CÉLIA SIQUEIRA FERRAZ

MITE DIVERSITY, NEW SPECIES DESCRIPTION AND EVALUATION OF PHYTOSEIID MITES AS BIOLOGICAL CONTROL AGENTS OF *Aceria litchii* (KEIFER) (ACARI: ERIOPHYIDAE) ON LITCHI PLANTS

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André Lage Perez

Felipe Colares Batista

José Henrique Schoereder
(Coorientador)

Manoel Guedes Correa Gondim Junior

Angelo Pallini Filho
(Orientador)

Dedico

A minha família, em especial aos meus pais, pelo amor incondicional.

Ao meu esposo Lucimar por compartilhar a vida comigo com amor e carinho.

"Olhar para trás após uma longa caminhada pode fazer perder a noção da distância que percorremos, mas se nos detivermos em nossa imagem, quando a iniciamos e ao término, certamente nos lembraremos o quanto nos custou chegar até o ponto final, e hoje temos a impressão de que tudo começou ontem. Não somos os mesmos, mas sabemos mais uns dos outros. E é por esse motivo que dizer adeus se torna complicado! Digamos então que nada se perderá. Pelo menos dentro da gente..."

João Guimarães Rosa

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BIOGRAFIA

CÉLIA SIQUEIRA FERRAZ, filha de Severino Ferraz de Valões e Maria da Soledade Siqueira de Valões, nasceu em São José do Belmonte-PE, em 04 de março de 1988. Cursou o nível fundamental no Colégio Municipal Dr. Arcôncio Pereira e o nível médio na Escola Professor Manoel de Queiroz, na mesma cidade, concluindo em 2005. Em agosto de 2006, ingressou no Curso de Agronomia da Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, onde foi bolsista de iniciação científica durante quase a totalidade do curso, sob a orientação dos Professores Cláudia Helena Cysneiros Matos de Oliveira e Carlos Romero Ferreira de Oliveira. Graduou-se Engenheira Agrônoma em março de 2011. No mesmo mês e ano iniciou o Curso de Mestrado em Produção Vegetal na Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, em Serra Talhada - PE, sob a orientação da Professora Cláudia Helena Cysneiros Matos de Oliveira, concluindo em fevereiro de 2013. Em agosto de 2013 iniciou o Doutorado no Programa de Pós-Graduação em Entomologia na Universidade Federal de Viçosa, em Viçosa – MG, sob a orientação do Professor Angelo Pallini, submetendo-se à defesa de tese em 28 de novembro de 2017.

ABSTRACT

FERRAZ, Célia Siqueira, D.Sc., Universidade Federal de Viçosa, November, 2017. **Mite diversity, new species description and evaluation of phytoseiid mites as biological control agents of Aceria litchii (Keifer) (Acari: Eriophyidae) on litchi plants**. Adviser: Angelo Pallini Filho. Co-advisers: Arnoldus Rudolf Maria Janssen, José Henrique Schoereder and Madelaine Venzon.

Aceria litchii (Keifer) (Acari: Eriophyidae) is considered one of the main pests of the litchi crop through the world. The control of this pest mite in the main production areas is done by pruning of the infested branches and application of pesticides. However, there is no pesticide registered in Brazil for control of this mite in the litchi crop and pruning is considered expensive by many growers. In addition, pesticides are often inefficient against most eriophyid mites since they frequently live under protective structures of the plant. This all reinforces the need of research for alternative control practices that are sustainable in the long term, such as biological control, which consists of the use of natural enemies to reduce the population density of pest species. Given this, initially, surveys were carried out in litchi orchards seeking natural enemy species with potential to control A. litchii. Later, two new species of the Iolinidae family, which were found during these survey, were described and illustrated. Then, two species from the Phytoseiidae family collected during the field survey were selected, Phytoseius intermedius Evans & MacFarlane and Amblyseius herbicolus (Chant), and the ability of these predators to control A. litchii in litchi plants was investigated under field conditions. Moreover, it was investigated whether the supplementation of the A. herbicolus population with alternative food (cattail pollen) could improve the control of A. litchii. Most of the mite species found belong to families that were predominantly predators. Phytoseiidae was the most diverse family of mites, with a total of 11 species. The two new species described and illustrated in the Iolinidae family were Pausia litchiae **n. sp.** and Pseudopronematulus nadirae **n. sp.**. Moreover, it was shown that the incidence of erinea on plants increased throughout the experiment on all treatments. In the same way, the pollen addition did not provide greater population growth for A. herbicolus on the litchi plants. In conclusion, these results showed the arthropod diversity found associated with the erinea induced by A. litchii, highlighting the large number of predatory mites. Furthermore, the description of the new species of mites in the Iolinidae family was also shown. In addition, it was observed that P. intermedius and A. herbicolus presented limitations to control the increase of the incidence of erinea induced by A. litchii.

