/pes_relatorios/2017_12_05_Invent%C3%A1rio_e_Estimativa_do_Cinturão_Citricola_2017-2018.pdf. Acess in 02 Jan 2019.

Gerson U (2003) Acarine pests of citrus: overview and non-chemical control. **Systematic and Applied Acarology** 8:3-12.

Grogan KA, Goodhue RE (2012) Spatial externalities of pest control decisions in the California citrus industry. **Journal of Agricultural and Resource Economics** 37: 156-179.

Helle W, Wysoki M (1983) The chromosomes and sex-determination of some actinotrichid taxa (Acari), with special reference to Eriophyidae. **International Journal of Acarology** 9:67–71.

Horn TB, Johann L, Ferla NJ (2011) Ecological interactions between phytophagous and predaceous mites in citrus agroecosystems in Taquari Valley, Rio Grande do Sul, Brazil. **Systematic and Applied Acarology** 16:133-144.

Hoy MA (Ed) (2016). Agricultural acarology: introduction to integrated mite management. Gainesville: University of Florida, 2016, 430p.

IBGE - Instituto Brasileiro de Geografia e Estatística - Produção agrícola-lavoura permanente. Available in: https://cidades.ibge.gov.br/brasil/pesquisa/15/11863. Acessed in 25 January 2019.

Kogan M (1998) Integrated pest management: historical perspectives and contemporary developments. **Annual Review of Entomology** 43:243-270.

Lillo E, Skoracka A (2010) What's "cool" on eriophyoid mites? **Experimental and Applied Acarology** 51:3-30.

Liu S, Li J, Guo K, Qiao H, Xu R, Chen J, Xu C, Chen J (2016) Seasonal phoresy as an overwintering strategy of a phytophagous mite. **Scientific Reports** 6:25483.

Maoz Y, Gal S, Argov Y, Domeratzky S, Melamed E, Gan-Mor S, Coll M, Palevsky E. (2014) Efficacy of indigenous predatory mites (Acari: Phytoseiidae) against the citrus rust mite *Phyllocoptruta oleivora* (Acari: Eriophyidae): augmentation and conservation biological control in Israeli citrus orchards. **Experimental and Applied Acarology** 63:295-312.

Marcic D (2012) Acaricides in modern management of plant-feeding mites. **Journal of Pest Science** 85:395-408.

Matioli AL, Ueckermann EA, Oliveira CD (2002) Some Stigmaeidae and Eupalopsellidae mites from citrus orchards in Brazil (Acari: Stigmaeidae and Eupalopsellidae). **International Journal of Acarology** 28:99-120.

McCoy CW (1996) Pathogens of eriophyoid mites. In: Lindquist EE, Bruin J, Sabelis (Eds). **World crop pests**. Amsterdam: Elsevier p. 481-490.

McCoy CW, Albrigo LG (1975) Feeding injury to the orange caused by the citrus rust mite, *Phyllocoptruta oleivora* (Prostigmata: Eriophyoidea). **Annals of Entomological Society of America** 68:289–297.

McCoy CW, Davis PL, Munroe, KA (1976) Effect of late season fruit injury by the citrus rust mite, *Phyllocoptruta oleivora* (Prostigmata: Eriophyoidea), on the internal quality of Valencia orange. **Florida Entomologist** 59:335-341.

McCoy CW (1976) Leaf injury and defoliation caused by the citrus rust mite, *Phyllocoptruta oleivora*. **Florida Entomologist** 59:403-410.

Michalska K, Skoracka A, Navia D, Amrine JW (2010) Behavioural studies on eriophyoid mites: an overview. **Experimental and Applied Acarology** 51:31-59.

Moreira PHR (1993) **Ocorrência, dinâmica populacional de ácaros predadores em citros e biologia de *Euseius citrifolius* (Acari: Phytoseiidae).** 110f. Dissertação (Mestrado em Agronomia)- Unesp, Jaboticabal.

Moricochi L, Neves EM, Araújo PF (1981) Pesquisa e assistência técnica na citricultura: custos e retornos sociais. **Brazilian Journal of Rural Economy and Sociology** 19:189-203.

Navia D, Ochoa R, Welbourn C, Ferragut F (2010) Adventive eriophyoid mites: a global review of their impact, pathways, prevention and challenges. **Experimental and Applied Acarology** 51:225-255.

Neves MF, Trombin VG (2017) Anuário da citricultura 2017. São Paulo: CitrusBR, 2017.

Neves MF, Trombin VG, Milan P, Lopes FF, Cressoni F, Kalaki R (2010) O retrato da citricultura brasileira. Ribeirão Preto: CitrusBR.

Nunes MA, Mineiro JLC, Rogerio LA, Ferreira LM, Tassi A, Novelli VM, Kitajima E, Freitas-Astúa J (2018) First report of *Brevipalpus papayensis* as vector of Coffee ringspot virus and Citrus leprosis virus C. **Plant disease** 102:1046-1046.

Oldfield GN (1970) Mite transmission of plant viruses. **Annual Review of Entomology** 15:343-380.

Omoto C, Alves EA (2004) Resistência dos ácaros a acaricidas em citros. **Visão Agrícola** 2:82-86.

Omoto C, Dennehy TJ, Mccoy CW, Crane SE, Long JW (1994) Detection and characterization of the interpopulation variation of citrus rust mite (Acari: Eriophyidae) resistance to dicofol in Florida citrus. **Journal of Economic Entomology** 87:566-572.

Palevsky E, Argov Y, David TB, Gerson U (2003) Identification and evaluation of potential predators of the citrus rust mite, *Phyllocoptruta oleivora*, in Israel. **Systematic and Applied Acarology** 8:39-48.

Picazo-Tadeo AJ, Reig-Martínez E (2006) Agricultural externalities and environmental regulation: evaluating good practice in citrus production. **Applied Economics** 38:1327-1334.

Reis PR, Chiavegato LG, Alves EB, Sousa, EO (2000) Ácaros da família Phytoseiidae associados aos citros no município de Lavras, sul de Minas Gerais. **Anais da Sociedade Entomológica do Brasi**l 29:95-104.

Rezzadori K, Benedetti S. Proposições para valorização de resíduos do processamento do suco de laranja. In: **International Workshop Advances in Cleaner Production.** São Paulo: UNIP, 2009. p. 1-11.

Rodrigues JCV, Oliveira CAL (2005) Ácaros fitófagos dos citros. In: Junior DM, Negri JD, Pio RM, Junior JP (Eds.) **Citros.** Campinas: Instituto Agronômico, p. 691-727.

Royalty RN, Perring TM (1996) Nature of damage and its assessment. In: Lindquist EE, Bruin J, Sabelis (Eds). **World crop pests**. Amsterdam: Elsevier, p.493-512.

Sabelis MW, Bruin J. Evolutionary ecology: life history patterns, food plant choice and dispersal (1996) In: Lindquist EE, Bruin J, Sabelis (Eds). **World crop pests**. Amsterdam: Elsevier, p. 329-366.

Sato ME, Raga A, Cerávolo LC, Rossi AC (1994) Predation mites in citrus orchards at Presidente Prudente, State of São Paulo. **Anais da Sociedade Entomológica do Brasil** 23:435-435.

Sharma DD (1991) Occurrence of *Cephaleuros virescens*, a new record of leaf-curl in litchi (*Litchi chinensis*). **Indian Journal of Agricultural Sciences** 61:446-448.

Silva MZ, Sato ME, Oliveira CAL (2012) Diversidade e dinâmica populacional de ácaros em pomar cítrico. **Bragantia** 71:210-218.

Smith D, Beattie, GA, Broadley R (1997) Citrus pests and their natural enemies: integrated pest management in Australia. Queensland: Dept. of Primary Industries, 272p.

Smith L, Lillo E, Amrine JW (2010) Effectiveness of eriophyid mites for biological control of weedy plants and challenges for future research. **Experimental and Applied Acarology** 51:115-149.

USDA - United States Department of Agriculture (2018) Citrus world market and trades. Available in <https://apps.fas.usda.gov/psdonline/circulars/citrus.pdf>. Acessed in 02nd Jan of 2018.

Vacante V (2010) Citrus mites: identification, bionomy and control. Walingford: CABI, 371p.

Van Leeuwen T, Witters J, Nauen R, Duso C, Tirry L (2010) The control of eriophyoid mites: state of the art and future challenges. **Experimental and Applied Acarology** 51:205-224.

Van Leeuwen T, Dermauw W (2016) The molecular evolution of xenobiotic metabolism and resistance in chelicerate mites. **Annual Review of Entomology** 61: 475-498.

Waite GK (1999) New evidence further incriminates honey-bees as vectors of lychee erinose mite *Aceria litchii* (Acari: Eriophyidae). **Experimental and Applied Acarology** 23:145-147.

Yang Y, Allen JC, Knapp JL, Stansly PA (1994) Citrus rust mite (Acari: Eriophyidae) damage effects on ‘Hamlin’ orange fruit growth and drop. **Environmental Entomology** 23:244–247.

Chapter 2 - Biological and demographic parameters of *Tegolophus brunneus* (Acari: Eriophyidae) in citrus

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Abstract

The brown citrus rust mite *Tegolophus brunneus* Flechtmann (Acari: Eriophyidae) causes citrus rust, similarly to *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae). As this damage has intensified in recent years and *T. brunneus* has been reported in high population levels in several regions of Brazil, this mite has caused concern to growers and technicians. Because *T. brunneus* has been little studied and its bioecological characteristics are unknown, this study investigated the biological and demographic parameters of *T. brunneus* on citrus fruits under laboratory conditions. Our results showed that the egg incubation period and viability were 3.0 days and 94.5%, respectively. The larval and nymphal stage durations were 1.1 and 2.8 days. The development time of the immature stage was 6.9 days, with 92.3% survival. When females and males were maintained together, the sex ratio of offspring was 0.7; virgin females produced only males. The pre-oviposition and total pre-oviposition periods were 1.6 and 8.5 days, respectively. Fecundity was 8.5 eggs, and female and male longevities were 13.2 and 11.4 days, respectively. The estimate of demographic parameters indicated that the R_o and T of *T. brunneus* were 6.45 offspring and 13.0 days, and r and λ were 0.142 and 1.153 day^{-1}, respectively. These results suggest that *T. brunneus* has high growth potential on citrus trees. Therefore, management strategies may be required to reduce the population levels and damage caused by *T. brunneus* in citrus groves.

Keywords: Citrus brown mite; *Citrus sinensis*; demographic parameters; integrated pest management

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Introduction

Among the mites of agricultural importance, those belonging to the family Eriophyidae attack fruits, leaves, and twigs, producing galls, erinium, and blister which affect the normal growth, development, and production of crops (Lindquist et al. 1996; Javadi-Khederi et al. 2018). Some eriophyid species may be vectors of plant viruses (Oldfield and Proeseler 1996; Lillo and Skoracka 2010; van Leeuwen et al. 2010; Mielke-Ehret et al. 2010), and others have high quarantine importance (Navia et al. 2010), which makes this mite group second only to the Tetranychidae in importance to agroecosystems (VanLeeuwen et al. 2010).

In Brazil, the largest producer and exporter of orange juice in the world, eriophyid mites have been a serious threat, since they colonize practically all vegetative and reproductive structures of citrus trees (Chiaradia 2001). The high incidence of rust mites reduces not only the quality and value of marketable fresh fruits due to the formation of dark spots on fruit skins (also known as russeting), but also the longevity and tolerance of plants to abiotic stresses (McCoy and Albrigo 1975; Koller 1994). Citrus trees infested by eriophyid mites also produce smaller and lighter fruits than healthy trees (McCoy and Albrigo 1975; Allen 1978; Oliveira et al. 1991; Yang et al. 1994; Hall et al. 2005). Furthermore, fruits with rust undergo changes in the contents of acid and soluble solids of the juice and their skin may harden due to lignin accumulation, damaging commercial juice-extracting machines (McCoy and Albrigo 1975; Zucchi et al. 1993). High rust-mite populations on citrus trees can cause production losses of up to 70% if control measures are not adopted (Gerson and Vacante 2011).

In Brazilian citrus groves, rust damage has been attributed only to the citrus rust mite *Phyllocoptruta oleivora* (Ashmead) (Rodrigues and Oliveira 2005; Carvalho et al. 2016). However, during the period when the symptoms of citrus rust were increasing, a new species of mite, the brown citrus rust mite *Tegolophus brunneus* Flechtmann (Acari: Eriophyidae) was discovered on leaves and fruits of sweet orange [*Citrus sinensis* (L.) Osbeck (Sapindales: Rutaceae)] in commercial citrus groves in São Paulo state (Flechtmann 1999; Albuquerque 2006). This mite was described in 1999 (Flechtmann 1999) and was subsequently reported in high population levels on sweet orange and ‘Murcott’ mandarin leaves (*Citrus sinensis* L.

Osbeck × *Citrus reticulata* Blanco) in northern and southern Brazil (Bobot et al. 2011; Horn et al. 2011; Bressan and Ott 2017). Thus, the increasing severity of citrus rust on leaves, fruits, and twigs is associated not only with *P. oleivora*, but also with the occurrence of *T. brunneus* in citrus groves. In Australia, severe damage was observed in citrus groves with high infestations of *Tegolophus australis* Keifer (Acari: Eriophyidae) associated with *P. oleivora* (Smith and Papacek 1991). In Brazil, the association of *P. oleivora* with *T. brunneus* has been observed in several citrus production areas, especially those located in the southwest region where environmental conditions seem to be more favorable for the development of these mites. Due to the population increase, acaricide sprayings were required to reduce the population levels and reduce the losses caused by these mites, causing an increase in production costs and reduction in the environmental sustainability of the system (VanLeewven et al. 2015; Nakano et al. 2016).

Despite the importance and population increases of *T. brunneus* on citrus trees in recent years, few studies have assessed the biotic potential of this mite for the agroecosystems. In addition, no study has investigated the demographic parameters of *T. brunneus* on citrus trees. According to Stark and Banks (2003), estimation of the demographic parameters, especially net reproductive rate (R_o), mean generation time (T), finite rate of increase (λ), and intrinsic rate of increase (r) is important to understand not only the potential for mite population growth, but also the possible implications for its management. In this study, the biological and demographic parameters of *T. brunneus* were assessed under laboratory conditions. Knowledge of the biological and demographic parameters of *T. brunneus* is essential to an understanding of the density and population dynamics of this mite in citrus groves. In addition, the results of this study will help to determine future management strategies for this mite in citrus production systems.

Material and methods

Mites

The *T. brunneus* colony was established from mites collected in an experimental grove of' ‘Tahiti’ acid lime (*Citrus latifolia* Tanaka) in the municipality of Jaboticabal, São Paulo, Brazil, that had not received any pesticide application in the previous six months. The species (*T. brunneus*) was confirmed by Dr. Carlos Holger Wenzel Flechtmann, using slide mounts. After confirmation, specimens of *T. brunneus* were collected and transferred onto green fruits of 'Pera' sweet orange [*Citrus sinensis* (L.) Osbeck] approximately 10 cm in diameter, coated with paraffin to reduce dehydration. The fruits were placed in plastic jars (400 mL) half filled with autoclaved sand. Strips of moistened hydrophilic cotton were placed around the fruits to maintain moisture levels and prevent the mites from escaping. The fruits were replaced whenever required. Rearing was conducted in a climate-controlled room with temperature 25 ± 1 °C, relative humidity (RH) 70 ± 10%, and 12 L: 12 D h photoperiod.

Development and reproduction of *Tegolophus brunneus*

In order to assess *T. brunneus* development and reproduction, green fruits of 'Pera' sweet orange approximately 10 cm in diameter were coated with paraffin to prevent dehydration as described above. Then, a circular area (~ 1 cm in diameter) was delimited on the fruit skin with moistened hydrophilic cotton and used as the experimental unit. Next, an adult *T. brunneus* female ~ 48 h old was transferred onto each experimental unit for oviposition. The fruits were placed in plastic jars (400 mL) and kept in a climate-controlled room as described above. The number of eggs laid by females in the experimental units was counted 12 h after the females were released. Afterward, one egg was randomly selected and maintained in each experimental unit in order to assess the egg duration and viability. Fifty-four experimental units (replicates) were used, and the bioassay was repeated twice over time.

The number of hatched larvae was assessed every 12 h for 7 days. The egg incubation period was determined based on the time between oviposition and larvae hatching, and the egg viability was calculated based on the ratio of the number of hatched larvae to the number of eggs assessed. The larvae were kept on the same experimental units to assess the duration and survival of larval and nymphal stages. These assessments were performed every 12 h. The survival of the larval and nymphal stages was determined based on the proportion of nymphs and adults obtained from the larvae and nymphs surviving in the experimental units, respectively. Mites that did not react to the touch of a fine brush were considered dead.

Assessment of the adult-stage parameters of *Tegolophus brunneus*

Sixty-eight females and 28 males, newly emerged (≤ 12 old), were transferred to new experimental units in order to assess the pre-oviposition period, fecundity, and female and male longevities. The mites were kept separately in each experimental unit, and the females were not fertilized. The dead mites and eggs laid by females in experimental units were counted and removed every 12 h. The fecundity was determined based on the total number of eggs laid by females during the entire adult period. The experiment ended when all individuals died.

Determination of the occurrence and type of parthenogenesis for *Tegolophus brunneus*

To determine the occurrence and type of parthenogenesis, one female *T. brunneus* (≤ 24 hold) was transferred to each 'Pera' sweet orange fruit for oviposition, following the same procedure described above. After oviposition, the females were removed, and one egg was randomly selected and maintained on each experimental unit (fruit). The eggs were assessed daily until the larvae emerged. The larvae were kept on the same experimental units and assessed daily until reaching the adult stage (F_1 generation). The obtained adults were kept on individual experimental units to prevent any possibility of mating and to allow the assessment of the occurrence and type of parthenogenesis. For this purpose, 100 females were assessed. When the F_1 generation females started oviposition, one egg of each female was randomly selected and assessed daily until reaching the adult stage (F_2 generation). Slides were prepared with these adults to separate the sexes and determine the occurrence and type of parthenogenesis of this eriophyid.

Estimate of the demographic parameters of *Tegolophus brunneus*

Based on the data for egg duration and viability, larval and nymphal stage duration and survival, sex ratio, pre-oviposition period, fecundity, and female and male longevities, the biological and demographic parameters of *T. brunneus* were estimated using all individuals tested (including females, males, and individuals that died during the immature stage) as proposed by Chi (1988). The original data for all individuals were analyzed according to the model proposed by Chi and Liu (1985), using the TWOSEXMSChart software (http://140.120.197.173/ecology/Download/TWOSEX-MSChart.rar) (Chi 2014). Based on the biological data, the values of the age-stage-specific survival rate (S_{xj}), where: x = age in days and j = development stage; age-specific survival rate (l_x); age-specific fecundity (m_x); age-stage-specific survival expectancy (e_{xj}); and age-stage-specific reproduction (v_{xj}) were calculated. In addition, the following demographic parameters were estimated:

Net reproductive rate (R_o):

(1)

$$R_o = \sum_{x=0}^{\infty} l_x m_x$$

intrinsic rate of increase (r):

(2)

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

mean generation time (T):

(3)

$$T = \ln R_o / r$$

and finite rate of increase (λ):

(4)

$$\lambda = e^r$$

The intrinsic rate of increase was estimated by the equation of Euler-Lotka [Eq. (2)] (Goodman 1982). The e_{xj} was calculated according to the method proposed by Chi and Su (2006). The mean and standard error of biological and demographic parameters were estimated by the bootstrap method, following the procedure proposed by Huang and Chi (2012). During the bootstrap procedure, the data for each biological and demographic parameter analyzed were re-sampled 80,000 times.

Results

Immature stage duration and survival of *Tegolophus brunneus*

The egg incubation period was 3.0 ± 0.09 days, with 94.4 ± 0.03% egg viability. The larval and nymphal stage durations were 1.1 ± 0.04 and 2.8 ± 0.09 days, respectively. The egg-adult development time was 6.9 ± 0.13 days, with 92.3 ± 3.68% survival. Due to the different development rates of mites, the different development stages of the mite overlapped (Figure 1). The probability of a newly hatched larva to survive until the adult stage was 0.65 for females and 0.27 for males (Figure 1).

Determination of the adult stage parameters of *Tegolophus brunneus*

When females and males were maintained together, the sex ratio was 0.7 ± 0.04, indicating a higher proportion of females than males in the population tested. The pre-oviposition period (from adult emergence to the first egg) was relatively short (1.6 ± 0.19 days), whereas the total pre-oviposition period (from egg to the first egg laid) was 8.5 ± 0.23 days. The mean female fecundity was 8.5 ± 0.64 eggs, with a maximum oviposition rate of 0.83 eggs day^{-1} (Figure 2). Female and male longevities were 13.2 ± 0.79 and 11.4 ± 1.36 days, respectively.

Life expectancy and reproductive value of *Tegolophus brunneus*

The data on age-stage-specific life expectancy indicated that the lifespan of a newly hatched mite reared on green fruits of 'Pera' sweet orange was 18.6 days (Figure 3). The reproductive value of a newly hatched female (v_1) is exactly the finite rate of increase (λ) of a population. Therefore, the reproductive peak (the period in which the females contribute most to the population growth of the mite) occurred at 9.0 days after the larvae hatched (Figure 4).

Determination of the occurrence and type of parthenogenesis for *Tegolophus brunneus*

Our results showed that *T. brunneus* has two types of reproduction, sexual and asexual. Based on the individuals analyzed, *T. brunneus* fertilized females originate females and males, while unfertilized females originate only males, indicating the occurrence of arrhenotokous parthenogenesis.

Estimate of the demographic parameters of *Tegolophus brunneus*

The estimates of the demographic parameters of *T. brunneus* reared on green fruits of 'Pera' sweet orange indicated that net reproductive rate (R_o) was 6.4 ± 0.84 offspring, with a mean generation time (*T*) of 13.0 ± 0.28 days. The intrinsic rate of increase (*r*) and the finite rate of increase (λ) were 0.142 ± 0.009 and 1.153 ± 0.011 days^{-1}, respectively.

Discussion

In this study, the biological and demographic parameters of the brown citrus rust mite *T. brunneus* reared on green fruits of 'Pera' sweet orange were demonstrated. The results showed that the egg incubation period was 3.0 days. This result is agreement with the egg incubation period observed for *P. oleivora* reared on fruits of 'Valencia' sweet orange (3.0 days) and *Aculus fockeui* (Nalepa and Troussant) (Acari: Eriophyidae) maintained on nectarine leaves (3.2 days) at 25 °C (Allen et al. 1995; Abou-Awad et al. 2010). Egg incubation periods slightly higher (from 3.4 to 3.5 days) than those obtained in the present study have been observed for other eriophyid mites: *Aceria guerreronis* Keifer reared on leaves of *Syagrus romanzoffiana* (Cham.) Glassman (Ansaloni and Perring 2004) and *Aculops pelekassi* (Keifer) (Acari: Eriophyidae) that developed on leaves of *Poncirus trifoliata* (L.) Rafinesque at 26 °C (Seo and Kim 2014a). The differences in the egg incubation period may be related to the physiological characteristics of the species, environmental factors (mainly temperature and relative humidity), and quality of the food consumed by females during the pre-imaginal period (Sternlicht 1970; Noronha et al. 2017). The shorter egg incubation period observed here demonstrates the potential of *T. brunneus* to rapidly colonize the vegetative and reproductive structures of citrus trees. The short incubation period also reduces exposure to egg predators and the effectiveness of acaricides with ovicidal properties.

The egg-adult biological cycle of *T. brunneus* was relatively short (7.0 days) due to the short development time of the embryo, larvae, and nymphs of this mite. A short egg-adult development period was also reported for other eriophyid species, including *P. oleivora* and *Aceria sheldoni* (Ewing) (Acari: Eriophyidae), which had an immature-stage development time of 7.0 days at 25 °C (Sternlicht 1970; Allen et al. 1995). However, an egg-adult development time of 9.3 days was observed for *Calacarus heveae* Feres (Acari: Eriophyidae) reared on rubber tree leaves at 28 °C (Ferla and Moraes 2003). Van der Merwe and Coates (1965) found an increase in the immature-stage development time of *Calacarus citrifolii* Keifer (Acari: Eriophyidae) reared on lemon seedlings in lower temperatures (from 6.0 to 13.0 days at 27 and 20 °C, respectively), indicating that temperature is one of the main factors that regulate the development of mites. Beyond temperature, the relative humidity also affects the development and survival of eriophyids. In general, high relative-humidity periods are more favorable than dry periods for eriophyid development (Jeppson et al. 1958; Sternlicht 1970; Hobza and Jeppson 1974; Xu et al. 2006; Seo and Kim 2014b).

In addition to the short egg-adult development period, the immature-stage survival rate of *T. brunneus* was higher than 92%. In general, immature stages of eriophyid mites have a high survival rate. For example, immature stages of *Tegolophus hassani* (Keifer) (Acari: Eriophyidae) and *Aceria ficus* (Cotte) (Acari:

Eriophyidae) reared on olive and fig leaves at 31 and 25 °C, respectively, showed no mortality during the entire development period (Abou-Awad et al. 2000; Abou-Awad et al. 2005). Therefore, under conditions of warm temperatures (≥ 25 °C) and high humidity (> 60%), *T. brunneus* and other eriophyid mites may reach high population levels in a short time and severely damage crops (Duso et al. 2010).

For the adult stage, our results showed a higher proportion of females than males in the population tested. Similarly, Sternlicht and Goldenberg (1971) found in a three-year assessment that the proportion of males in *A. sheldoni* ranged from 8 to 36% at different times of the year. In the field, 80% of specimens of *T. brunneus* assessed were females, confirming the higher proportion of females in the population (data not shown). In haplodiploid species, the females control the sex of the progeny and determine the sex ratio in populations (Filia et al. 2015). Ding et al. (2018) demonstrated that in arrhenotokous species, when females mate often, they produce more females than males. Our results showed that *T. brunneus* is capable of arrhenotokous parthenogenesis, because only males were produced when the females were not fertilized. Arrhenotokous parthenogenesis has also been reported for other eriophyid species, including *P. oleivora* (Swirskii and Amitai 1957), *A. sheldonii* (Sternlicht and Goldenberg 1971), *Leipothrix dipsacivagus* Petanovic and Rector (Acari: Eriophyidae) (Stoeva et al. 2011), and *A. mangiferae* (Abou-Awad et al. 2011), which indicates that arrhenotoky seems to be common in this mite group. Likewise, Helle and Wyisock (1983), using a karyotype test for eight eriophyid species, demonstrated that eggs that originated males had half the number of chromosomes (n = 2) than those that originated females (n = 4).

The occurrence of parthenogenesis affords the evolutionary advantage of colonizing an area from only one female, which then initiates bisexual reproduction when this female is fertilized by males of the progeny produced, a phenomenon known as oedipal mating (Tomlinson 1966; Filia et al. 2015; Ding et al. 2018). On the other hand, sexual reproduction also helps to maintain population levels, genetic variability and population stability of mites in ecosystems (Agrawal 2001). The reproductive characteristics of eriophyids help to explain the wide distribution of these mites and their success in colonizing new habitats, which account for their high agricultural and quarantine importance (Navia et al. 2010). Furthermore, the selection of pesticide-resistant individuals occurs more rapidly in haplodiploid species (Denholm et al. 1998; Carrière 2003; Filia et al. 2015), requiring special care in using acaricides to control these mites.

The pre-oviposition period was 1.6 days at 25 °C. This period is longer than those observed for females of *Phyllocoptes adalius* (Keifer) (Acari: Eriophyidae) (1.0 day) (Druciareck et al. 2014), and shorter than those recorded for females of *T. hassani* (3.2 days) (Abou-Awad et al. 2005), *Aceria mangiferae* (Sayed) (Acari: Eriophyidae) (4.7 days), and *Metaculus mangiferae* (Attiah) (Acari: Eriophyidae) (2.4 days) reared in the same environmental conditions (Abou-Awad et al. 2011). The short pre-oviposition period of *T. brunneus* suggests that the reproductive apparatus develops completely soon after the adult emerges.

Egg production by the *T. brunneus* females was relatively low (8.5 eggs female^{-1}) in relation to the other eriophyid species, such as *T. hassani* (11.2 eggs female^{-1}) (Abou-Awad et al. 2005), *P. oleivora* (11.2 eggs female^{-1}) (Allen et al. 1995), and *Aculops lycopersici* (Massee) (Acari: Eriophyidae) (51.0 eggs female^{-1}) (Haque and Kawai 2003). Peak reproduction occurred at 9.0 days for *T. brunneus* females, suggesting that females at this age contributed most to the population growth. The low fecundity of *T. brunneus* females recorded here may be related to the method of keeping the females individually on the experimental units, without fertilization. According to Druciarek et al. (2014), *P. adalius* fertilized females produced more eggs than unfertilized females. Although the fecundity was low, *T. brunneus* showed high egg viability, which may also contribute to the rapid population increases of this mite on citrus trees.

The estimated age-stage-specific life expectancies (the expected lifetime of an individual of a specific age and stage) of a newly hatched female and male of *T. brunneus* were 15.1 and 12.6 days, respectively, gradually decreasing with the age of the mite. *Tegolophus brunneus* females and males showed a longer life expectancy than females (9.7 days) and males (5.4 days) of *Abacarus hystrix* Nalepa (Acari: Eriophyidae) (Skoracka and Kuczynski 2004). These results indicate the high potential of this mite to remain on citrus trees for a long period of time, which increases its capacity to damage the fruits.

Concerning the demographic parameters, our results showed a higher net reproductive rate (R_o = 6.4 offspring) and intrinsic rate of increase (*r* = 0.142 day^{-1}) and a longer mean generation time (*T* = 13.0 days) for *T. brunneus* than those observed for *P. oleivora* (R_o = 2.7 offspring; *r* = 0.0971 day^{-1}; *T* = 10.2 days) (Allen et al. 1995). However, these authors assumed a sex ratio of 0.50 for *P. oleivora*, which does not correspond to most eriophyid mites. Li et al. (1989) found higher *r* values for *P. oleivora* reared at the same temperature (*r* = 0.16–0.21 day^{-1}). High *r* values were also recorded for *A. fockeui* reared on nectarine leaves (*r* = 0.147 day^{-1}) and for the olive rust mite *Tegolophus hassani* (Keifer) (Acari: Eriophyidae) at 25 °C (*r* = 0.116 day^{-1}) that developed on olive leaves (Abou-Awad et al. 2005, Abou-Awad et al. 2010). The *r* is the most important demographic parameter because it represents the growth potential of a species (Carey 1993). Regarding the *T* value obtained here, our results suggest that *T. brunneus* in citrus groves tends to have fewer generations than *P. oleivora*. Therefore, under equal conditions, *T. brunneus* may have a higher population growth potential and cause greater damage to citrus trees than *P. oleivora*, or may cause similar damage even at significantly lower population levels than *P. oleivora*. Therefore, the relationship between population levels and damage should be further investigated, to determine economic thresholds as a way of determining the best time to implement control measures.

Due to the rapid biological development of *T. brunneus*, the occurrence of sexual and asexual reproduction, and the high values of demographic parameters (R_o, *r,* and *T*), *T. brunneus* has high potential as a citrus pest because of its capacity to rapidly colonize plants and to reach high population levels in a short time. Additional laboratory and field studies to assess the ecological relationships of *T. brunneus* with other eriophyid species (especially *P. oleivora*) and predatory mites should be carried out to determine not only the adaptive capacity of this mite, but also to help to define management strategies to reduce population levels and damage in citrus groves.

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References

Abou-Awad BA, AL-Azzazy MM, El-Sawi SA (2010) The life – history of the peach silver mite, *Aculus fockeui* (Acari: Eriophyidae) in Egypt. Arch Phytopathol Plant Prot 43:384–389

Abou-Awad BA, El-Sawaf BM, Reda AS, Abdel-Khalek AA (2000) Environmental management and biological aspects of the two eriophyoid fig mites *Aceria ficus* (Cotte) and *Rhyncaphytoptus ficifoliae* Keifer in Egypt. Acarologia 40:419–429

Abou-Awad BA, Metwally AM, Al-Azzazy MM (2005) Environmental management and biological aspects of two eriophyid olive mites in Egypt: *Aceria oleae* and *Tegolophus hassani*. Z Pflanzenkd Pflanzen Umwelt 112:287–303

Abou-Awad BA, Metwally AS, Al-Azzazy MM (2011) Environmental management and biological aspects of two eriophyid mango mites in Egypt: *Aceria mangiferae* and *Metaculus mangiferae*. Acarologia 51:481–497

Agrawal AF (2001) Sexual selection and the maintenance of sexual reproduction. Nature 411:692

Albuquerque FA (2006) Diversidade de ácaros em cultivo orgânico de citros e na vegetação natural circundante, e perspectivas para a criação massal de *Iphiseiodes zuluagai* (Acari: Phytoseiidae). Dissertation, São Paulo State University

Allen JC (1978) The effect of citrus rust mite damage on citrus fruit drop. J Econ Entomol 71: 746–750

Allen JC, Yang Y, Knapp JL (1995) Temperature effects on development and fecundity of the citrus rust mite (Acari: Eriophyidae). Environ Entomol 24:996–1004

Ansaloni T, Perring TM (2004) Biology of *Aceria guerreronis* (Acari: Eriophyidae) on queen palm, *Syagrus romanzoffiana* (Arecaceae). Int J of Acarol, 30:63–70

Bobot TDE, Franklin E, Navia D, Gasnier TRJ, Lofego AC, Oliveira BMD (2011) Mites (Arachnida, Acari) on *Citrus sinensis* L. Osbeck orange trees in the state of Amazonas, Northern Brazil. Acta Amazon 41:557–566

Bressan LR, Ott AP (2017) Distribuição intra-planta e flutuação populacional de *Tegolophus brunneus* (Acari: Eriophyidae: Phyllocoptinae) em tangerineira. Cad Pesq 29:23–29

Carey JR (1993) Applied demography for biologists: with special emphasis on insects. Oxford University Press, New York

Carrière Y (2003) Haplodiploidy, sex, and the evolution of pesticide resistance. J Econ Entomol 96:1626–1640

Carvalho HWLD, Martins CR, Teodoro AV, Soares-Filho WDS, Passos OS (2016) Agronomical performance of 'Piemonte' mandarin grafted on several rootstocks in the Brazilian Coastal Tablelands. Pesq Agropec Bras 51:1830–1838

Chi H (1988) Life-table analysis incorporating both sexes and variable development rates among individuals. Environ Entomol 17:26–34

Chi H (2014) TWOSEX-MSCHART: A computer program for the age-stage two-sex life table analysis. http://140.120.197.173/ecology/Download/TWOSEXMSChart.rar

Chi H, Liu H (1985) Two new methods for the study of insect population ecology. Bull Inst Zool Acad Sin 24:225–240

Chi H, Su HY (2006) Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. Environ Entomol 35:10–21

Chiaradia LA (2001) Flutuação populacional do ácaro da falsa-ferrugem *Phyllocoptruta oleivora* (Ashmead, 1879) (Acari, Eriophyidae) em pomares de citros da região oeste catarinense. Pesq Agrop Gaúcha 7:111–120

Denholm I, Cahill M, Dennehy TJ, Horowitz AR (1998) Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. Philos Trans R Soc Lond B Biol Sci 353:1757–1767

Ding T, Chi H, Gökçe A, Gao Y, Zhang B (2018) Demographic analysis of arrhenotokous parthenogenesis and bisexual reproduction of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Sci Rep 8:1–10

Druciarek T, Lewandowski M, Kozak M (2014) Demographic parameters of *Phyllocoptes adalius* (Acari: Eriophyoidea) and influence of insemination on female fecundity and longevity. Exp App Acarol 63:349–360

Duso C, Castagnoli M, Simoni S, Angeli, G (2010) The impact of eriophyoids on crops: recent issues on *Aculus schlechtendali*, *Calepitrimerus vitis* and *Aculops lycopersici*. Exp App Acarol, 51: 151–168

Ferla NJ, Moraes GJ (2003) Ciclo biológico de *Calacarus heveae* Feres, 1992 (Acari, Eriophyidae). Rev Bras Entomol 47:399–402

Filia AG, Bain SA, Ross L (2015) Haplodiploidy and the reproductive ecology of Arthropods. Curr Opin Insect Sci 9:36–43

Flechtmann CH (1999) *Tegolophus brunneus* n. sp., a new citrus rust mite from Brazil (Acari: Eriophyidae). Int J Acarol 25:265–267

Gerson U, Vacante V (2011) Acari. In: Vacante V, Gerson U (eds) Integrated control of citrus pests in the Mediterranean region. Bentham Science Publishers, Sharjah, United Arab Emirates, pp. 88–108

Goodman D (1982) Optimal life histories, optimal notation, and the value of reproductive value. Am Nat 119:803–823

Hall DG, Childers CC, Eger JE (2005) Effects of reducing sample size on density estimates of citrus rust mite (Acari: Eriophyidae) on citrus fruit: simulated sampling. J Econ Entomol 98:1048–1057

Haque MM, Kawai A (2003) Effect of temperature on development and reproduction of the tomato russet mite, *Aculopslycopersici* (Massee) (Acari: Eriophyidae). Appl Entomol Zool 38:97–101

Helle W, Wysoki M (1983) The chromosomes and sex-determination of some actinotrichid taxa (Acari), with special reference to Eriophyidae. Int J Acarol 9:67–71