RESUMO

FERRAZ, Célia Siqueira, D.Sc., Universidade Federal de Viçosa, novembro de 2017. **Diversidade de ácaros, descrição de novas espécies e avaliação de ácaros fitoseídeos como agentes de controle biológico de Aceria litchii (Keifer) (Acari: Eriophyidae) em plantas de lichia**. Orientador: Angelo Pallini Filho. Coorientadores: Arnoldus Rudolf Maria Janssen, José Henrique Schoereder e Madelaine Venzon.

Aceria litchii (Keifer) (Acari: Eriophyidae) é uma das principais pragas que ocorrem na cultura da lichia no mundo. O controle desse ácaro nos principais países produtores dessa fruta é realizado através da aplicação de acaricidas e poda. No entanto, não há nenhum acaricida registrado no Brasil para o controle de A. litchii e a poda é considerada por muitos produtores um manejo dispendioso. Além disso, o controle químico é frequentemente ineficaz contra muitos ácaros eriofídeos, pelo fato de os mesmos viverem sob estruturas da planta que os protegem. Isso tudo reforça a pesquisa de práticas alternativas de controle e que sejam sustentáveis a longo prazo, como por exemplo, o controle biológico, que consiste no uso de inimigos naturais para reduzir a densidade populacional de espécies praga. Diante disso, inicialmente, foi realizado um levantamento em pomares de lichia buscando-se espécies de inimigos naturais com potencial para controle de A. litchii. Posteriormente, duas novas espécies da família Iolinidae, encontradas durante o levantamento de campo, foram descritas e ilustradas. Em seguida, foram selecionadas duas espécies de ácaros da família Phytoseiidae encontradas durante o levantamento de campo, Phytoseius intermedius Evans & MacFarlane e Amblyseius herbicolus (Chant), para investigar sua capacidade em controlar A. litchii sob condições de campo. Além disso, foi investigado se a suplementação da população de A. herbicolus com alimento alternativo (pólen de taboa) poderia aumentá-la e melhorar o controle de A. litchii. Foi observado que a maioria das espécies coletadas no levantamento de campo pertenceram a famílias de ácaros constituídas predominantemente de predadores. A família Phytoseiidae foi a mais diversa, com um total de 11 espécies. As duas novas espécies descritas da família Iolinidae foram Pausia litchiae **n. sp.** e Pseudopronematulus nadirae **n. sp.**. Adicionalmente, foi demonstrado que a incidência de erinea nas plantas de lichia aumentou durante todo o experimento em todos os tratamentos. Como também, a adição de pólen não proporcionou maior crescimento para a população de A. herbicolus em plantas de lichia. Em conclusão, esses resultados mostraram a grande diversidade da comunidade de artrópodes encontrada associada às erinea induzidas por A. litchii, com destaque para o grande número de espécies de ácaros predadores, incluindo a descrição

das novas espécies de ácaros da família Iolinidae. Além disso, observamos que os ácaros predadores P. intermedius e A. herbicolus apresentaram limitações para controlar o aumento da incidência de erinea induzidas por A. litchii.

SUMÁRIO

GENERAL INTRODUCTION

The litchi (Litchi chinensis Sonnerat) is a tropical and subtropical fruit tree of the Sapindaceae family, native to Southeastern China and Northeast Vietnam, where it is considered the national fruit (Menzel 2002). It has been widely cultivated for many years in Asian countries, especially China, but it is relatively new elsewhere. The litchi plants were introduced to Africa and the Americas only in the 19th century (Carr and Menzel 2013). In Brazil, litchi trees began to be commercially produced in the western part of the state of São Paulo in the 1970s and since then have been spreading through the other states due to its profitability (Yamanishi et al. 2000). The statistics about the planted area and production of the crop are out of date, but it is estimated that São Paulo is the largest producer in the country. In the harvest of 2009/2010, this state was responsible for 77% of national yield followed by Minas Gerais with 12%. São Paulo has 44.93% of the litchi trees, while Minas Gerais has 43.07%, showing that the production in the state of Minas Gerais also presents potential to increase (Yoneya 2010; Bacci 2011).

The litchi production can be affected by several factors, and among them the infestation by pests, which attack the plants and fruits at different stages of growth. Some Lepidoptera larvae have been reported causing damage to commercial crops (Srivastava et al. 2016). However, the litchi erinose mite, Aceria litchii (Keifer) (Acari: Eriophyidae), is one of the main pests of the crop worldwide (Waite and Gerson 1994; Sabelis and Bruin 1996; Mitra 2002; De Azevedo et al. 2014; Srivastava et al. 2016). In Brazil, despite of the interest of growers in the litchi crop, the knowledge about control methods of A. litchii is still scarce. Aceria litchii induces a gall called erienum (pl. erinea) through its feeding, which in addition of the leaves, it could occur in the newly formed leaf bud, inflorescences, and fruits (Westphal and Manson 1996; Waite and Hwang 2002).

The erineum consists of an abnormal growth of trichomes (hairs) on the surface of the plant epidermis, which makes leaves distorted or curled. Its structure has several diagnostic aspects, including position on the leaves, density of trichomes, and colour. Trichomes may be elongated, clavate, capitate, unicellular, or multicellular, depending on the mite species (Keifer et al. 1982). In litchi, the erineum patch occurs on the abaxial surface of leaves, which appear blister-like on the upper surface, and sometimes may cover almost the entire leaf surface. In the early stages of development, the trichomes are whitish and later become reddish brown, looking like a patch of felt (Nishida and Holdaway 1955; Jeppson et al. 1975; Keifer et al. 1982; Westphal and Manson 1996; Waite and Hwang 2002).

There is no information about the level of infestation on litchi plants to implement control measures for A. litchii on commercial crops. In general, what some growers do is a preventive control to avoid the establishment of this pest in their orchard. The pruning of infested branches, often followed by the chemical applications, is the most adopted control method in the main producing areas of litchi (Srivastava et al. 2016). Nevertheless, there are no pesticides registered for this mite in Brazil (Brasil 2016) and many producers consider the pruning an expensive management tool. Therefore, alternative strategies of pest regulation should be studied with the intention of achieving a more sustainable production.

Biological control consists of the use of natural enemies to reduce the population density of pest species and is one of the best alternatives to chemical control when it comes to pest management (DeBach 1964; Bale et al. 2008). This management tactic has some advantages compared to the pesticide use because in many cases the natural enemies are naturally occurring organisms, in addition they usually are specific in the range of prey that will attack and can actively seek out pests. Moreover, pests are unlikely to develop resistance against the natural enemies, which make the biological control more sustainable in the long term. Biological control is also less aggressive to the environment and to the health of the growers and food consumers, when compared to the control based on chemical pesticides application (Bale et al. 2008).

However, biological control depends on extensive preliminary studies to understand what is going on within the system. Given the recent emergence of A. litchii in the country, a process of colonization by the natural enemy species associated to this mite might be still in progress. Thus, the search for these organisms associated with the pest in the field is still necessary. Some surveys have already been carried out on litchi orchards, which reported mites of the Phytoseiidae family associated to A. litchii (Waite and Gerson 1994; Picoli et al. 2010; De Azevedo et al. 2014). But, in Brazil, these studies are restricted to the state of São Paulo. The Phytoseiidae family represents the most significant predators of eriophyoid mites, which have been extensively used in many biological control programs (Sabelis 1996; Gerson et al. 2003; Sabelis et al. 2008). Moreover, laboratory studies have shown that A. litchii is a suitable prey for some phytoseeid species (Azevedo et al. 2016; personal observation). Nevertheless, the effect these predatory mites on the population of A. litchii has not been well investigated.