Hobza RF, Jeppson, LR (1974). A temperature and humidity study of citrus rust mite employing a constant humidity air-flow technique. Environ Entomol 3:813–822

Horn TB, Johann L, Ferla NJ (2011) Ecological interactions between phytophagous and predaceous mites in citrus agroecosystems in Taquari Valley, Rio Grande do Sul, Brazil. Syst Appl Acarol 16:133–144

Huang YB, Chi H (2012) Age-stage, two-sex life tables of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying female age-specific life tables to insect populations. Insect Sci 19:263–273

Javadi-Khederi S, Khanjani M, Gholami M, Panzarino O, Lillo E (2018) Influence of the erineum strain of *Colomerusvitis* (Acari: Eriophyidae) on grape (*Vitis vinifera*) defense mechanisms. Exp Appl Acarol 75:1–24

Jeppson LR, Jesser MJ, Complin, JO (1958) Factors affecting populations of the citrus bud mite in southern California lemon orchards and acaricide treatments for control of this eriophyid. J Econ Entomol 51:657–662

Koller OC (1994) Citricultura: laranja, limão e tangerina. Porto Alegre: Rigel, 446p

Li LS, Huang FN, Chen JL (1989) Ecological characteristics of the citrus rust mite, *Phyllocoptruta oleivora* (Ashm.). Acta Entomol Sinica 32:184–191

Lillo E, Skoracka A (2010) What's "cool" on eriophyoid mites? Exp Appl Acarol 51:3–30

Lindquist EE, Bruin J, Sabelis MW (1996) Eriophyoid mites: their biology, natural enemies and control. Elsevier, Amsterdam

McCoy CW, Albrigo LG (1975) Feeding injury to the orange caused by the citrus rust mite, *Phyllocoptruta oleivora* (Prostigmata: Eriophyoidea). Ann Entomol Soc Am 68:289–297

Mielke-Ehret N, Thoma J, Schlatermund N, Muehlbach HP (2010) Detection of European mountain ash ringspot-associated virus-specific RNA and protein P3 in the pear leaf blister mite *Phytoptus pyri* (Eriophyidae). Arch Virol 155:987–991

Nakano VE, Kussumi, T A, LEMES, VRR, Kimura, I DA, Rocha SB, Alaburda J, Oliveira MCC, Ribeiro RA, Faria AL, Waldhelm KC (2016) Evaluation of pesticide residues in oranges from São Paulo, Brazil. Food Sci Technol 36:40–48

Navia D, Ochoa R, Welbourn C, Ferragut F (2010) Adventive eriophyoid mites: a global review of their impact, pathways, prevention and challenges. Exp Appl Acarol 51:225–255

Noronha ACS, Ferreira CT, Tavares EJM, Lima DB (2017) Fertility life table of *Tetranychus palmarum* Flechtmann & Noronha (Acari: Tetranychidae) in oil palm. Neotrop Entomol https://doi.org/10.1007/s13744-017-0550-y

Oldfield GN, Proeseler G (1996) Eriophyoid mites as vectors of plant pathogens. World Crop Pests, Amsterdam

Oliveira CAO, Sala I, Santos-Junior JE (1991) Ácaro da falsa ferrugem dos citros: resultados de 61 ensaios de campo visando seu controle. Jaboticabal: FUNEP, 50p

Rodrigues JCV, Oliveira CAL (2005) Ácaros fitófagos dos citros. In: Mattos-Júnior D, de Negri JD, Pio RM, Pompeu-Júnior J (eds) Citros. Instituto Agronômico/Fundag, Campinas

Seo YD, Kim, DS (2014a) Temperature-driven models of *Aculops pelekassi* (Acari: Eriophyidae) based on its development and fecundity on detached citrus leaves in the laboratory. J Asia Pac Entomol 17:135–142

Seo YD, Kim DS (2014b) The effects of humidity and citrus leaf age on the multiplication of *Aculops pelekassi* (Acarina: Eriophyoidae) and seasonal population abundances in citrus orchards. Korean J Appl Entomol 17:135–142

Skoracka A, Kuczynski L (2004) Demography of the cereal rust mite *Abacarus hystrix* (Acari: Eriophyoidea) on quack grass. Exp Appl Acarol 32:231–242

Smith D, Papacek DF (1991) Studies of the predatory mite *Amblyseius victoriensis* (Acarina: Phytoseiidae) in citrus orchards in south-east Queensland: Control of *Tegolophus australis* and *Phyllocoptruta oleivora* (Acarina: Eriophyidae), effect of pesticides, alternative host plants and augmentative release. Exp Appl Acarol 12:195–217

Stark JD, Banks JE (2003) Population-level effects of pesticides and other toxicants on arthropods. Annu Rev Entomol 48:505–519

Sternlicht M (1970) Contribution to the biology of the citrus bud mite, *Aceria sheldoni* (Ewing) (Acarina: Eriophyidae). Ann Appl Biol 2:221–230

Sternlihcht M, Goldenberg S (1971) Fertilisation, sex ratio and postembryonic stages of the citrus bud mite *Aceria sheldoni* (Ewing) (Acarina, Eriophyidae). Bull Entomol Res 60:391–397

Stoeva A, Rector BG, Harizanova V (2011) Biology of *Leipothrix dipsacivagus* (Acari: Eriophyidae), a candidate for biological control of invasive teasels (*Dipsacus* spp.). Exp Appl Acarol 55:225–232

Swirski E, Amitai S (1957) Techniques for breeding the citrus rust mite (*Phyllocoptruta oleivor*a Ashm. Acarina: Eriophyidae). Bull Res Coun Israel B 6:251–252

Tomlinson J (1966) The advantages of hermaphroditism and parthenogenesis. J Theor Biol 11:54–58

van der Merwe TJ, Coates TJ (1965) Biological study of the grey mite *Calacarus citrifolii* Keifer. S Afr J Agric Sci 8:817–824

van Leeuwen T, Tirry L, Yamamoto A, Nauen R, Dermauw W (2015) The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pestic Biochem Physiol 121:12–21

van Leeuwen T, Witters J, Nauen R, Duso C, Tirry L (2010) The control of eriophyoid mites: state of the art and future challenges. Exp Appl Acarol 51:205–224

Xu X, Li ., Wang D, Hong X, Wu J, Yuan, Y, Yongda Y, Xie, X (2006) Effect of temperature and relative humidity on development and reproduction of the tomato russet mite, *Aculops lycopersici* (Massee) (Acarina, Eriophyidae). Acta Entomol Sin 49:816–821

Yang Y, Allen JC, Knapp JL, Stansly PA (1994) Citrus rust mite (Acari: Eriophyidae) damage effects on ‘Hamlin’ orange fruit growth and drop. Environ Entomol 23:244–247

Zucchi AR, Silveira-Neto S, Nakano O (1993) Guia de identificação de pragas agrícolas. Piracicaba: FEALQ. 139p

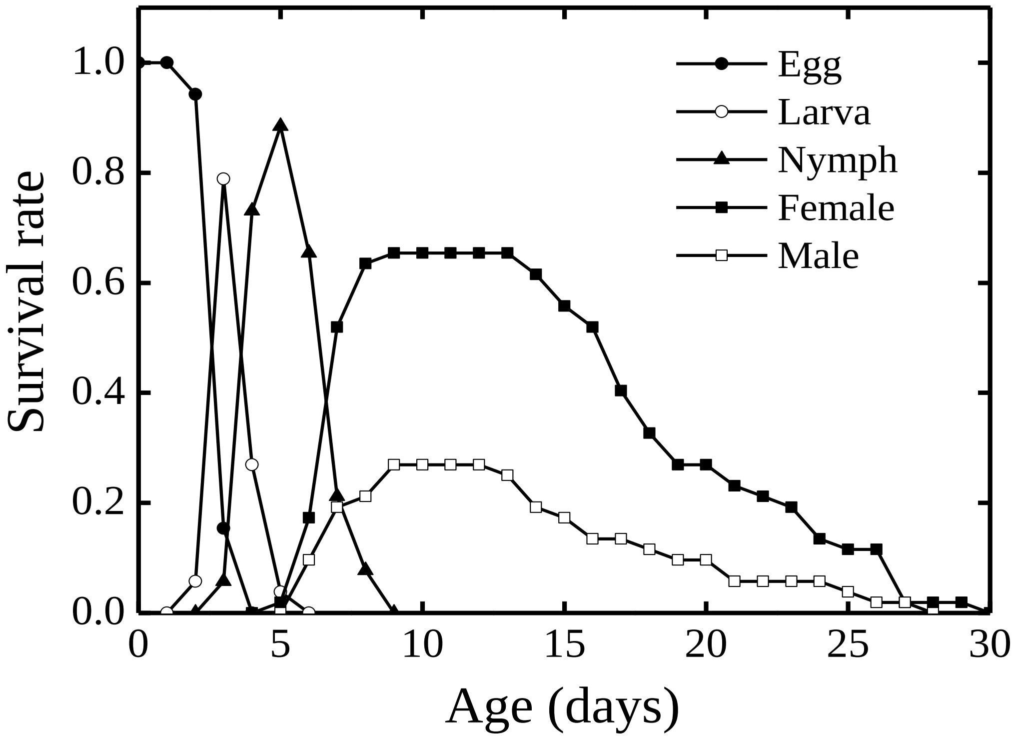

Figure 1. The age-stage-specific survival rate of *Tegolophus brunneus* reared on green fruits of 'Pera' sweet oranges. Temperature 25 ± 1 °C, relative humidity 70 ± 10%, and 12L: 12D h photoperiod.

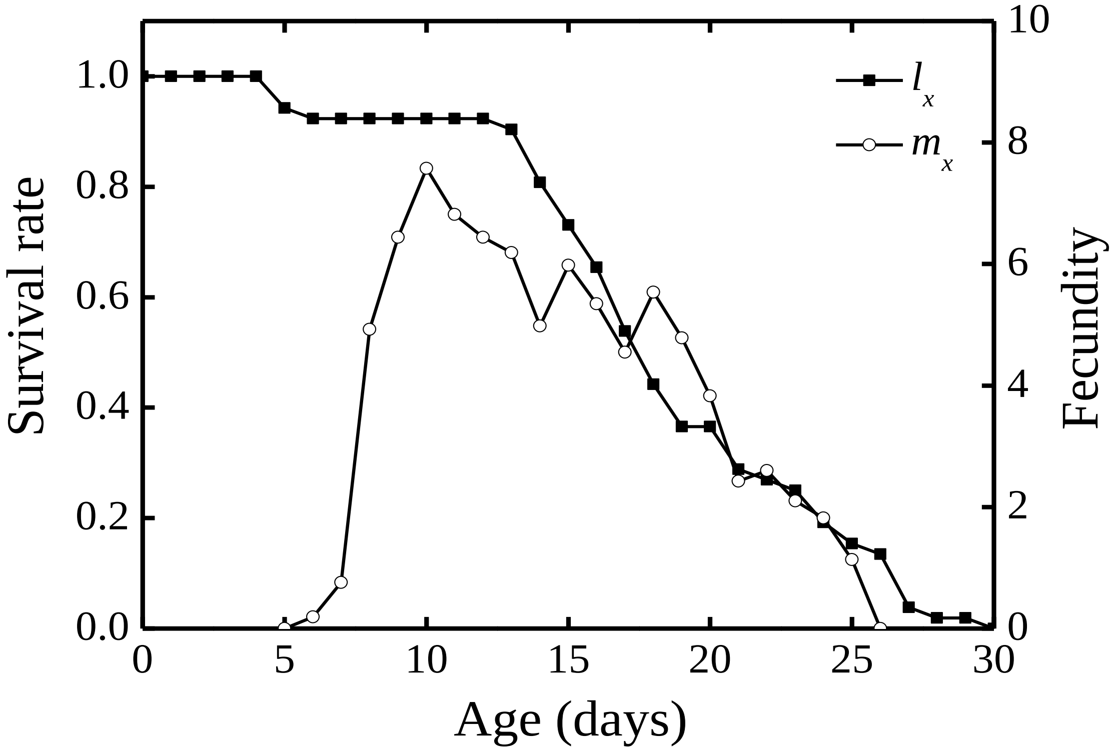

Figure 2. Age-specific survival rate (*l_x*) and age-specific fecundity (*m_x*) of *Tegolophus brunneus* reared on green fruits of 'Pera' sweet oranges. Temperature 25 ± 1 °C, relative humidity 70 ± 10%, and 12L: 12D h photoperiod.

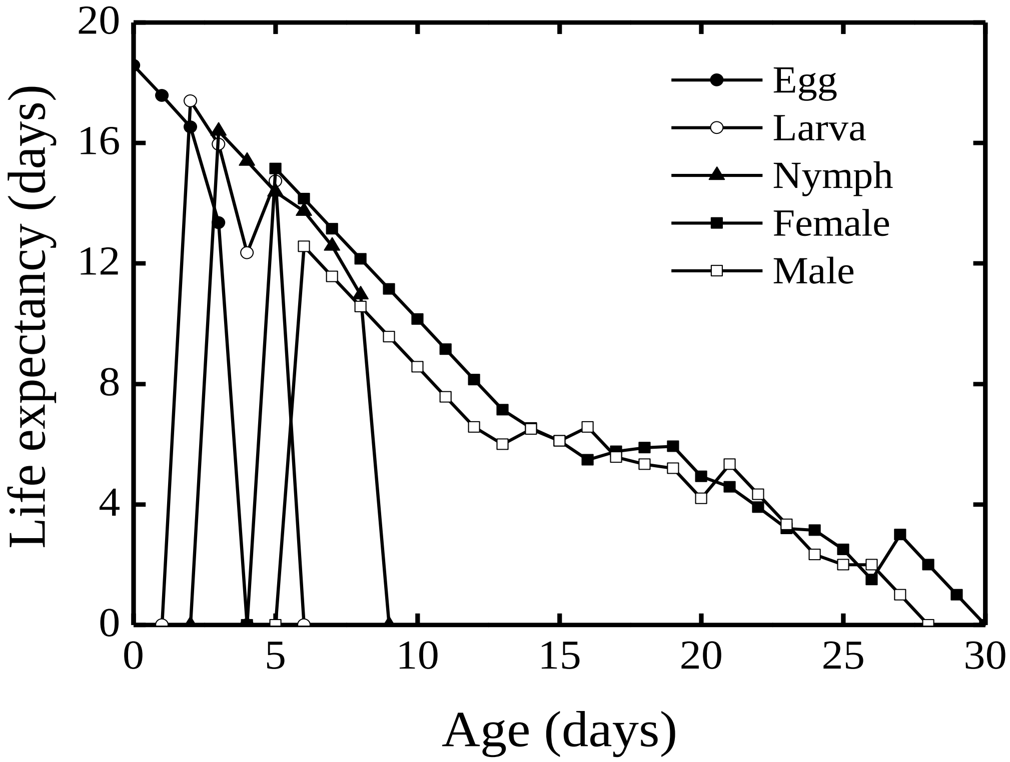

Figure 3. Age-stage-specific life expectancy of *Tegolophus brunneus* reared on green fruits of 'Pera' sweet oranges. Temperature 25 ± 1 °C, relative humidity 70 ± 10%, and 12L: 12D h photoperiod.

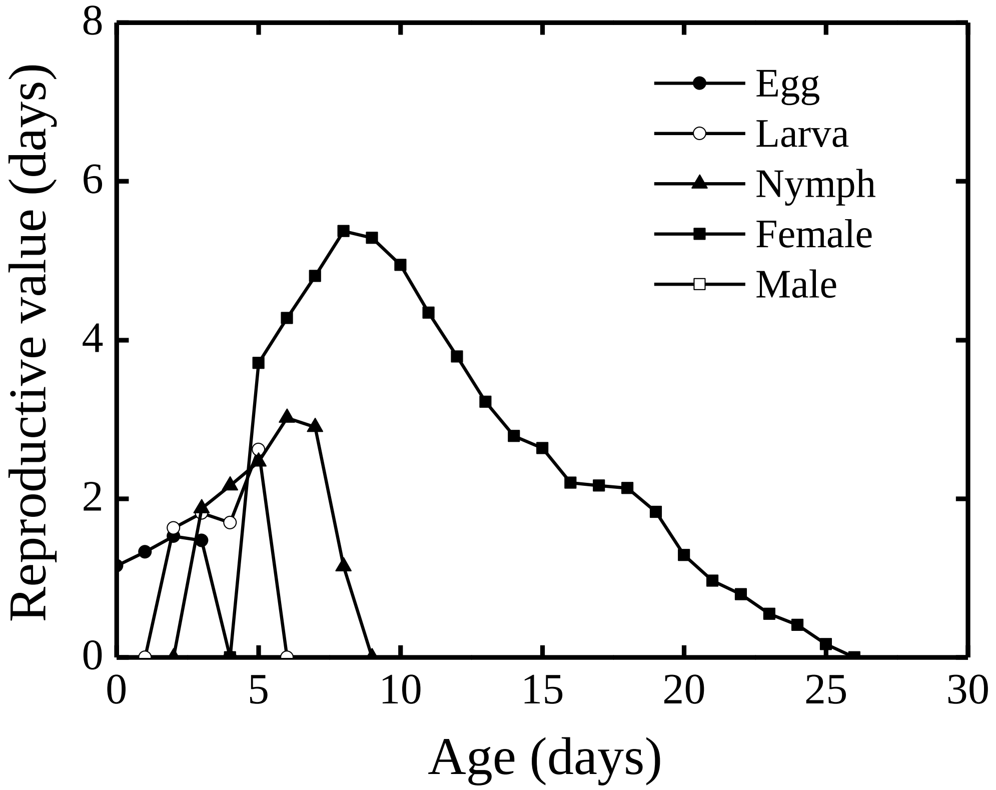

Figure 4. Age-stage-specific reproduction of *Tegolophus brunneus* reared on green fruits of 'Pera' sweet oranges. Temperature 25 ± 1 °C, relative humidity 70 ± 10%, and 12L: 12D h photoperiod.

Chapter 3 - Distribution of *Tegolophus brunneus* Flechtmann and *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) on the main citrus production region of Brazil and differential toxicity of the acaricides sulfur and abamectin to these species

Abstract - Many mites of the family Eriophyidae are important pests worldwide, especially the species *Phyllocoptruta oleivora*. The pest's injuries cause darkening of leaves, twigs and fruits, making the fruits unfit for sale at *in natura* market, besides affecting plant productivity. Another species that causes the same symptoms was described in Brazil recently: the citrus brown mite *Tegolophus brunneus*. Although studies have not been performed with this species, growers and technicians have attributed the rising in rust damages to *T. brunneus*, affirming that this mite is more aggressive and resistant to acaricides than *P. oleivora*. Based on that, the aim of this work was to study the distribution of *T. brunneus* in São Paulo and Minas Gerais State and also to study the differential toxicity of the acaricides sulfur and abamectin to both species. Infested fruits were collected from different orchards in many municipalities, including the main citrus species and varieties. It was observed that only plants of 'Tahiti' acid lime were infested by *T. brunneus*, while *P. oleivora* infested all citrus varieties and species. *Tegolophus brunneus* was 13 times more tolerant to abamectin than *P. oleivora*, while the toxicity of sulfur was the same to both species. These results showed *T. brunneus* specificity to infest 'Tahiti' acid lime, causing important damages to this crop, and suggest that attention should be paid to the management of this mite using abamectin.