Despite being known to be mainly predators of small arthropods, many phytoseiid mites presents a great variety of feeding habits, consuming food items such as fungi, plant exudates, pollen, and even being able to extract liquid from leaf cells (McMurtry et al. 2013). The omnivorous feeding habit of these predators gives them advantage for the biological control because they can persist on plants longer than those that are strictly carnivorous, by feeding on alternative foods when prey is absent or scarce (Symondson et al. 2002; Nomikou et al. 2002; Duarte et al. 2015; Janssen and Sabelis 2015). Thus, pollen has an important role in maintaining rearings in lab as well as for persistence and population growth of predators in the field, which could help prevent pest outbreaks (Nomikou et al. 2002; Duarte et al. 2015). The availability of an extra food resource may increase predator densities and subsequently cause a reduction of the pest populations, improving biological control by these predators (van Rijn and Tanigoshi 1999; Nomikou et al. 2002; van Rijn et al. 2002; Gnanvossou et al. 2005; Nomikou et al., 2010; Duarte et al. 2015; Janssen et al. 2015).

The key problem in effectively controlling litchi erinose mite is that, just like other eriophyid mites, its small size enables them to hide inside refuges, where they find protection from biotic and abiotic stresses (Sabelis and Bruin, 1996). Although the erineum may serve as protection for A. litchii against their natural enemies, these mites can be more easily preyed when they are exposed, moving from an older erineum to infest new leaves (De Azevedo et al. 2014). Population outbreaks of A. litchii are favoured when there is the emergence of new shoots, which occurs especially in the runup to the flowering (Nishida and Holdaway 1955; Alam and Wadud 1963). Thus, predators with a generalist diet, including pollen, should be better adapted to persist and increase on plants, being in high density at the time of migration of A. litchii, these predators could control it more easily, since it is harder to get access to it inside the erineum.

Despite the potential to expand the production area of litchi in Brazil and the reports of orchards infested by A. litchii, few studies for the management of this mite have been carried out so far, even in the main producing regions. The infestation levels at which the litchi plants have been reported are considered sufficiently high to cause losses (Azevedo et al. 2014).

Thus, in this thesis, it was investigated the occurrence of natural enemies associated with A. litchii and strategies to use predatory mites of the Phytoseiidae family for control of this pest in litchi plants. In Chapter 1, a survey was carried out in commercial and non-commercial litchi orchards infested by A. litchii, searching for species of natural enemies with potential for controlling it. In Chapter 2, it was presented the description and illustration of two new species of the Iolinidae family associated with A. litchii, which deserve future studies to assess their potential as predators of A. litchii.

And in Chapter 3, the capacity of the predatory mites Phytoseius intermedius Evans & MacFarlane and Amblyseius herbicolus (Chant) (Acari: Phytoseiidae) to control of A. litchii on litchi plants under field conditions was investigated. Additionally, it was also evaluated if by supplementing the population of A. herbicolus with pollen the population of this predator would increase and achieve a better control of A. litchii.

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CHAPTER I

Arthropods inhabiting galls induced by Aceria litchii on litchi trees

Abstract

This study aimed to describe the arthropods associated with galls of Aceria litchii (Keifer) (Acari: Eriophyidae) and identifying natural enemies associated with this mite at the field. Surveys were carried out in commercial and non-commercial litchi orchards. Arthropods found in association with A. litchii were collected and identified. A total of 985 and 1,872 specimens of mites were identified in commercial and non-commercial areas, respectively. The collected mites belonged to the families Cheyletidae, Cunaxidae, Eupodidae, Iolinidae, Stigmaeidae, Phytoseiidae, Tarsonemidae, Tenuipalpidae, Tetranychidae, Tryophtydeidae, Tuckerellidae, Tydeidae, Winterschmidtiidae and Xenocaligonellidae and the suborder Oribatida. In addition, 249 and 173 specimens of hexapods belonging to orders Collembola, Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Psocoptera and Thysanoptera were collected in commercial and non-commercial areas, respectively. We observed a great diversity and abundance of mites and hexapods in the galls induced by A. litchii, many of these mites are predators, which could have potential to control it. Among the predators, Phytoseiidae was the most diverse family with a total of 11 species. Phytoseius intermedius Evans & MacFarlane was the most abundant with 34.3% and 49.7% of the phytoseiids collected in the commercial and non-commercial areas, respectively. Thus, studies to test the potential of these species and the adoption of management practices that enhance this ecological service must be carried out to promote satisfactory control of the litchi erinose mite.

Key words: diversity, Litchi chinensis, litchi erinose mite, predators, taxonomy.

Introduction

Litchi (Litchi chinensis Sonnerat (Sapindaceae)) is a tropical and subtropical fruit tree native to Southeastern China and Northeast of Vietnam (Menzel 2002). Commercial production of litchi only started in Brazil at the western part of the state of São Paulo in the 1970s. Due to the high profitability of this crop, it expanded to other parts of the country (Yamanishi et al. 2000). The state of São Paulo is the largest producer with about 77% of the national yield followed by Minas Gerais with 12% in the 2009/2010 harvest. São Paulo has 44.93% of the litchi trees, while Minas Gerais has 43.07%, showing that the production in the state of Minas Gerais has potential to increase (Yoneya 2010; Bacci 2011).

Many factors affect the litchi production and among them the infestation by pests, such as the litchi erinose mite Aceria litchii (Keifer) (Eriophyidae), considered the main pest of the crop in several countries (Sabelis and Bruin 1996; Mitra 2002; De Azevedo et al. 2014). This mite was first recorded in Brazil in 2008 at the state of São Paulo (Raga et al. 2010) and since then it has spread through all the litchi producing areas.

Aceria litchii induces the formation of a gall called erineum (pl. erinea) as the result of its feeding. The erineum consist of an abnormal growth of trichomes on the under surface of the plant epidermis. The affected leaves may become distorted or curled. In the early stages of development, the trichomes are whitish and later become reddish brown, looking like a patch of felt. Aceria litchii starts its infestation in young tissues of the plant and it can remain in the same plant year-round, migrating from older galls to new shoots. It also infests inflorescences and fruits, which can directly compromise the yield and/or making the fruit unfit for consumption or depreciating its commercial value (Jeppson et al. 1975; Keifer et al. 1982; Westphal and Manson 1996; Waite and Hwang 2002).

Among the measures to control of A. litchii, the biological control appears as an alternative available for managing this mite due to its sustainability. The advantages of its use are expressed mainly by comparisons with chemical control, which is one of the main forms indicated for control this mite in other countries (Waite and Hwang 2002). Such benefits refer to the fact that, in many cases, natural enemies are naturally occurring with the prey. In addition, they actively seek out their prey and is unlikely to the prey develop resistance to a biological control agent, thus, in several cases, the control can be maintained over long-term. Furthermore, this method leaves no toxic waste to the environment and are harmless to the growers (Bale et al. 2008). In addition, there is no pesticide registered for this mite in Brazil (Brasil 2016).

However, the biological control depends on extensive preliminary studies to understand what is going on within the system. The first step is the identification of natural enemies associated with the pest in the field. In Brazil, some surveys have already been carried out on litchi orchards, but are restricted to the state of São Paulo (De Azevedo et al. 2014). As the emergence of A. litchii is relatively recent in the country, a process of colonization by the natural enemy species associated to this mite might be still in progress. Therefore, the aim of this study was to search for species of natural enemies with potential for controlling A. litchii, through diagnostic surveys, in order to update the arthropod fauna in litchi orchards in Brazil.

Materials and Methods

The survey was carried out in two areas with different management practices. The first was a commercial plantation (area with 100 plants), var. Brewster, located in Buieié Site (20º 42' 22.3" S; 42º 50' 55.5" W; 703.8 m altitude), which was maintained with spontaneous vegetation between rows and received all cultural practices, except the application of pesticides during the survey. The second was a non-commercial area located at the Campus of the Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil (20º 45' 32.2" S; 42º 52' 05.9" W; 624.5 m altitude), whose plants were kept without any cultural practices and it was pesticide-free. The litchi trees were on average 8.5 years old and had 7.0 meters high on both sites. The climate classification of the region is type "Cwa", according to Köppen and Geiger, characterized by altitude tropical climate with rainy summer and dry winter.