Keywords: Chemical control, varieties, 'Tahiti' acid lime, citrus rust mite

1. Introduction

Mites of the family Eriophyidae are very important citrus pests, as they infest this crop every year, requiring costly management measures to control mite populations (Van Leeuwen et al., 2010). *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae), the citrus rust mite, is the most important species of this family to citrus production worldwide and in Brazil (Gerson, 2003, Rodrigues and Oliveira, 2005).

Recently, another species which causes russeting in citrus, the citrus brown mite *Tegolophus brunneus* Flechtmann (Acari: Eriophyidae), was described in Brazil (Flechtmann, 1999), concerning growers and technicians. This mite is similar to *Tegolophus australis* Keifer (Acari: Eriophyidae), a species restricted to Australia (Smith and Papacek, 1991; Smith et al., 1997; Flechtmann, 1999). *Tegolophus brunneus* could be differentiated from *P. oleivora* in the field by presenting a brownish-purple color, while *P. oleivora* presents a yellowish coloration, and also for showing the frontal lobe more elongated than *P. oleivora*. Furthermore, *T. brunneus* has a ridge along the dorsal part of the tanossome (Flechtmann, 1999), while *P. oleivora* has a furrow (Vacante, 2010).

In Brazil, this mite was reported in surveys in citrus in São Paulo, Rio Grande do Sul and Amazonas States (Albuquerque, 2006, Bobot et al., 2011; Horn et al., 2011; Bressan and Ott, 2017). However, so far there are no studies on this mite's distribution, biology, host preference, damage potential and importance to citrus production.

In São Paulo and in the west-southwest Minas Gerais citrus belt, the main citrus production region of Brazil, responsible for more than 80% of national citrus production (Neves and Trombin, 2017), the damage and losses caused by citrus rust mites have increased in the last decade, and many growers are attributing this rise to the citrus brown mite. Even without scientific proof, many growers and technicians believe that *T. brunneus* causes higher damage than *P. oleivora* and is more tolerant to acaricides than *P. oleivora*. However, these relationships have not been studied so far.

The discussion about *T. brunneus* as a potential pest in Brazil is justified, since *T. australis*, a species very similar to *T. brunneus*, is a major pest of citrus in Australia (Smith and Papacek, 1991; Smith et al., 1997). Also, the quarantine importance of *T. brunneus* should be highlighted, as Brazil exports *in natura* citrus fruits to many countries (Embrapa, 2017). The tiny size of Eriophyidae mites makes them difficult to detect, and they could be carried over long distances and establish population in many countries (Navia et al., 2010).

Information about the occurrence and damage potential of *T. brunneus* is urgently needed and is very important to citrus integrated pest management (IPM), as the precise identification of mite pests and its potential to damage the plants are some of the bases for IPM (Smith et al., 1997; Kogan, 1998).

Thus, the aim of this work was to investigate the distribution of the citrus brown mite *T. brunneus* in the main Brazilian citrus production region, in different municipalities and production systems, on the man scion and rootstock varieties planted, to determine the real pest status of this species to citrus production. Furthermore, a study of differential toxicity between *T. brunneus* and *P. oleivora* with the main acaricides used to control Eriophyidae mites in citrus was performed.

2. Material and Methods

2.1. Areas and period of collection

Sampling of citrus material infested with eriophyid mites were performed during the crop season 2016/2017 and 2017/2018, starting in February 2016 and finishing in July 2018. The collection areas were located in São Paulo and in the west-southwest Minas Gerais citrus belt, selecting the municipalities most representative in each microregion, to represent the greater part of citrus belt production (Figure 1). The orchards were carefully selected to cover the main citrus species and varieties planted, different climatic conditions, and production systems (conventional, organic, dooryard plants and in a varietal block).

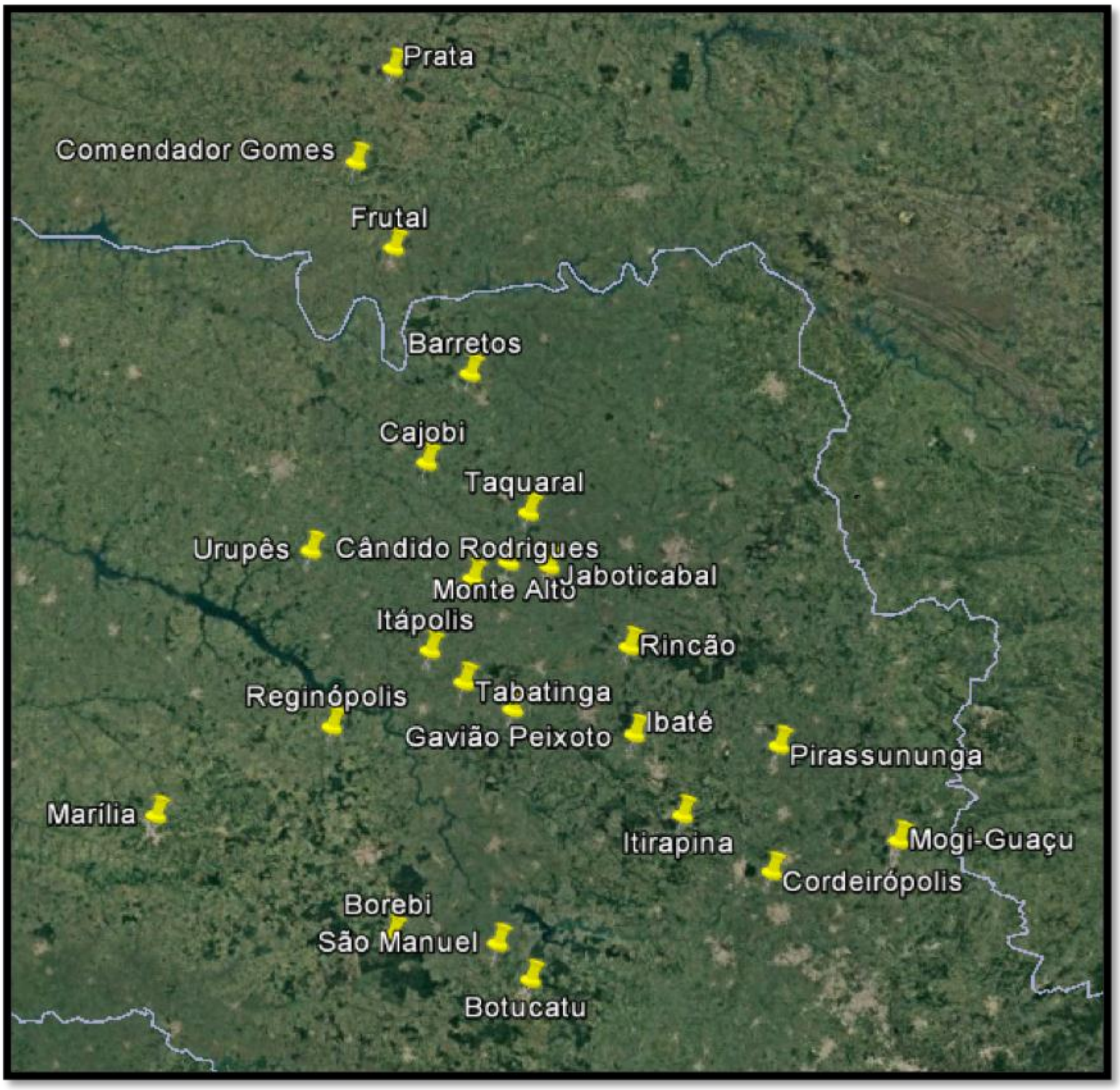

Figure 1. Municipalities of São Paulo and Minas Gerais states where citrus material was collected to map the distribution of *Tegolophus brunneus*

2.2. Sampling units

Each sampling unit consisted of 10 fruits infested by rust mites in each area, previously checked in the field using a hand lens of 10x magnification. These fruits were placed in styrofoam boxes hermetically closed and immediately taken to the Laboratory of Acarology of UNESP/FCAV.

The citrus scion species surveyed were the sweet oranges [*Citrus sinensis* (L.) Osbeck)] of the varieties Natal, Valencia, Pera, Hamlim, Baianinha, Westin, Folha Murcha, and 'Tahiti' acid lime [*Citrus latifolia* (Tanaka)], Eureka lemon (*Citrus limonia* Osbeck), Rangpur lime (*Citrus limonia* Osbeck), 'Murcott' tangor (*Citrus sinensis* ×

Citrus reticulata) and citron (*Citrus medica*) with different combinations of the rootstocks Rangpur lime, 'Sunki' mandarin (Citrus sunki Horte x Tanaka), Cleopatra mandarin (*Citrus reshini* Hort) and Swingle citrumel (*Citrus paradisi* × *Poncirus trifoliata*).

2.3. Mite identification

Specimens of eriophyid mites were mounted on slides containing a droplet of Berlese Modified medium and covered with a coverslip (Krantz and Walter, 2009). The slides were then heated on a heating block for diaphanization for at least 8 hours. Afterwards the slides were sealed using colorless enamel. The mites were identified using a microscope with phase contrast, according to Flechtmann (1999) and Vacante (2010). At the beginning of the study, *T. brunneus* slides were sent to Dr. Carlos H. W. Flechtmann, descriptor of the species, for confirmation.

Images of *T. brunneus* and *P. oleivora* were recorded using a high resolution, Hirox microscope to distinguish the species *in vivo*, being possible to observe morphological details with high resolution (Figure 2). In addition, adults of *T. brunneus* and *P. oleivora* were mounted on metal 'stubs' to study using Scanning Electron Microscopy. For that study, the mites were fixated in osmium tetroxide (OsO_4) 2% for 12 hours and kept in a glass dehumifier using silica gel to maintain the relative humidity near 0%. Afterwards, the mites were fixated in ethanol 70%, dehydrated in an ethylic series of 80, 90, 95 and 100% (three times each), and dried in a dryer equipment to critical point Leica EM CPD 300. After that, the mites were mounted in "stubs" with an adhesive double side carbon tape, coated with gold in a 'sputter coater' Baltec SDC 050, and examinated in MEV JEOL JSM IT 300. The images were obtained digitally (Figure 3 and 4).

The main characteristics observed to separate the species were mite coloration, ornamentation of prodorsal shield, frontal lobe elongation and presence of a ridge or furrow along the thanossome. *Phyllocoptruta oleivora* presents a yellowish color and a characteristic ornamentation on the prodorsal shield due to the presence of median and lateral lines, short frontal lobal elongation and the presence of a furrow on the dorsal face of tanossome (Vacante, 2010) (Figure 2a and 4). In contrast, *T. brunneus* presents a brownish-purple color, presence of punctuations on the prodorsal shield, high elongation of frontal lobe and the presence of a ridge along the tanossome (Flechtmann, 1999) (Figures 2b and 3).

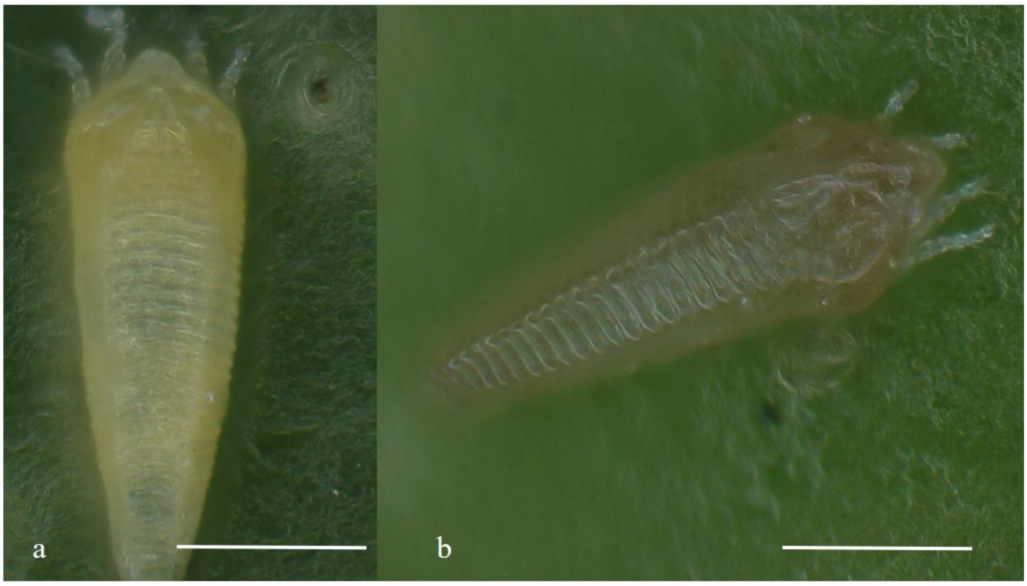

Figure 2. a) Adult *Phyllocoptruta oleivora* (a) (magnification 1500 x) and b) *Tegolophus brunneus* (magnification 1500 x). Scale bar: 50 µm (a). Scale bar: 50 µm (b). Images obtained using high resolution Hirox microscope.

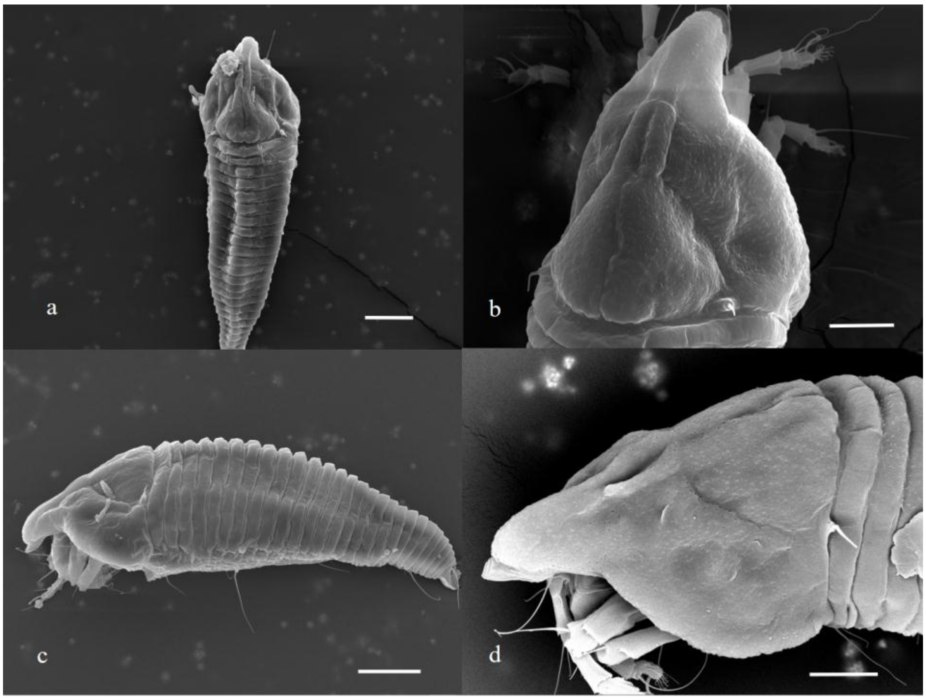

Figure 3. Adult *Tegolophus brunneus* (**a-**dorsal view; **b-** Detail of prodorsal shield and gnatossoma; **c-** lateral view; **d-** Prodorsal shield and gnatossoma in lateral view. Images obtained using JEOL JSM IT 300 microscope. **a**) Scale bar: 20 µm; **b)** Scale bar: 10 µm; **c**) Scale bar: 20 µm; d) Scale bar: 10 µm.

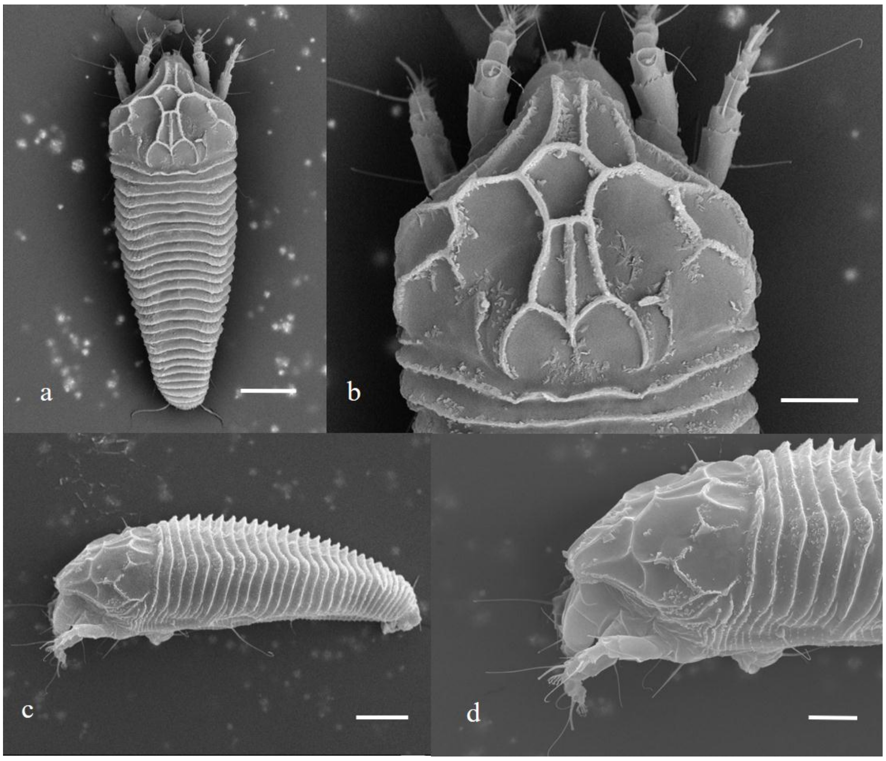

Figure 4. Adult *Phyllocoptruta oleivora* (**a-** dorsal view; **b-** Details of prodorsal shield and gnatossoma; **c-** lateral view; **d)** Prodorsal shield and gnatossoma in lateral view. Images obtained using Scanning Eletron Microscopy JEOL JSM IT 300. **a**) Scale bar: 20 µm; **b)** Scale bar: 10 µm; **c**) Scale bar: 20 µm; d) Scale bar: 10 µm.

2.4. Estimation of Lethal Concentration 50 (LC50) and 80 (LC80)

The mites used in the experiments were obtained from a colony maintained in the laboratory during six months in green fruits of the sweet orange ‘Pera’ partially coated with paraffin (Morais et al., 2019). The rearing was initiated from mites collected in plants of ‘Tahiti’ acid lime in the campus of Faculdade de Ciências Agrárias e Veterinárias, Unesp, campus de Jaboticabal-SP (UNESP/FCAV) (S21°14’34.6”W48°17’03.2”), that had never received pesticide application.