To evaluate the presence of arthropods associated with A. litchii on litchi, five and four plants were sampled monthly from commercial and non-commercial area, respectively, between April and November 2015. Four branches with 0.30 meters from each plant were sampled, each branch was taken from each one of the cardinal directions (North, South, East, West). They were located between 1.5 and 3.0 meters above ground. Two leaves were collected from each third of the branch (apical, middle, and basal). These samples were put in a tagged paper bag, in a cooler with ice packs and transported to the laboratory.

In the laboratory, the samples stored in individual paper bags were put in polyethylene bags and kept in a refrigerator at 10 ± 3° C. Within four days, the samples were evaluated under a stereomicroscope (Zeiss® Stemi 2000-c). One leaflet with erineum from each leaf collected was chosen randomly and analysed to verify the presence of arthropods. Mites and hexapods found were collected with a thin hair brush (Condor® #000) and transferred to a plastic vial (2.0 mL) with alcohol 70% for later identification. The mites were then mounted on microscope slides using Hoyer's medium and examined under a phase-contrast microscope (Olympus BX41) for identification, which was carried out in the Acarology Laboratory of the Federal Rural University of Pernambuco by Professor Dr. Manoel Guedes C. Gondim Junior. The specimens were deposited at the Laboratory of Acarology (Department of Entomology, Federal University of Viçosa).

The presence of A. litchii in the erinea was evaluated after the collection of other arthropods. The leaflets were placed again in paper bags and left for a period of four to five hours, at 25° C; this allowed the mites to exit the erinea and we then assess their presence/absence under stereomicroscope. Some specimens of A. litchii were collected and mounted on slides.

Results

The Subclass Acari (mites) was the most abundant group found in the two collection areas 985 and 1,872 specimens in the commercial and non-commercial areas, respectively) (Table 1). In addition, 163 and 142 specimens of the Class Insecta; 86 and 31 specimes of the Class Entognatha (springtails) were collected in the commercial and non-commercial areas, respectively (Table 2). It was not possible to identify 31 specimens because of their bad state of conservation. Of all mite species, 57.9% were collected on the two sampling sites (Table 1), while 87.5% of the hexapods (Classes Insecta and Entognata) also occurred on both locations (Table 2).

Most of the mite species belong to families composed predominantly of predators (Cheyletidae, Cunaxidae, Iolinidae, Phytoseiidae and Stigmaeidae) while the remaining mite species belong to families of phytophagous mite (Tenuipalpidae, Tetranychidae, Tuckerellidae and Xenocaligonellidae) and families of diverse or inaccurate feeding habits (Eupodidae, Tarsonemidae, Tryophtydeidae, Tydeidae and Winterschmidtiidae) and the Suborder Oribatida. These organisms may be detritivorous, algivorous, fungivorous, bacteriophagous and pollenophagous (Walter and O'Dowd 1995; Krantz and Walter 2009). The new records from this survey include two new species from Iolinidae family, which were described in another manuscript (Chapter 2).

Phytoseiidae was the most diverse family of mites, with a total of 11 species (Table 1). Among these species, Phytoseius intermedius Evans & MacFarlane, Phytoseius woodburyi De Leon, Iphiseiodes zuluagai Denmark & Muma and Amblyseius herbicolus (Chant) were the most abundant on both areas. Phytoseius intermedius represented 34.3%

and 49.7% of the total of phytoseiids collected in the commercial and non-commercial areas, respectively.

The phytophagous species collected were not so abundant and diverse as the other groups and represented 3.7 and 1.3% of the mites collected in the commercial and non-commercial areas, respectively. The specimens with varied feeding habits, showed a high representation in the samples, with 40.2% and 16.2% of the mites collected in the commercial and non-commercial areas, respectively. The most abundant from this group in the commercial area were Tarsonemus sp. 1 (Tarsonemidae) (14.0%), Suborder Oribatida (9.0%) and Czenspinskia sp. (Winterschmidtiidae) (8.6%). While at the non-commercial area were Brachytydeus sp. (Tydeidae) (4.7%), Czenspinskia sp. (3.6%) and Eupodidae sp. (3.1%) with their respective percentages of the total number of mites collected.

Regarding the hexapods present in our study, in the commercial area the order Diptera was the most abundant (81.0%), followed by Thysanoptera (8.6%) and Psocoptera (7.4%). Whereas at the non-commercial area, 50.0% of the specimens were from the order Diptera, 31.7% Psocoptera, and 12.7% Thysanoptera. All specimens of Entognatha collected on the two sampling sites were from the Order Collembola, which are represented by the springtails.