The lethal concentrations (LC50 and LC80) of the acaricides abamectin (Vertimec® 18EC, Syngenta) and sulfur (Kumulus®DF, Basf) were estimated to adult mites of *P. oleivora* and *T. brunneus*.

A circular area with 2 cm of diameter was delimited in green fruits of sweet orange ‘Pera’ with approximately 10 cm in diameter and sprayed with a Potter Tower using the volume of 2 mL of solution per fruit. After the residues dried, ten mites from the colony were transferred per fruit, using ten replicates for each concentration of abamectin and 20 replicates for sulfur. Eight concentrations were used per acaricide ranging from 150-2400 ppm for sulfur and 0.56-36 ppm for abamectin. Mortality was evaluated 48 hours after transference, and the mites that did not react to the touch of a fine brush were considered dead.

For estimation of the lethal concentrations (LCs) was used a binomial model with a linking function log-log complement (gompit model), using the Probit Procedure of Software SAS version 9.2 (SAS Institute, 2011)

3. Results

Tegolophus brunneus was only found on plants of ‘Tahiti’ acid lime (Tabela 1) in the municipalities of Jaboticabal, Fernando Prestes, Itápolis, Urupês, Tabatinga, Monte Alto and Cândido Rodrigues, in São Paulo State. In the other hand, *P. oleivora* infested all citrus species and varieties (Table 1).

The mites *P. oleivora* and *T. brunneus* have never been observed infesting the same fruit. In April 2018 two samples were collected in the same area of ‘Tahit’ acid lime production and, one of them was infested by *P. oleivora* and the other by *T. brunneus*.

The symptoms caused in Tahiti acid lime by *T. brunneus* were similar to those caused by *P. oleivora*, characterized by silvering of fruit skin in the beginning of infestation which progresses to cause dark spots similar to russeting. In the leaves, the damages caused by these mites were spots of dark coloration, predominantly in the border and abaxial part of leaves (Figure 5).

Phyllocoptruta oleivora was 13 times more susceptible to abamectin than *T. brunneus* (Table 2), while the toxicity to sulfur between both species was equal (Table 3).

Figure 5. Damages caused by *Tegolophus brunneus* in fruits and leaves of 'Tahiti' acid lime in Itápolis-SP (S21°45'20.711"W48°45'20.711)

Table 1. Sampling areas for mapping the occurrence of *Tegolophus brunneus*

Municipality	Geographic coordinates		Altitude (m)	Scion	Rootstock	Number of evaluated mites	% *P. oleivora*	% *T. brunneus*
February 2016	S	W						
Reginópolis-SP	21°48’38’’	49°9’05’’	474	Hamlim	Swingle	214	100	0
São Manuel-SP	22º45'19.692"	48º28'2.305"	530	Valencia	Swingle	897	100	0
Cordeirópolis-SP	22º27'45.709"	47º24'39.859"	709	Pera	Rangpur lime	35	100	0
June 2016								
Pirassununga-SP	-	-		Valencia	Rangpur lime	35	100	0
Marília-SP	22º16’49.8”	50º04’35.0”	468	Valencia	Rangpur lime	10	100	0
Monte Alto-SP	21°12’36”	48°35’29”	589	Murcott		786	100	0
July 2016								
Gavião Peixoto-SP	21°44’43.43”	48°27’34.80”	575	Pera	Sunki	163	100	0
Itirapina-SP	22°12’16.13”	47°44’04.26”	793	Valencia	Rangpur lime	122	100	0
August 2016								
Gavião Peixoto-SP	21°44’43.43”	48°27’34.80”	575	Pera	Sunki	301	100	0
Itirapina-SP	22°12'16.13"	47°44'04.26"	793	Valencia	Rangpur lime	106	100	0
September 2016								
Gavião Peixoto-SP	21°44’43.43”	48°27’34.80”	575	Pera	Sunki	326	100	0
Itirapina-SP	22°12'16.13"	47°44'04.26"	793	Valencia	Rangpur lime	03	100	0
October 2016								
Itirapina-SP	22°12'16.13"	47°44'04.26"	793	Valencia	Rangpur lime	14	100	0
February 2017								
Rincão-SP	21°32'49.99"	48° 5'49.32"	565	Valencia	Swingle	710	100	0

Cajobi-SP	20°49'20.43"	48°48'21.51"	573	Natal	Swingle	1050	100	0
Frutal-SP	20°8'5.02"	48°47'4.02"	498	Natal	Rangpur lime	376	100	0
Prata-MG	19°22'59,36"	48°46'42,44"	668	Valencia	Swingle	486	100	0
Comendador Gomes-MG	19°40'28.05"	48°56'1,79"	714	Valencia	Cleopata	467	100	0
Barretos-SP	20°29'23,09"	48°35'13.60"	550	Valencia	Rangpur lime	1102	100	0
Borebi-SP	22°43'32.97"	49° 5'26.13"	637	Baianinha	Swingle	173	100	0
March 2017								
Rincão-SP	21°32'49.99"	48° 5'49.32"	563	Valencia	Swingle	911	100	0
Barretos-SP	20°29'33.45"	48°34'51.42"	553	Valencia	Rangpur lime	1295	100	0
Prata-MG	19°22'59.36"	48°46'42.44"	668	Valencia	Swingle	1019	100	0
Cajobi-SP	20°50'14.77"	48°49'29.55"	551	Valencia	Swingle	328	100	0
Rincão-SP	21°31'32.25"	48°7'22.61"	570	Folha Murcha	Swingle	233	100	0
Prata-MG	19°22'59.36"	48°46'42.44"	668	Valencia	Swingle	452	100	0
Mogi-Guaçú-SP	22°12'18.88"	47°10'17.29"	654	Valencia	Rangpur lime	514	100	0
Santo Antônio de Posse-SP	22°34’07.69”	46°59’13.84”	615	Pera	Rangpur lime	151	100	0
Santo Antônio de Posse-SP	22°34’07.69”	46°59’13.84”	615	Murcott	Rangpur lime	233	100	0
May 2017								
Rincão-SP	21°33'4.18"	48°4'50.56"	574	Folha Murcha	Volkamericano	853	100	0
Barretos-SP	20°29'8.04"	48°35'20.17"	555	Valencia	Rangpur lime	219	100	0
Mogi-Guaçú-SP	22°12'18.88"	47°10'17.29"	654	Valencia	Rangpur lime	783	100	0
June 2017								
Rincão-SP	21°32'22.09"	48° 6'30.25"	556	Valencia	Rangpur lime	773	100	0
July 2017								
Rincão-SP	21°32'49.99"	48° 5'49.32"	565	Valencia	Rangpur lime	440	100	0
Mogi-Guaçú-SP	22°12'18,88"	47°10'17.29"	654	Valencia	Rangpur lime	1405	100	0
August 2017								

Rincão	21°33'43.89"	48°6'0.80"	570	Valencia	Swingle	211	100	0
Mogi-Guaçú-SP	22°12'18.88"	47°10'17.29"	647	Valencia	Rangpur lime	1060	100	0
October 2017								
Mogi-Guaçú-SP	22°12'18,88"	47°10'17.29"	651	Valencia	Rangpur lime	734	100	0
Rincão-SP	21°32'31,11"	48° 8'16.38"	590	Hamlim	Cleopata	277	100	0
Mogi-Guaçú-SP	22°12'18,88"	47°10'17.29"	654	Valencia	Rangpur lime	463	100	0
November 2017								
Rincão-SP	21°32'31.11"	48° 8'16.38"	589	Hamlim	Cleopata	77	100	0
January 2018								
Rincão-SP	21°31'56.04"	48° 8'18.79"	542	Valencia	Rangpur lime	466	100	0
Mogi-Guaçú	22°12'18.88"	47°10'17.29"	653	Valencia	Rangpur lime	1402	100	0
Mogi-Guaçú	21°31'46.83"	48° 7'26.75"	570	Pera	Rangpur lime	1083	100	0
March 2018								
Borebi-SP	22°42'49.33"	49° 3'40.16"	632	Valencia	Swingle	845	100	0
Borebi-SP	22°42'12.01"	49° 4'36.81"	652	Natal	Swingle	1257	100	0
Botucatu-SP	22°42'48,00"	48°23'15.58"	498	Hamlim	Swingle	82	100	0
Botucatu-SP	22°42'59.55"	48°22'59.05"	512	Hamlim	Sunki	356	100	0
Botucatu-SP	22°42'39.82"	48°22'55.31"	511	Hamlim	Swingle	122	100	0
Rincão-SP	21°32'58.09"	48°7'2.16"	558	Natal	Swingle	2415	100	0
Jaboticabal-SP	21°14'38''	48°17'19.1"	562	Tahiti lime	Rangpur lime	190	0	100
Fernando Prestes-SP	21º16'44.256"	48º40'59.466"	547	Tahiti lime	Rangpur lime	523	0	100
April 2018								
Rincão-SP	21°32'58.09"	48°7'2.16"	563	Natal	Swingle	1247	100	0
Urupês-SP	21º8'51.691"	49º13'45.505"	460	Tahiti lime	Rangpur lime	274	0	100
Urupês-SP	21º8'51.691"	49º15'11.934"	436	Tahiti lime	Rangpur lime	891	100	0
Urupês-SP	21º8'45.586"	49º13'43.043"	460	Tahiti lime	Rangpur lime	2117	100	0
Cândido Rodrigues-SP	21°19'44.45"	48°37'50.82"	586	Tahiti lime	Rangpur lime	122	0	100

Jaboticabal-SP	21°14’34.6”	48°17’03.2”	562	Tahiti lime	Rangpur lime	249	0	100
Jaboticabal-SP	21°14’34.6”	48°17’03.2”	562	Cidre	-	102	100	0
Jaboticabal-SP	21°14’34.6”	48°17’03.2”	562	Rangpur lime	-	79	100	0
Tabatinga-SP	21º39'19.382”	48º34'39.05"	565	Tahiti lime	Rangpur lime	1093	0	100
Itápolis-SP	21°31’54.433”	48°45’20.711”	502	Tahiti lime	Rangpur lime	1602	0	100
May 2018								
Ibaté-SP	21°57'48.13"	48°7'38.03"	550	Eureka lemon	Rangpur lime	623	100	0
Rincão-SP	21°32'48.82"	48°7'15.32"	562	Natal	Swingle	1150	100	0
Tabatinga-SP	21º39'19.382”	48º34'39.05"	565	Tahiti lime	Rangpur lime	2006	0	100
Tabatinga-SP	21º39'19.382	48º34'39.05"	565	Murcott	Rangpur lime	322	100	0
Tabatinga-SP	21º39'19.382”	48º34'39.05"	565	Tahiti lime	Rangpur lime	736	0	100
Tabatinga-SP	21º39'57.679”	48º36'57.55"	549	Tahiti lime	Rangpur lime	50	0	100
July 2018								
Taquaral-SP	21º0'43.888"	48º25'36.268"	578	Natal	Swingle	52	100	0
Itápolis-SP	21°31’54.433”	48°45’20.711”	502	Tahiti lime	Rangpur lime	291	0	100

Table 2. Estimation of lethal concentration 50 (LC50) and 80 (LC80) (mg a.i. L-1) and confidence interval to the acaricide sulfur (Kumulus® DF AG) on adults of *Phyllocoptruta oleivora* and *Tegolophus brunneus* after 48 h of exposure to pesticides residue.

Species	Exposure time (h)	N[1]	*Slope* ± EP (*P* value)	LC_{50} (CI)[2]	LC_{80} (CI)[2]	[3]χ^2	d.f.	h[4]
Phyllocoptruta oleivora	48	1600	3,27 ± 0,49 (*P* < 0,0001)	1,44 (1,14-1,69)	2,60 (2,16-3,59)	9,40	5	1,88
Tegolophus brunneus	48	1600	3,98 ± 0,30 (*P* < 0,0001)	1,04 (0,62-1,25)	1,87 (1,32-2,46)	6,82	5	1,36

[1]N: number of mites tested;
[2]IC: confidence interval at 95% error probability;
[3]χ^2: Value of Pearson square;
[4]h.: Heterogenity factor.

Table 3. Estimation of lethal concentration 50 (LC50) and (LC80) and confidence interval of the acaricide abamectin (Vertimec® 18 EC) (mL p.c. L-1) on adults of *Phyllocoptruta oleivora* and *Tegolophus brunneus* after 48 hours of exposure to pesticides residues.

Species	Exposure time (h)	N[1]	*Slope* ± EP (*P* value)	LC_{50} (CI)[2]	LC_{80} (CI)[2]	[3]χ^2	d.f.	h[4]
Phyllocoptruta oleivora	48	800	2,90 ± 0,25 (*P* < 0,0001)	0,003 (0,002-0,004)	0,008 (0,005-0,012)	7,20	4	1,44
Tegolophus brunneus	48	800	1,97 ± 0.21 (*P*< 0.0001)	0,04 (0.01 -0.06)	0,10 (0.06 -0.15)	7,04	4	1,76

[1]N: number of mites tested;
[2]IC: confidence interval at 95% error probability;
[3]χ^2: Value of Pearson square;
[4]h.: Heterogenity factor.

4. Discussion

The results presented here are the first report on the distribution of the citrus brown mite *T. brunneus* in the main citrus region of Brazil, as well as this mite's infestation on different citrus species and varieties. Among the species evaluated, *T. brunneus* infested only 'Tahiti' lime acid, while *P. oleivora* infested all evaluated species. Similar results were found by Oliveira (2007), in which this author for three crop years did not collect *T. brunneus* on sweet orange plants 'Valencia' in surveys conducted in Mogi-Guaçu, São Paulo State. Ferreira et al. (2018) during a two-year survey in Amazonas State and Silva et al. (2012) during a yearlong collection in Descalvado-SP did not collect *T. brunneus* infesting sweet orange Pera.

On the other hand, *T. brunneus* was collected in sweet orange 'Pera' and tangor 'Murcott' by other authors in São Paulo, Rio Grande do Sul and Amazonia States (Flechtmann, 1999; Albuquerque, 2006; Bobot et al., 2011; Horn et al., 2011; Bressan and Ott, 2017).

Probably, peculiarities in each production system can help explain the occurrence of *T. brunneus* in sweet orange 'Pera' and tangor 'Murcott' by these authors, contrasting with the results obtained in this work. The chemical control in these orchards can play a key role to explain the infestation of *T. brunneus* in these citrus varieties, as *T. brunneus* is 13 times more tolerant than *P. oleivora* to abamectin. Thus, in areas with high application of acaricides and fungicides containing abamectin, *T. brunneus* can present a competitive advantage in relation to *P. oleivora*, allowing *T. brunneus* to infest citrus species and varieties that *P. oleivora* would infest without the selection pressure of abamectin application.

Furthermore, pesticide application can be harmful to the natural mortality agents of eriophyids, which can cause changes on this mite population. The main biological control of these mites are entomopathogenic fungi and predatory mites of the Phytoseiidae and Stigmaeidae families (Mccoy, 1996; Moraes and Zacarias, 2002); and the negative effects of abamectin to this organisms should be highlighted, being harmful to the main natural enemies of Eriophyidae mites in the field (Ibrahim and Yee, 2000; Oliveira and Neves, 2004; Lima et al., 2016).

The deleterious effects on higher trophic levels can affect the second trophic level through top down regulation, changing the frequencies of phytophagous species which compete by the same resource (Messelink et al., 2012). The 13 times higher tolerance of *T. brunneus* compared to *P. oleivora* to abamectin and the side effects of this pesticide to the main natural enemies of this mites in the field can play a key role in these mite infestation levels in the field. Alternation in the ecological dominance among arthropod species due to pesticides action is reported in the literature (Sun et al., 2013; Cordeiro et al., 2014), however, studies involving mites are scarce (Venzon et al., 2001).

Besides the 'aesthetic' damage, other harmful effects could be caused by *T. brunneus* in 'Tahiti' acid lime, as negative effects in juice quality, defoliation, reduction in photosynthetic potential, and lignin accumulation in the fruit skin in high infested plants. These damages were well determined to *P. oleivora* infesting citrus and could occur in plants infested by *T. brunneus* in different intensities, due to the similarities in the feeding behavior of these mites (McCoy and Albrigo, 1975; Sabelis and Bruin, 1996).

The results obtained indicated the necessity of performing studies to determine the economic damage threshold of *T. brunneus* in 'Tahiti' acid lime as determined by McCoy and Albrigo (1975) in sweet orange 'Valencia' with *P. oleivora*. These authors determined that in this variety the economic threshold level is 70-80 mites/cm^2 of fruit surface.

Based on collections performed in a citrus varietal block at UNESP/FCAV and in an orchard where plants of 'Tahiti' acid lime and tangor Murchott were cultivated in consortium, it was possible to confirm the specificity of *T. brunneus* to infest 'Tahiti' acid lime. In these areas, even with different citrus species and varieties very near one another, *T. brunneus* was observed infesting only 'Tahiti' lime. However, Morais et al. (2019) verified that *T. brunneus* developed in fruits of sweet orange 'Pera'. These authors observed that in this substrate this mite species showed a biological cycle of seven days, adult longevity of 11 to 13 days and the females oviposit on average 8.5 eggs during its life.

Host specificity is a common characteristic of Eriophyidae and the majority of species is restricted to a plant genus or even to a single species (Lillo et al., 2018). However, the mechanisms which explain the infestation of *T. brunneus* only in 'Tahiti' lime should be investigated.

It should be emphasized that in none of the fruits collected the two eriophyids mites were observed infesting together. One possible explanation could be due to recognition of volatiles emitted from the plants infested by one of the species, which make other species avoid infesting this plant due to competition and/or exposition to constitutive defenses produced by the plant previously attacked by the other herbivore (Pallini et al., 1997; Dicke, 2000; Wei et al., 2014).

However, more research is needed to better comprehend the host mite relationships between *T. brunneus* and 'Tahiti' acid lime, the main mortality agents of this mite in the field, population dynamics, as management measures to control rust mites in citrus are based on *P. oleivora* characteristics, which could worsen the situation of citrus rust mite management in this crop.

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6. References

Albuquerque FA (2006) **Diversidade de ácaros em cultivo orgânico de citros e na vegetação natural circundante, e perspectivas para a criação massal de *Iphiseiodes zuluagai* (Acari: Phytoseiidae)**. 95f. Tese (Doutorado em Agronomia-Entomologia Agrícola)- Unesp de Jaboticabal.

Bobot TE, Chilson EF, Navia D, Gasnier TRJ, Lofego AC, Oliveira BM (2011) Mites (Arachnida, Acari) on *Citrus sinensis* L. Osbeck orange trees in the state of Amazonas, Northern Brazil. **Acta Amazonica** 41:557-566.

Bressan LR, Ott AP (2017) Distribuição intra-planta e flutuação populacional de *Tegolophus brunneus* (Acari: Eriophyidae: Phyllocoptinae) em tangerineira. **Caderno de Pesquisa** 29:23-29.

Cordeiro EMG, Corrêa, AS, Guedes RNC (2014) Insecticide-mediated shift in ecological dominance between two competing species of grain beetles. **Plos One**, 9: e100990.

Dicke M (2000) Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. **Biochemical Systematics and Ecology**, 28: 601-617.

EMBRAPA (2017) **Destinos das exportações brasileiras de limão em 2017**. Cruz das Almas: Embrapa: Mandioca e Fruticultura, 4p.

Ferreira CT, Krug C, Garcia MVB, Moraes GD (2018) Leprosis mite and other mite species (Acari) associated to orange groves in Brazilian Central Amazon. **Systematic and Applied Acarology** 23:449-462.

Flechtmann CH (1999) *Tegolophus brunneus* n. sp., a new citrus rust mite from Brazil (Acari: Eriophyidae). **International Journal of Acarology** 25:265-267.

Gerson U (2003) Acarine pests of citrus: overview and non-chemical control. **Systematic and Applied Acarology** 8:3-12.

Horn TB, Johann L, Ferla NJ (2011) Ecological interactions between phytophagous and predaceous mites in citrus agroecosystems in Taquari Valley, Rio Grande do Sul, Brazil. **Systematic and Applied Acarology** 16:133-144.

Kogan M (1998) Integrated pest management: historical perspectives and contemporary developments. **Annual Review of Entomology** 43:243-270.

Krantz GW, Walter DEA (2009) Collection, rearing and preparation for studying .In.: Krantz GW, Walter DEA (Eds). **A Manual of Acarology**. Lubbock: Texas Tech University Press, p. 83-96.

Ibrahim YB, Yee TS (2000) Influence of sublethal exposure to abamectin on the biological performance of *Neoseiulus longispinosus* (Acari: Phytoseiidae). **Journal of Economic Entomology** 93:1085-1089.

Lillo E, Pozzebon A, Valenzano D, Duso, C (2018) An intimate relationship between eriophyoid mites and their host plants–a review. **Frontiers in Plant Science** 9:1-14.

Lima, DB, Melo JW, Gondim MG, Guedes RN, Oliveira JE (2016). Population-level effects of abamectin, azadirachtin and fenpyroximate on the predatory mite *Neoseiulus baraki*. **Experimental and Applied Acarology** 70:165-177.

Messelink GJ, Sabelis MW, Janssen A (2012) Generalist predators, food web complexities and biological pest control in greenhouse crops. In: Soloneski S (Ed) **Integrated Pest Management and Pest Control-Current and Future Tactics**. Rijeka: InTech, p.191-214.

Mccoy CW (1996) Pathogens of eriophyoid mites. In.: Lindquist EE, Sabelis MW, Bruin J (Eds.) **World Crop Pests**. Amsterdam: Elsevier, p. 481-490.

Mccoy CW, Albrigo LG (1975) Feeding injury to the orange caused by the citrus rust mite, *Phyllocoptruta oleivora* (Prostigmata: Eriophyoidea). **Annals of the Entomological Society of America** 68:289-297.

Moraes GJ, Zacarias MS (2002) Use of predatory mites for the control of eriophyid mites. In: PROCEEDINGS OF THE INTERNATIONAL WORKSHOP ON COCONUT MITE. **Anais eletrônicos…**Sri Lanka: Coconut Research Institute.

Morais MR, Zanardi OZ, Andrade DJ (2019) Biological and demographic parameters of *Tegolophus brunneus* (Acari: Eriophyidae) in citrus. **Experimental and Applied Acarology.** (in press).

Navia D, Ochoa R, Welbourn C, Ferragut F (2010) Adventive eriophyoid mites: a global review of their impact, pathways, prevention and challenges. **Experimental and Applied Acarology** 51:225-255.

Neves MF, Trombin VG (2017) **Anuário da citricultura**. São Paulo: CitrusBR, 60p.

Oliveira WP (2007) **Flutuação e densidade populacional de ácaros (Acari) em três sistemas de produção de citros**. 143f. Tese (Doutorado em Ecologia Aplicada)-CENA- ESALQ/USP, Piracicaba.

Oliveira RCD, Neves PM (2004) Compatibility of *Beauveria bassiana* with acaricides. **Neotropical Entomology** 33:353-358.

Pallini A, Janssen A, Sabelis MW (1997) Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. **Oecologia** 110: 179-185.

Rodrigues JCV, Oliveira CAL (2005) Ácaros fitófagos dos citros. In: Junior DM, Negri JD, Pio RM, Junior JP (Eds.) **Citros.** Campinas: Instituto Agronômico, p. 691-727.

Sabelis MW, Bruin J. Evolutionary ecology: Life history patterns, food plant choice and dispersal (1996) In: Lindquist EE, Bruin J, Sabelis (Eds). **World crop pests**. Amsterdam: Elsevier, p. 329-366.

SAS Institute. 2011. Statistical analysis system: getting started with the SAS learning. Version 9.2. Cary: SAS Institute.

Silva MZ, Sato ME, Oliveira CAL (2012) Diversidade e dinâmica populacional de ácaros em pomar cítrico. **Bragantia** 71: 210-218.

Smith D, Beattie, GA, Broadley R (1997). **Citrus pests and their natural enemies: integrated pest management in Australia**. Queesland: Dept. of Primary Industries, 272p.

Smith D, Papacek DF (1991) Studies of the predatory mite *Amblyseius victoriensis* (Acarina: Phytoseiidae) in citrus orchards in south-east Queensland: control of *Tegolophus australis* and *Phyllocoptruta oleivora* (Acarina: Eriophyidae), effect of pesticides, alternative host plants and augmentative release. **Experimental and Applied Acarology** 12:195-217.

Sun DB, Liu YQ, Qin L, Xu J, Li FF, Liu SS (2013) Competitive displacement between two invasive whiteflies: insecticide application and host plant effects. **Bulletin of Entomological Research** 103:344-353.

Vacante V (2010) **Citrus mites: identification, bionomy and control**. Walingford: CABI, 371p.

Van Leeuwen T, Witters J, Nauen R, Duso C, Tirry L (2010) The control of eriophyoid mites: state of the art and future challenges. **Experimental and Applied Acarology**, 51: 205-224.

Venzon M, Janssen A, Sabelis MW (2001) Prey preference, intraguild predation and population dynamics of an arthropod food web on plants. **Experimental and Applied Acarology** 25:785-808.

Wei J, van Loon JJ, Gols R, Menzel TR, Li, Kang L, Dicke M (2014) Reciprocal crosstalk between jasmonate and salicylate defence-signalling pathways modulates plant volatile emission and herbivore host-selection behaviour. **Journal of Experimental Botany** 65:3289-3298.

Chapter 4 - Predatory mites collected in a conventional and in an organic citrus orchard and the functional response of Phytoseiidae mites preying on *Phyllocoptruta oleivora* (Ashmead) and *Tegolophus brunneus* Flechtmann (Acari: Eriophyidae)

Abstract - Among the arthropods that are pests worldwide, the mites should be highlighted. In Brazil and in other main citrus grower's countries, one of the key pests of this crop is the citrus rust mite *Phyllocoptruta oleivora*, due to the damages caused to fruits, leaves and twigs, which depreciate citrus production, causing defoliation, increase in transpiration rates, and negative effects in juice quality. In 1999, the brown citrus mite *Tegolophus brunneus* Flechtmann (Acari: Eriophyidae), which also causes rust damages in citrus, was described in Brazil and caused concern to growers and technicians, as failures to control rust mites have been recorded. The management of this species is performed through chemical control, using acaricides. As a result of decades of pesticide use, it is possible that these mite populations have been selected for resistance. Therefore, the study of new management strategies to control rust mites is needed. Thus, a study was conducted to determine the main mite predatory species in citrus orchards and the functional response of these species on *P. oleivora* and *T. brunneus*. Surveys were conducted in both a conventional and an organic citrus orchard for one year, and the functional responses of the predators *Iphiseiodes zuluagai*, *Euseius concordis*, *Euseius citrifolius* and *Amblyseius acalyphus* to both prey species were estimated. In the organic orchard, *I. zuluagai* (77.27%), *E. concordis* (18.7%) and *Proprioseiopsis neotropicus* (4.54%) were collected, while in the conventional area were collected *I. zuluagai* (71.53%) and *E. concordis* (4.54%). *Euseius concordis* showed a type II functional response preying on *P. oleivora*, while the others predators showed a type III functional response on this mite. When preying on *T. brunneus*, *E. concordis* and *E. citrifolius* showed a type III functional response, while *A. acalyphus* and *I. zuluagai* a type II functional response. *Amblyseius acalyphus*, *E. concordis*, *I. zuluagai* and *E. citrifolius* preyed on average 150, 70, 115 and 21 *T. brunneus* adult mites and 155, 100, 87 and 19 *P. oleivora* adult mites, respectively. These predators showed potential to control eriophyid mites in citrus, mainly *A. acalyphus*, which showed the highest predation.

Keywords: Biological control, integrated pest management, predator diversity

1. Introduction

One of the main citrus pests worldwide is the citrus rust mite *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae), being the key pest in many countries (Childers and Acor, 1999; Gerson, 2003; Vacante, 2010). The feeding behavior of these mites on the plants causes darkening of fruits, leaves and twigs, producing damage known as ‘russeting’, harming the plants qualitatively and quantitatively (Mccoy and Albrigo, 1975). Another eriophyid species that can inflict similar damage in citrus is *Tegolophus australis* Keifer (Acari: Eriophyidae) in Australia (Smith et al., 1997), being a major pest to citrus production in that country. A similar species to *T. australis* described in Brazil in 1999, the citrus brown mite *Tegolophus brunneus* Flechtmann (Acari: Eriophyidae), was reported infesting sweet oranges plants in the main citrus production region of São Paulo State, and in high population levels in the North and South regions of the country in the sweet orange Pera and tangor ‘Murcott’ (Flechtmann, 1999; Horn et al., 2011; Bobot et al., 2011, Bressan and Ott, 2017).

The control of these species has relied exclusively on chemical pesticides, using the same active ingredients, abamectin and sulfur, over time (Omoto and Alves, 2004). Due to failures in controlling these mites’ population in the field, it is speculated that populations of these pests could have been selected for resistance, as previously reported for *P. oleivora* (Omoto et al., 1994; Berg et al., 1999).

New management strategies should be sought to prevent injuries of these rust mites for citrus production, and one of the most promising is biological control. Few studies have been conducted on the action of predatory mites to control *P. oleivora* (Argov et al., 2002; Palevsky et al., 2003; Maoz et al., 2014), and none were conducted with *T. brunneus* so far. Furthermore, predatory potential of the main predatory mites collected in citrus in Brazil to control rust mites has never been tested.

In both conventional and organic citrus orchards in Brazil, the predatory mite species most frequently collected were *Iphiseiodes zuluagai* Denmark, Muma (Acari: Phytoseiidae), *Euseius concordis* (Chant) (Acari: Phytoseiidae) and *Euseius citrifolius* Denmark, Muma (Acari: Phytoseiidae) (Sato et al., 1994; Alburquerque, 2006; Horn et al., 2011; Bobot et al., 2011; Silva et al., 2012; Ferreira et al., 2018). However, even though high population levels were collected in many orchards, these predatory mites were not able to prevent pest population growth and associated damages.

Studies are needed to select and introduce predatory mite species with potential to control rust mites in citrus orchards. Among these potential species that should be highlighted is *Amblyseius acalyphus* Denmark, Muma (Acari: Phytoseiidae), since this mite was observed preying on *T. brunneus* in a ‘Tahiti’ acid lime field without pesticide application (Personal communication).

Thus, the functional responses of *I. zuluagai*, *E. concordis*, *E. citrifolius* and *A.acalyphus* on different densities of *T. brunneus* and *P. oleivora* adults were estimated in order to evaluate the predation potential of this species to control citrus rust mites.

2. Material and Methods

2.1. Sampling areas

Surveys were performed monthly in both a conventional and an organic citrus orchard. The predatory mites were collected between April, 2016 and July, 2017. The organic orchard consisted of sweet orange ‘Pera’ grafted on ‘Rangur’ lime *Citrus limonia* Osbeck, planted in July, 2013 and located in Itirapina-SP (22°12'16.13"S47°44'04.26"W). In this area pesticides were not applied. The conventional system orchard consisted of sweet orange ‘Valencia’ grafted on ‘Sunki’ mandarin *Citrus sunki* Hort. Ex Tan., planted in August, 2009 and located in Gavião Peixoto-SP (21°44’43.43”S 48°27’34.80”W), and this orchard were heavily sprayed with pesticides.

2.2. Sample units

Each sample unit was represented by sixteen completely expanded leaves and sixteen green fruits with diameters between 1.5 and 6.0 cm. Eight leaves and fruits were collected from the external part of plant canopy and eight from the interior part in the

North, South, West and East quadrants. The samples were collected randomly in ten plants in the area. The vegetal structures were put in paper bags and placed in Styrofoam boxes hermetically closed and taken immediately to the laboratory for evaluation. The fruits and leaves were analyzed in the laboratory using a stereoscopic microscope looking for predatory mites, which were mounted in Hoyer medium on a slide and placed in an oven at 45 °C (Krantz and Walter, 2009). Afterwards, the slides were sealed using colorless enamel.

The mites were identified using dichotomous keys not published and available at the Summer Acarology Program at Ohio State University, Columbus, United States (“The Ohio State University Summer Acarology Program,” Columbus, Ohio, United States of America). The species were confirmed by Dr. Gilberto José de Moraes from Escola Superior de Agricultura “Luiz de Queiróz”, Universidade de São Paulo (ESALQ/USP), Piracicaba-SP.

2.3. Mite colony

The rearing of *E. citrifolius* and *E. concordis* was started from mites collected at the campus of ESALQ/USP, in avocado plants and in *Jatropha curcas* nuts, respectively. *Iphiseiodes zuluagai* and *A. acalyphus* were collected on *Pachystachys lutea* Nees and ‘Tahiti’ acid lime plants, respectively, on the campus of Faculdade de Ciências Agrárias e Veterinárias of UNESP (UNESP/FCAV), in Jaboticabal-SP.

The predatory mites of all species were maintained in colonies using the methodology proposed by McMurtry and Scriven (1965), consisting of a tray containing a polyetilen foam for its base, on which a 11 x 7 x 0.2 cm (Paviflex®) resin board was placed. The species of the genus *Euseius* were kept in plastic trays on leaves of *Canavalia ensiformis* L. put over a foam saturated with filtered water to maintain humidity and to prevent the mites from escaping.

Euseius citrifolius and *E. concordis* were fed using castor bean pollen *Ricinus communis* L, while *A. acalyphus* and *I. zuluagai* were fed with a mixture of different stages of *Thyreophagus cracentiseta* Barbosa, O’Connor (Acari: Acaridae), castor bean pollen, and honey diluted to 10%. The rearing was maintained in controlled conditions (26 ± 1 °C, 60 ± 10% RH, in the dark).

2.4. Functional response

The functional responses of *E. citrifolius*, *E. concordis, A. acalyphus* and *I. zuluagai* on adults of *P. oleivora* and *T. brunneus* were evaluated in the laboratory for different prey densities. In an expanded leaf of sweet orange 'Pera' was demarcated a circle of 2 cm, and adult mites for both species were transferred, and this circle was surrounded by humid cotton to produce a barrier to prevent the mites from escaping. An adult female of each species was released in the arena, and predation was allowed for 24 hours for the densities of 5, 10, 20, 40, 80 and 120 adult mites of *P. oleivora* and *T. brunneus* per arena to the species *E. concordis*, *E. citrifolius* and *I. zuluagai* and the densities of 5, 10, 20, 40, 80, 120 and 160 of *P. oleivora* and *T. brunneus* per arena for *A. acalyphus.*

The functional responses were estimated in two steps: first, the shape of the curve of each functional response was determined through a logistic regression of proportion of mites consumed in function of original density of mites by predator using ProcCADMOD of SAS Software (SAS Institute, 2011). Initially a cubic model was tested due to the capacity of capture putative variation on the curve of functional response (Juliano, 1993). The signal of linear term of equation generated from proportion of predated mites was used to determine the type of functional response. The linear term when non-significant indicates a Type I functional response. When the term is significant and negative, it indicates a Type II functional response and when positive the functional response is type III.

The second step was to determine the parameters handling time (Th) and attack rate (a') of functional response. These parameters were estimated using a non-linear regression method using the method of minimum squares (Juliano, 1993).

3. Results

In both organic and conventional areas, all predatory mites collected were of the Phytoseiidae family. However, the organic area presented higher number of species than the conventional area, with three species collected: *I. zuluagai* (77.27%), *Proprioseiopsis neotropicus* (Ehara) (Acari: Phytoseiidae) (18.19%) and *E. concordis* (4.54%) (Table 1).

Tabela 1. Phytoseiidae predatory mite species collected in an organic citrus orchard in Itirapina-SP, on sweet orange *Citrus sinensis* 'Pera' grafted on 'Rangpur' lime *Citrus limonia*

Species	Collection date
Euseius concordis	04/12/2016
Iphiseiodes zuluagai	07/14/2016
Iphiseiodes zuluagai	07/14/2016
Iphiseiodes zuluagai	07/17/2016
Iphiseiodes zuluagai	07/14/2016
Iphiseides zuluagai	08/12/2016
Iphiseiodes zuluagai	08/12/2016
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Iphiseiodes zuluagai	07/27/2017
Proprioseiopsis neotropicus	07/27/2017
Proprioseiopsis neotropicus	07/27/2017

In the conventional area two species were collected: *I. zuluagai* (71.43%) and *E. concordis* (28.57%) (Table 2).

Table 2. Phytoseiidae predatory mite species collected in a convencional area of citrus production in Gavião Peixoto-SP, on sweet orange *Citrus sinensis* 'Valencia' grafted on 'Sunki' mandarin *Citrus sunki*.

Species	Collection date
Euseius concordis	04/12/2016
Euseius concordis	04/12/2016
Euseius concordis	05/14/2016
Euseius concordis	05/14/2016
Iphiseiodes zuluagai	04/12/2016
Iphiseiodes zuluagai	12/12/2016
Iphiseiodes zuluagai	04/12/2016
Iphiseiodes zuluagai	05/14/2016
Iphiseiodes zuluagai	08/12/2016
Iphiseiodes zuluagai	07/17/2017
Iphiseiodes zuluagai	07/17/2017
Iphiseiodes zuluagai	07/17/2017
Iphiseiodes zuluagai	07/17/2017
Iphiseiodes zuluagai	07/17/2017

Regarding functional response to *A. acalyphus*, *E. citrifolius* and *I. zuluagai*, the linear coefficient of logistic regression to *P. oleivora*, was positive and significant ($P < 0.05$), indicating a type II functional response, while *E. concordis* showed linear coefficient negative and significative, showing a type III functional response (Table 5). When preying on *T. brunneus*, *A. acalyphus* and *I. zuluagai* showed a type III functional response, while *E. concordis* and *E. citrifolius* a type II functional response (Table 6).