Aceria litchii was also abundant in our survey. It was present throughout the collection period with 62.5 and 67.9 % of infestation on the samples collected in the commercial and non-commercial areas, respectively.

Discussion

The arthropods community associated to erinea induced by A. litchii in our study was quite diverse showing that the ecological interactions involving galls can be complex. The environment created by the erinea might increase the availability of food resources in addition to providing shelter and protection against adverse environmental conditions and natural enemies, influencing the distribution and abundance of the organisms on this community (Price et al. 1980; Jones et al. 1994, 1997; Fukui 2001; Crawford et al. 2007; Cornelissen et al. 2016).

The mites were the most abundant organisms in our collections and among them the Phytoseiidae family had the highest specimens abundance and species diversity. Phytoseiid mites are predators that feed on phytophagous mites and insect eggs, but most of them may also have other feeding habits, consuming fungi, plant exudates and pollen (Mcmurtry et al. 2013). Moreover, as the structure of the erineum induced by A. litchii persists on the leaflets after the mites leave it, the old erineum can be used as resource by other organisms, which could also serve as alternative feeding for predators species found on the samplings (Jones et al. 1994, 1997; Fukui 2001; Crawford et al. 2007; Cornelissen et al. 2016).

Despite the profitability of litchi crop, in Brazil few studies have been conducted on biological, taxonomic, or ecological aspects, focusing on diversity of arthropods present on it. The survey conducted by Mineiro and Raga (2003) on five municipalities of the State of São Paulo was the first in litchi orchards in Brazil. However, their study did not record the presence of A. litchii. The authors recorded the occurrence of 19 mite species belonging to nine families. The Phytoseiidae family also presented the greatest species diversity, with five species, three of them we also found in our study as A. herbicolus, Euseius concordis (Chant) and I. zuluagai.

Two new surveys carried out in litchi orchards few years later reported a different composition of the predatory mites of the Phytoseiidae family that could be associated with A. litchii (Picoli et al. 2010; De Azevedo et al. 2014). The species of the Phytoseiidae family cited in the studies from Picoli et al. (2010) and De Azevedo et al. (2014) were also found in our research, these being Amblyseius compositus Denmark & Muma, A.

herbicolus, Euseius alatus De Leon, E. concordis, I. zuluagai, P. intermedius and P. woodburyi. However, the species diversity found in our study was higher.

In our survey, we observed that from all predator species sampled, P. intermedius had the greatest abundance in both sampling areas. This predator was first recorded in Brazil in 2007 in the western part of the State of São Paulo. It was collected from seven plant species, all of them known to present high densities of trichomes over their leaves, confirming the preference of this species of the subfamily Phytoseiinae by plants with these foliar structures (Demite et al. 2008; McMurtry et al. 2013; Tixier and Kreiter 2014).

Due the fact that species of Phytoseiinae are usually smaller than the two other subfamilies of Phytoseiidae, have a loss of soma setae, and some of the dorsal shield setae are stout and usually serrate have suggested that such traits could improve their movement among the trichomes (McMurtry et al. 2013; Tixier and Kreiter 2014). This could allow P. intermedius to prey on A. litchii, even with the barriers that the erinea might represent. Given that A. litchii has been reported as a suitable prey for P. intermedius on laboratory studies (Azevedo et al. 2016), its high population associated with A. litchii, in the state of São Paulo and thereafter in our study, suggests that this predator could be a potential biological control agent for A. litchii (Picoli et al. 2010; De Azevedo et al. 2014).

Although the erineum serve as protection for A. litchii against their natural enemies, these mites can be more easily reached when they are exposed, leaving from an older erineum to infest new young leaves (De Azevedo et al. 2014). Population outbreaks of A. litchii are favoured when there is the emergence of new shoots, especially in the runup to the flowering and it can cause serious loss to crop yield (Alam and Wadud 1963). This occurs because organisms that induce galls must synchronize their activities of colonization with the phenology of the tissues of its host to obtain the morphological and physiological conditions for colonization, survival, and growth, due to their close association with the host plants (Oliveira et al. 2016).