On *E. concordis* occurred an increase in consumption of *T. brunneus* and *P. oleivora* until a density of 80, being not possible to observe a stabilization on the curve with the evaluated densities (Figure 1 and 2). Curve stabilization was also not observed for *A. acalyphus* to both species until the density of 160 mites per arena^{-1} (Figures 1 and 2), and consumption stabilization was not observed. Based on the linear coefficient, functional response type II was observed for these predatory mites (Tables 5 and 6).

Iphiseiodes zuluagai consumed on average 80 adult mites of both preys in a 24-hour period, with posterior stabilization of consumption. *Euseius citrifolius* presented the lowest predation potential on both species, with an increasing consumption until the density of 20 mites, with posterior stabilization and a drop in consumption afterwards (Figures 1 and 2). *Amblyseius acalyphus* showed the highest consumption among predators, preying on average 155 and 149 adults of *P. oleivora* and *T. brunneus*, respectively, in a 24-hour period (Tables 3 and 4).

Table 3 – Average values (confidence interval 95%) of attack rate (a') and handling time (Th) of *Amblyseius acalyphus, Euseius citrifolius*, *Euseius concordis* and *Iphiseiodes zuluagai* preying on *Tegolophus brunneus* and estimated number of prey consumed in 24 h.

Species	a'	T_h	T/T_h
Amblyseius acalyphus	0.1572 a[1]	0.0067 d	149.25 a
	(0.1152 – 0.1992)	(0.0064 – 0.0069)	(144.9275– 149.2537)
Euseius citrifolius	0,0053 b	1.1473 a	20.92 d
	(0.00113 – 0.00947)	(0.9522 – 1.3424)	(17.8784 – 25.2048)
Euseius concordis	0.0237 b	0.3350 b	71.64 c
	(-0.0481 – 0.0954)	(0.2955 – 0.3744)	(64.1026 – 81.2182)
Iphiseiodes zuluagai	0.0059 b	0.2089 c	114.89 b
	(0.0046 – 0.0072)	(0.1997 – 0.2181)	(110.0413 – 120.1803)

[1] Averages followed by the same letter in the column did not differ when there is sobreposition on confidence interval.

Table 4 – Average values (confidence interval 95%) of attack rate (a') and handling time (Th) of *Amblyseius acalyphus, Euseius citrifolius*, *Euseius concordis* and *Iphiseiodes zuluagai* preying on *Plyllocoptruta oleivora* and number of estimated numbers of prey consumed in 24 h.

Species	a'	T_h	T/T_h
Amblyseius acalyphus	0.0923 a[1]	0.00644 c	155.28 a
	(0,0677-0.1170)	(0.00601-0.00686)	(145.7726-166.3893)
Euseius citrifolius	0.0058 bc	1.2749 a	18.82 c
	(0,00159 – 0.00997)	(1.0829 – 1.4669)	(16.3610 – 22.1627)
Euseius concordis	0.0013 bc	0.2409 b	99.62 b
	(0.000853 – 0.00185)	(0.1856 – 0.2962)	(81.0263 – 129.3103)
Iphiseiodes zuluagai	0.0066 b	0.2769 b	86,67 b
	(0.00308 – 0.0101)	(0.2514 – 0.3024)	(79.3651 – 95.4654)

[1] Averages followed by the same letter in the column did not differ when there is sobreposition on confidence interval.

Table 5. Estimated parameters of logistic regression of adult proportion of *Phyllocoptruta oleivora* preyed by *Amblyseius acalyphus, Euseius citrifolius, Euseius concordis* and *Iphiseiodes zuluagai*

Species	Parameters	Value ± SE	DF	χ^2	P
Amblyseius acalyphus	Intercept	0.8783 ± 0.3222	1	7.43	0.0064
	Linear	0.1392 ± 0.0196	1	50.65	< 0.0001
Euseius citrifolius	Intercept	0.1616 ± 0.2149	1	0.57	0.4522
	Linear	0.0142 ± 0.0109	1	1.69	0.1935
	Quadratic	-0.00042 ± 0.00011	1	14.77	0.0001
Euseius concordis	Intercept	3.0564 ± 0.3259	1	87.95	< 0.0001
	Linear	-0.1759 ± 0.0197	1	79.84	< 0.0001
	Quadratic	0.00302 ± 0.000323	1	87.26	< 0.0001
	Cubic	-0.00001 ± $1.533E^{-6}$	1	89.05	< 0.0001
Iphiseiodes zuluagai	Intercept	2.3320 ± 0.6432	1	13.14	0.0003
	Linear	0.1246 ± 0.0461	1	7.29	0.0069
	Quadratic	-0.0026 ± 0.0008	1	11.31	0.0008
	Cubic	0.00001 ± $3.695E^{-6}$	1	11.47	0.0007

[1] Averages followed by the same letter in the column did not differ when there is sobreposition on confidence interval.

Table 6. Estimated parameters of logistic regression of adult proportion of *Tegolophus brunneus* adults preyed by *Amblyseius acalyphus, Euseius citrifolius, Euseius concordis* and *Iphiseiodes zuluagai*

Species	Parameters	Values ± SE	DF	χ^2	P
Amblyseius acalyphus	Intercept	3.0738 ± 0.3443	1	79.69	< 0.0001
	Linear	0.0184 ± 0.00813	1	5.15	0.0232
	Quadratic	-0.00016 ± 0.000041	1	15.66	< 0.0001
Euseius citrifolius	Intercept	2.2782 ± 0.4896	1	21.65	< 0.0001
	Linear	-0.2026 ± 0.0542	1	13.97	0.0002
	Quadratic	0.0054 ± 0.0016	1	11.94	0.0006
	Cubic	-0.00004 ± 0.00001	1	12.46	0.0004
Euseius concordis	Intercept	3.1470 ± 0.4875	1	41.68	< 0.0001
	Linear	-0.0715 ± 0.0311	1	5.29	0.0214
	Quadratic	0.00175 ± 0.000527	1	11.00	0.0009
	Cubic	-0.00001 ± $2.542E^{-6}$	1	20.07	< 0.0001
Iphiseiodes zuluagai	Intercept	1.2128 ± 0.4384	1	7.65	0.0057
	Linear	0.1234 ± 0.0357	1	11.98	0.0005
	Quadratic	-0.0018 ± 0.0006	1	8.21	0.0042
	Cubic	$6.506E^{-6}$ ± $3.085E^{-6}$	1	4.46	0.0346

Values (± standard error), DF = degrees of freedom, χ^2 = Qui-square, P = P value

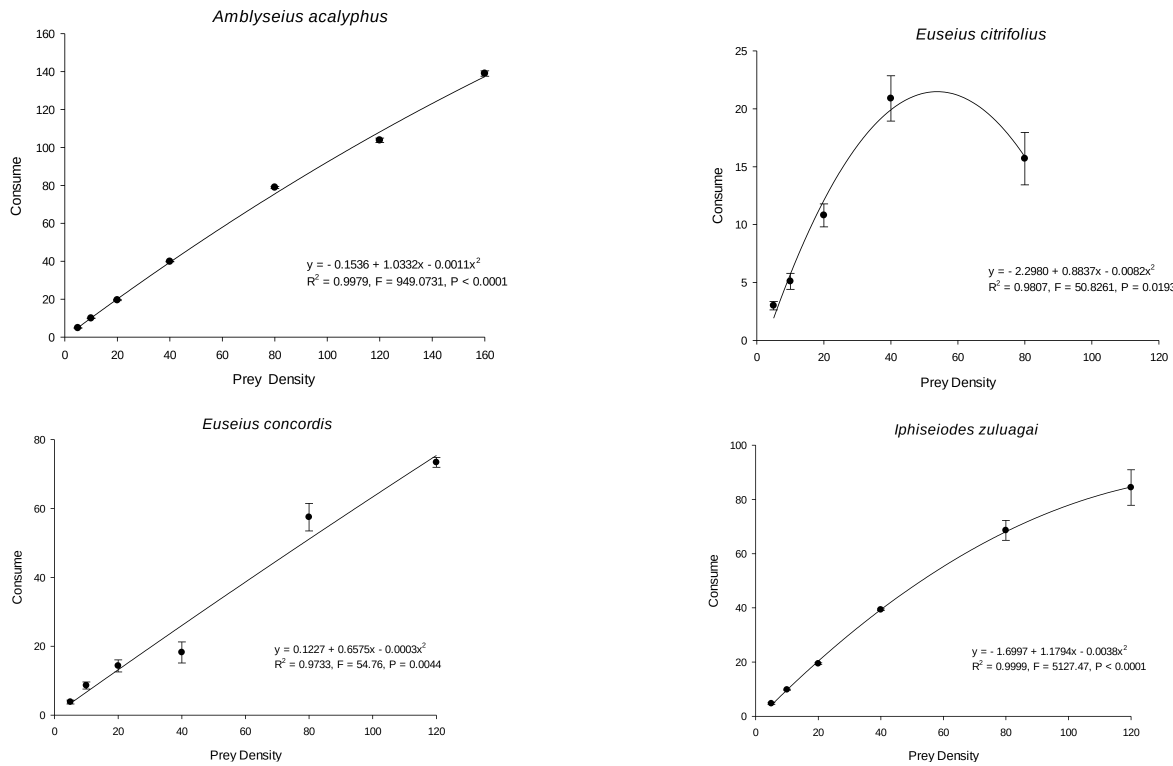

Figure 1. Average number of *Phyllocoptruta oleivora* adults consumed by *Amblyseius acalyphus*, *Euseius citrifolius*, *Euseius concordis* and *Iphiseiodes zuluagai* in different densities on 24h.

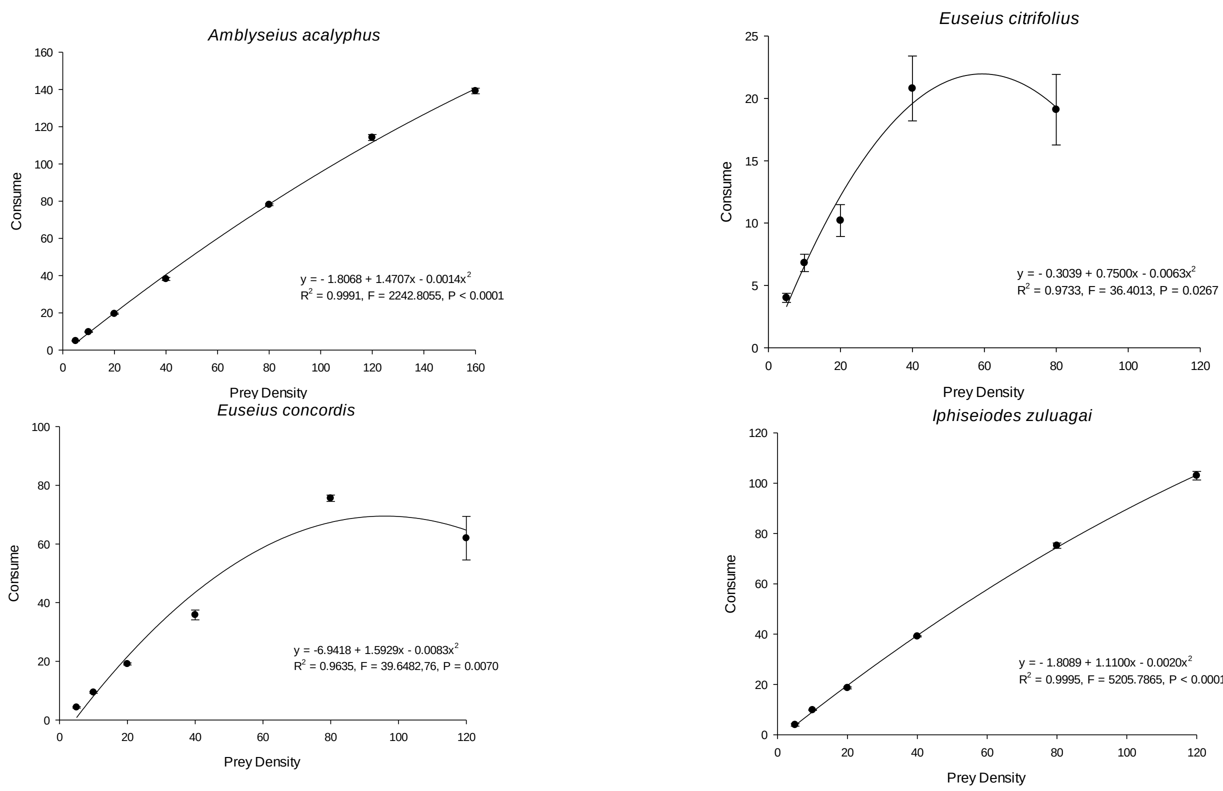

Figure 2. Average number of *Tegolophus brunneus* adults consumed by *Amblyseius acalyphus, Euseius citrifolius*, *Euseius concordis* and *Iphiseiodes zuluagai* at different densities on 24h.

The handling time did not differ among species when preying on *T. brunneus*. On the other hand, *A. acalyphus* showed the lowest handling time, followed by *I. zuluagai*, *E. concordis* and *E. citrifolius* (Tables 3 and 4).

5. Discussion

The results of this presented study reveal new information about the main predatory mite species present in a citrus organic and conventional orchard, as well as the results of a functional response of Phytoseiidae species preying on *T. brunneus* and *P. oleivora*.

Concerning the predatory mites found in citrus, similar results were obtained by Oliveira (2007) in surveys performed in citrus orchards in Aguaí-SP and Mogi-Guaçú-SP, where *I. zuluagai* was the predominant species, followed by *E. concordis* and *E. citrifolius*. Moreira (1993) also found that *I. zuluagai* was the predominant species in an orchard in Jaboticabal-SP, representing 88% of predatory mites collected.

On the other hand, Silva et al. (2012) observed a great predominance of *E. concordis* (98.3%) in an organic orchard in Descalvado-SP and Sato et al. (1994) in Presidente Prudente-SP observed that *E. concordis* and *E. citrifolius* were the predominant species, with lower occurrence of *I. zuluagai* (5%).

The main surveys showed that *I. zuluagai* and *E. concordis* are the predominant species in citrus, and that species usually alternated as the dominant species in different surveys. Many factors may help to explain the occurrence of these species in the orchards, such as the chemical management used, climatic conditions, presence of alternative food and intraguild predation (Liang and Huang, 1994; Venzon et al., 2001; Vis et al., 2006; Messelink et al., 2012).

The organic area showed higher diversity of predatory mite species, as besides *I. zuluagai* and *E. concordis*, *P. neotropicus* was also collected, though it was absent in the conventional area. Some species of *Proprioseiopsis* are predators of mites of the Tydeidae family, which were observed in high number in the organic area, although absent in the conventional orchards, possibly due to the high application of acaricides, as these mites are very susceptible to the pesticides (Knopp and Hoy, 1983; Castagnoli et al., 2005). Oliveira (2007) found *P. neotropicus* in citrus in Aguaí-SP and Mogi-Guaçú-SP and Bobot et al. (2011) in Amazonia State. The potential of this species to control Eriophyidae should be further explored, as *Proprioseiopsis badri* (Yousef and El-Borolossy) (Acari: Phytoseiidae) showed excellent development preying on the Eriophyidae *Cisaberopteros kenya* Keifer, *Aceria olivae* (Zaher and Abou-Awad), *Aculops lycopersici* (Masse) and *Aceria dioscoridis* (Soliman and Abou-Awad) (Momen et al., 2014). *Proprioseiopsis neotropicus* was also collected in fragment forests and in rubber, coffee and apple trees (Feres et al., 2005; Ferla and Moraes, 1998; Mineiro et al., 2008).

Silva et al. (2012), found a high predominance of the species *E. concordis* (98%) in orchards located in Descalvado-SP, negatively correlated with *P. oleivora*. This predator, although in high population levels in the area (approximately 1.5 mites per leaf), was not able to prevent the development of *P. oleivora* high populations, which was also the case for the use of other predatory mite species to control rust mites (Argov et al., 2002; Palevsky et al., 2003; Maoz et al., 2014).

Concerning the functional response, *A. acalyphus*, *E. citrifolius* and *I. zuluagai* presented functional response type III, while *E. concordis* showed a type II functional response preying on *P. oleivora.* Preying on *T. brunneus, A. acalyphus* and *I. zuluagai* showed a type III functional response, while *E. concordis* and *E. citrifolius* a type II. This is the response most commonly observed for Phytoseiidae predatory mites (Sabelis, 1986). Similar responses were observed for *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) preying on *Oligonychus punicae* (Hirst) (Acari: Tetranychidae) (Sandness and McMurtry, 1972), mobile stages of *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) (Morell et al., 2010) and eggs of *Raoiella indica* Hirst (Acari: Tenuipalpidae) (Carrillo and Peña, 2012). Predators with a type II functional response are effective to control preys at low population levels, being an important trait for a natural enemy, as the action of these mites at low pest population levels can prevent the pests of reaching the economic threshold level (Sabelis, 1986).

On the other hand, predators which present a type III functional response show lower predation rates at low prey population levels. The same result was obtained by Reis et al. (2003) for *I. zuluagai* preying on adults of *Brevipalpus* sp. The lower proportion of prey consumed at low population levels can be explained by the stimulation/interference model proposed by Sandness and McMurtry (1970), in which low prey densities are not capable of stimulating predatory mites to initiate searching behavior. Thus, it is important that natural enemies with different predatory behaviour coexist in the area, as the occurrence of predators which act at different populational levels of prey can cause a synergistic effect on pest control (Symondson et al., 2002; Snyder and Ives, 2003).

This is the first work evaluating predation of *I. zuluagai* in Eriophyidae mites in citrus. Vis et al. (2006) showed low oviposition of *I. zuluagai* preying on *Calacarus heveae* Feres (Acari: Eriophyidae) on rubber trees. Villanueva and Childers (2014) showed that a mite of the same genus *Iphiseiodes quadripilis* (Banks) (Acari: Phytoseiidae) did not complete development when fed with *P. oleivora*. Furthermore, these authors verified that *I. quadripilis* prefer to prey on *A. lycopersici* rather than *P. oleivora*. Thus, it is important to conduct predation tests with choice between *P. oleivora* and *T. brunneus* to verify if there is preference to prey on *P. oleivora* or *T. brunneus*, as *I. zuluagai* was the predatory mite most commonly found in the organic and conventional orchard and could play an important role in this mite’s populational dynamic.

Amblyseius acalyphus should be highlighted by the higher number of mites consumed, with around 140 adults of *P. oleivora* and *T. brunneus*, showing high attack rates and lower handling times. This mite was collected in high populational levels in an organic orchard in Jaguariúna (Albuquerque, 2006) and in low populational levels by Bressan and Ott (2017) in an organic orchard of tangor ‘Murcott’ in Rio Grande do Sul, but was absent in many surveys conducted in citrus orchards (Bobot et al., 2011; Silva et al., 2012; Horn et al., 2011; Ferreira et al., 2018).

It should be emphasized that *A. acalyphus* has been more commonly collected in areas of natural vegetation (Feres et al., 2005; Lofego and Moraes, 2006) or in agricultural areas adjacent to these areas with low pesticide application or organic orchards, as rubber trees (Demite and Feres, 2007; Demite and Feres, 2008) and coffee crops (Silva et al., 2010).

Euseius concordis preyed on average 80 adult mites of both prey species. In contrast, *E. citrifolius* showed the lowest predation among predators, preying on average 20 adult mites of both species, with higher handling times and lower attack rates. *Euseius concordis* is an important predator of Eriophyidae mites in other crops, as *Aculops lycopersici* (Massee) (Acari: Eriophyidae) in tomato plants (Lopes, 2015). However, this is the first work to show *E. concordis* potential to control *P. oleivora* and *T. brunneus*, which is very important, as in a survey conducted by Sato (1994) this species represented 98% of predatory mites collected in a citrus orchard in Presidente Prudente-SP.

The high consumption of evaluated species could be due to the reduced size of eriophyid mites compared to these predators, being necessary a high number of mites to satiate these organisms, which is advantageous to biological control (Van Rijn et al., 2005). Thus, it was observed that even after offering 160 adult mites of *P. oleivora* to *A. acayphus*, stabilization of the consumption curve in a plateau did not occur, which indicates predator satiation. The same was observed with *I. zuluagai* when offered *T. brunneus* in the density of 80 mites. It should be stressed that the mites used in our experiments were not starved previously, and the predation can be even higher than what was observed.

Despite present in the areas in high population levels, these mites have not been capable of maintaining these eriophyid populations below economic threshold levels. The evaluated mites are generalist predators which can choose to feed in food resources other than eriophyid mites (Furtado and Moraes, 1998; Vis et al., 2006). In this way, there is not a relationship of density-dependence between the prey and predator as occur for specialist predatory mites of type I, according to classification of McMurtry et al. (2013) for Phytoseiidae, which feed exclusively in mites of *Tetranychus* genus.

The generalist predators present the advantage of maintaining its population in the area preying on alternative food supply, as in the cases of predatory type IV, and can act in initial infestation of pests (Symondson et al., 2002; Harmon and Andow, 2004). Thus, the best strategy is to integrate natural enemies that act in different niches and show different predation behaviors (Losey and Deno, 1998; Sih et al., 1998). Additional studies are necessary to determine which predatory mites prefer to feed on Eriophyidae to act in association with generalist predatory mites. More studies should be conducted with *A. acalyphus*, as this mite was observed in the field at high populational levels, and the eriophyid population was low, despite the optimal conditions for eriophyid development. In addition, *P. neotropicus*, the second most encountered species in the organic orchard, also deserves further attention, due to excellent development of a species of the same genus, *P. carbonatus*, in four important eriophyid mite species (Momen et al., 2014).

The control of *T. australis* was demonstrated in Australia by Smith and Papacek (1991) by *Euseius victoriensis* (Acari: Phytoseiidae), with a provision of alternative food resources to the mite along the year by Rhode grass *Clorys guayana* Kunth, which provide pollen to these predators, allied with the use of selective pesticides (Smith et al., 1997). Based on this experience, mite management in Brazillian citrus orchards can be improved, following the study of biodiversity to determine effective natural enemies integrated with agricultural practices that favor the action and permanence of these organisms in agroecosystems.

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6. References

Albuquerque FA (2006) **Diversidade de ácaros em cultivo orgânico de citros e na vegetação natural circundante, e perspectivas para a criação massal de**

***Iphiseiodes zuluagai* (Acari: Phytoseiidae)**. 95f. Tese (Doutorado em Agronomia-Entomologia Agrícola)- Unesp de Jaboticabal.

Argov Y, Amitai S, Beattie GAC, Gerson U (2002) Rearing, release and establishment of imported predatory mites to control citrus rust mite in Israel. **BioControl** 47:399-409.

Bergh J, Rugg D, Jansson, RK, McCoy CW, Robertson JL (1999) Monitoring the susceptibility of citrus rust mite (Acari: Eriophyidae) populations to abamectin. **Journal of economic entomology** 92:781-787.

Bobot TE, Chilson EF, Navia D, Gasnier TRJ, Lofego AC, Oliveira BM (2011) Mites (Arachnida, Acari) on *Citrus sinensis* L. Osbeck orange trees in the state of Amazonas, Northern Brazil. **Acta Amazonica** 41:557-566.

Bressan LR, Ott, AP (2017) Distribuição intra-planta e flutuação populacional de *Tegolophus brunneus* (Acari: Eriophyidae: Phyllocoptinae) em tangerineira. **Caderno de Pesquisa**: 23-29.

Carrillo D, Peña JE (2012) Prey-stage preferences and functional and numerical responses of *Amblyseius largoensis* (Acari: Phytoseiidae) to *Raoiella indica* (Acari: Tenuipalpidae). **Experimental and Applied Acarology** 57:361-372.

Castagnoli M, Liguori M, Simoni S, Duso C (2005) Toxicity of some insecticides to *Tetranychus urticae*, *Neoseiulus californicus* and *Tydeus californicus*. **BioControl**: 50:611-622.

Childers CC, Achor DS. The eriophyoid mite complex on Florida citrus (Acari: Eriophyidae and Diptilomiopidae). In: PROCEEDINGS-FLORIDA STATE HORTICULTURAL SOCIETY. 1999. p. 79-87.

Demite PR, Feres RJ (2007) Ocorrência e flutuação populacional de ácaros associados a seringais vizinhos de fragmentos de Cerrado. **Neotropical Entomology** 36:117-127.

Demite PR, Feres RJF (2008) Influência de fragmentos de cerrado na distribuição de ácaros em seringal. **Neotropical Entomology** 37:196–204.

Ferla NJ, Moraes GD (1998) Ácaros predadores em pomares de maçã no Rio Grande do Sul. **Anais da Sociedade Entomológica do Brasil** 27:649-654.

Feres RJF, Lofego AC, Oliveira AR (2005) Ácaros plantícolas (Acari) da "Estação Ecológica do Noroeste Paulista", estado de São Paulo, Brasil. **Biota Neotropica** 5:43-56.

Ferreira CT, Krug C, Garcia MVB, Moraes GD (2018) Leprosis mite and other mite species (Acari) associated to orange groves in Brazilian Central Amazon. **Systematic and Applied Acarology** 23:449-462.

Flechtmann, CHW (1999). *Tegolophus brunneus* n. sp., a new citrus rust mite from Brazil (Acari: Eriophyidae). **International Journal of Acarology** 25:265-267.

Furtado IP, Moraes GD (1998) Biology of *Euseius citrifolius*, a candidate for the biological control of *Mononychellus tanajoa* (Acari: Phytoseiidae, Tetranychidae). **Systematic and Applied Acarology** 3:43-48.

Gerson, U (2003) Acarine pests of citrus: overview and non-chemical control. **Systematic and Applied Acarology** 8:3-12.

Harmon J, Andow DA (2004) Indirect effects between shared prey: predictions for biological control. **BioControl** 49:605-626.

Horn TB, Johann L, Ferla NJ (2011) Ecological interactions between phytophagous and predaceous mites in citrus agroecosystems in Taquari Valley, Rio Grande do Sul, Brazil. **Systematic and Applied Acarology** 16:133-144.

Juliano SA (1993) Nonlinear curve fitting: predation and functional response curves. In.: **Design and analysis of ecological experiments** (SCHEINER, S.M and GUREVITCH, J., Eds.). Chapman and Hall, New York, pp.159-182.

Knop N, Hoy M (1983) Biology of a tydeid mite, *Homeopronematus anconai* (n. comb.)(Acari: Tydeidae), important in San Joaquin Valley vineyards. **Hilgardia** 51:1-30.

Krantz GW, Walter DEA (2009) Collection, rearing and preparation for studying .In.: Krantz GW, Walter DEA (Eds). **A Manual of Acarology**. Lubbock: Texas Tech University Press, p. 83-96.

Liang W, Huang, M (1994) Influence of citrus orchard ground cover plants on arthropod communities in China: a review. **Agriculture, Ecosystems and Environment** 50:29-37.

Lofego AC, Moraes GJ (2006) Ácaros (Acari) associados a mirtáceas (Myrtaceae) em áreas de cerrado no estado de São Paulo com análise faunística das famílias Phytoseiidae e Tarsonemidae. **Neotropical Entomology** 35:731-746.

Lopes, PC. **Eficiência de populações de *Euseius concordis* (Acari: Phytoseiidae) e espécies afins no controle do microácaro-do-tomateiro, *Aculops lycopersici*** (Acari: Eriophyidae). 2015. Tese de Doutorado. Universidade de São Paulo.

Losey JE, Denno RF (1998) Positive predator–predator interactions: enhanced predation rates and synergistic suppression of aphid populations. **Ecology** 79:2143-2152.

Maoz Y, Gal S, Argov Y, Domeratzky S, Melamed E, Gan-Mor S, Coll M, Palevsky E. (2014) Efficacy of indigenous predatory mites (Acari: Phytoseiidae) against the citrus rust mite *Phyllocoptruta oleivora* (Acari: Eriophyidae): augmentation and conservation biological control in Israeli citrus orchards. **Experimental and Applied Acarology**, 63: 295-312.

Mccoy CW, Albrigo LG (1975) Feeding injury to the orange caused by the citrus rust mite, *Phyllocoptruta oleivora* (Prostigmata: Eriophyoidea). **Annals of the Entomological Society of America** 68:289-297.

McMurtry JA, Moraes GJD, Sourassou NF (2013) Revision of the lifestyles of Phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. **Systematic and Applied Acarology** 18:297-321

McMurtry JA, Scriven GT (1965) Insectary production of phytoseiid mites. **Journal of Economic Entomology** 58:282-284.

Messelink GJ, Sabelis MW, Janssen A (2012) Generalist predators, food web complexities and biological pest control in greenhouse crops. In: Soloneski S (Ed)

Integrated Pest Management and Pest Control-Current and Future Tactics. Rijeka: InTech, p.191-214.

Mineiro JLC, Sato ME, Raga A, Filho, MFS, Spongoski, S (2008) Incidência de ácaros em cafeeiro cv. Catuaí Amarelo. **Bragantia** 67:197-201.

Momen FM, Metwally AEM, Nasr AEK, Abdallah AA, Saleh KM (2014) Life history of *Proprioseiopsis badri* feeding on four eriophyid mite species (Acari: Phytoseiidae and Eriophyidae). **Phytoparasitica** 42:23-30.

Moreira PHR (1993) **Ocorrência, dinâmica populacional de ácaros predadores em citros e biologia de *Euseius citrifolius* (Acari: Phytoseiidae)**. Dissertação (Mestrado em Agronomia (Entomologia Agrícola)- Unesp, Jaboticabal.

Morell HR, Miranda I, Ramos M, Badii MH (2010) Functional and numerical responses of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) on *Polyphagotarsonemus latus* (Banks)(Acari:Tarsonemidae) in Cuba. **International Journal of Acarology** 36:371-376.

Oliveira WP (2007) **Flutuação e densidade populacional de ácaros (Acari) em três sistemas de produção de citros**. 143f. Tese (Doutorado em Ecologia Aplicada)- CENA- ESALQ/USP, Piracicaba.

Omoto C, Dennehy TJ, McCoy CW, Crane SE, Long JW (1994) Detection and characterization of the interpopulation variation of citrus rust mite (Acari: Eriophyidae) resistance to dicofol in Florida citrus. **Journal of Economic Entomology** 87:566-572.

Omoto C, Alves EA (2004) Resistência dos ácaros a acaricidas em citros. **Visão Agrícola** 2:82-86.

Palevsky E, Argov Y, David TB, Gerson U (2003) Identification and evaluation of potential predators of the citrus rust mite, *Phyllocoptruta oleivora*, in Israel. **Systematic and Applied Acarology** 8:39-48.

Reis PR, Sousa EO, Teodoro AV, Pedro Neto M (2003) Effect of prey density on the functional and numerical responses of two species of predaceous mites (Acari: Phytoseiidae). **Neotropical Entomology** 32:461-467.

Sabelis MW (1986) The functional response of predatory mites to the density of two-spotted spider mites. **Dynamics of Physiologically Structured Populations** 68:298-321.

Sandness JN, McMurtry JA (1970) Functional response of three species of Phytoseiidae (Acarina) to prey density. **Canadian Entomologist** 102:692-704.

Sandness JN, McMurtry JA (1972) Prey consumption behavior of *Amblyseius largoensis* in relation to hunger. **Canadian Entomologist** 104:461-470.

SAS Institute. 2011. Statistical analysis system: getting started with the SAS learning. Version 9.2. Cary: SAS Institute.

Sato, ME, Raga A, Cerávolo LC, Rossi AC (1994) Predation mites in citrus orchards at Presidente Prudente, State of São Paulo. **Anais da Sociedade Entomologica do Brasil**, 23:435-435.

Sih A, Englund G, Wooster D (1998). Emergent impacts of multiple predators on prey. **Trends in Ecology and Evolution** 13:350-355.

Silva EA, Reis PR, Zacarias MS, Marafeli PP (2010) Fitoseídeos (Acari: Phytoseiidae) associados a cafezais e fragmentos florestais vizinhos. **Ciencia Agrotécnica** 34:1146-1153.

Silva MZ, Sato ME, Oliveira CAL (2012) Diversidade e dinâmica populacional de ácaros em pomar cítrico. **Bragantia** 71:210-218.

Smith D, Beattie, GAC, Broadley R (1997). **Citrus pests and their natural enemies: integrated pest management in Australia**. Queesland: Dept. of Primary Industries, 272p.

Smith D, Papacek DF (1991) Studies of the predatory mite *Amblyseius victoriensis* (Acarina: Phytoseiidae) in citrus orchards in south-east Queensland: Control of *Tegolophus australis* and *Phyllocoptruta oleivora* (Acarina: Eriophyidae), effect of pesticides, alternative host plants and augmentative release. **Experimental and Applied Acarology** 12:195-217.

Snyder WE, Ives AR (2003) Interactions between specialist and generalist natural enemies: parasitoids, predators, and pea aphid biocontrol. **Ecology** 84:91-107.

Symondson WOC, Sunderland KD, Greenstone MH (2002) Can generalist predators be effective biocontrol agents? **Annual Review of Entomology** 47:561-594.

Van Rijn PCV, Bakker FM, Hoeven WAVD, Sabelis MW (2005) Is arthropod predation exclusively satiation-driven? **Oikos** 109:101-116.

Vacante V (2010) **Citrus mites: identification, bionomy and control**. Walingford: CABI, 371p.

Venzon M, Janssen A, Sabelis MW (2001) Prey preference, intraguild predation and population dynamics of an arthropod food web on plants. **Experimental and Applied Acarology** 25:785-808.

Villanueva RT, Childers CC (2014) Development of *Iphiseiodes quadripilis* (Banks)(Acari: Phytoseiidae) on pollen or mite diets and predation on *Aculops pelekassi* (Keifer)(Acari: Eriophyidae) in the laboratory. **Environmental Entomology** 36:9-14.

Vis RMJ, Moraes GJ, Bellini MR (2006) Initial screening of little known predatory mites in Brazil as potential pest control agents. **Experimental and Applied Acarology** 39:115-125.

Chapter 5 - FINAL CONSIDERATIONS

The scenario of citrus production in Brazil has changed considerably after the report of citrus plants infected with Huanglongbing (HLB) in 2014 in São Paulo State. The focus and justified effort to fight that disease caused an increase of the use of pesticides in citrus production, mainly to control the citrus psyllidae *Diaphorina citri*.

In contrast, the management of other key pests of citrus, among them the citrus rust mite *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae), received less attention during the last decade and, at the same time, citrus rust mites damages intensified and the failures to control these mites increased, concerning growers and technicians. As citrus rust mites are little studied in Brazil, it was difficult to point to the main causes for damage increases by these mites.

Thus, many people involved in citrus production alleged the citrus brown mite *Tegolophus brunneus* Flechtmann (Acari: Erophyidae) was responsible for symptom intensification and increased management difficulty. This mite was described in citrus in orchards in São Paulo State just ten years before citrus rust mite infestation increased and a relationship of cause-consequence was established, although without scientific proof.

Thus, we decided to investigate the biological development, distribution on the main citrus production region of Brazil, host specificity, toxicity to the main acaricides used and the natural enemies associated to *T. brunneus*, also including *P. oleivora* in these experiments.

We found that the biological development of *T. brunneus* was similar to *P. oleivora* in citrus fruits and showed the predatory potential of the Phytoseiidae mites *Iphiseiodes zuluagai* Denmark and Muma, *Euseius concordis* (Chant), *Euseius citrifolius* Denmark and Muma and *Amblyseius acalyphus* (Acari: Phytoseiidae), with high predation on both species.

The main result of this work was to show that *T. brunneus* infests only ‘Tahiti’ acid lime in the main Brazillian citrus production region, causing high damages to the species, while *P. oleivora* infest all citrus species and varieties. Furthermore, we showed that *T. brunneus* is 13 times more tolerant to sulfur than *P. oleivora*, while the toxicity of abamectin is similar to both species.

The work rejected the presumption that damage intensification by citrus rust mites in sweet oranges is due to *T. brunneus* infestation, as well as in mandarins, lemons, citron and other citrus species, but 'Tahiti' acid lime. These results open many research opportunities on eriophyidae management in 'Tahiti' acid lime, as according to the surveys and damages observed in the field, *T. brunneus* is a key pest of this crop.

Thus, many studies are necessary in 'Tahiti' acid lime involving *T. brunneus*, as field surveys in the main producing areas are needed to determine the predominance of this species compared to *P. oleivora*, the population dynamics of *T. brunneus* and the determination of economic threshold level in that crop. Some peculiarities of management in 'Tahiti' acid lime could lead to important challenges in the control of *T. brunneus*, such as the intense flushing pattern of that citrus species throught the year, allowing the development of high populations of that species. Also, this flushing requires a high pressure by abamectin and neonicotinoids pesticides for preventing infestation of mining and sucking pests, which can select mite populations resistant to these compounds. Also, copper is constantly applied in that crop to manage citrus canker, which can eliminate the entomopathogenic fungi that are key mortality factors for eriophyid mites in the field. Future research should take into consideration the production characteristics of this crop, which are different from other citrus species and bring the information needed to control this mite wisely.