There are also reports of predatory mites from other families preying on eriophyoid mites, such as Cheyletidae, Cunaxidae, Stigmaeidae, and Tarsonemidae (Perring and McMurtry 1996). In addition to the mites, several insect orders such as Diptera, Coleoptera, Hemiptera, Neuroptera and Thysanoptera were already reported feeding on eriophyoid mites (Perring and McMurtry 1996). Thus, A. litchii can serve as prey for these mites and insects justifying the occurrence of all these families and orders in our survey. It is known that some members of these groups are predators of other small arthropods. Thus, they could be there to feed not only on A. litchii, but also on other organisms that inhabit the galls induced by A. litchii (Walter et al 2009; Triplehorn and Jonnson 2011).

Mite species of the families Tetranychidae and Tenuipalpidae are obligatory phytophagous of great economic importance because many species are cosmopolitan, polyphagous, and occur in various plants of agricultural importance (Walter et al. 2009). Even though these species have been reported in low population in our study, their monitoring must be carried out since they may represent potential pests for this crop under some conditions.

In our research, we also recorded two new species of mites (Chapter 2). They belong to Iolinidae family, mites that are not always considered in field surveys on agricultural crops perhaps because little is known about their taxonomy, biology, and effectiveness on biological control of phytophagous mites. They are generally also not considered as phytophagous species that could perform any economic damage to agricultural crops. Some species of this family have also been found in close association with insects, but other are free living in soil and on plants. Among free living iolinid mites, there are species feeding on honeydew, fungi, and dead arthropods, but also preying on eriophyoid mites, as well as serving as alternative prey for phytoseiid mites (Jeppson et al. 1975; Lindquist 1983; Hessein and Perring 1986; Ferragut et al. 2008;

Walter et al. 2009; Horn et al. 2011). Whereas gall inducing mites inhabit environments to protect themselves from predators, the small size of the iolinid mites could grant them an advantage to feed on mites inducing erinea, such as A. litchii (Perring and McMurtry 1996). However, there are no reports of studies with these organisms so far.

We also observed that in part of the samples that had erinea A. litchii was no longer present, possibly due to the fact the mites migrated to infest new shoots. A. litchii leaving the erinea provides physical structures that did not exist before its presence in the litchi tree to other organisms to live there. So, in this case, the litchi erinose mite acts as an ecosystem engineer and it could be affecting the community of arthropods associated with the erinea, by creating a favourable habitat for them (Jones et al. 1994, 1997; Fukui 2001; Crawford et al. 2007; Cornelissen et al. 2016).

The mites of suborder Oribatida and of families Winterschmidtiidae, Tarsonemidae, Eupodidae and Tydeidae, and hexapods of the orders Collembola and Psocoptera present several feeding habits such as detritivorous, algivorous, fungivorous, bacteriophagous and pollenophagous (Walter and O'Dowd 1995; Krantz and Walter 2009). These organisms have been found in large numbers inhabiting the galls of A. litchii, probably because the galls induced by A. litchii provide shelter and food for them. It is necessary to emphasize the great diversity of mites and hexapods in the erinea induced by A. litchii, many of which being predators. Nevertheless, A. litchii still reached high infestations in the litchi plants (approximately 65% of the samples) and the effect of these natural enemy species on populational dynamic of A. litchii is still poorly understood. The small size of A. litchii and its ability to live hidden inside the erineum seem to be the key to their ecological success, enabling them to live in habitats small enough to be free of predation by natural enemies and to reach high populations (Alam and Wadud 1963; Sabelis and Bruin 1996).

Our study on litchi showed a diverse occurrence of mites and hexapods in this crop. In addition, among the species found, many of them were of predatory mites, being a larger number than the studies already carried out in this crop in Brazil. Furthermore, this is the first reported research on this fauna for the State of Minas Gerais, which is one of the largest litchi producers in the country. Further studies to test control strategies to improve the use of the natural enemy species found in our study as biological control agents for litchi erinose mite should be encouraged, since the control practices used by litchi growers have not yet reached satisfactory control of this mite.

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Table 1. Taxonomic classification of mites (Subclass Acari) associated with galls of Aceria litchii (Keifer) (Acari: Eriophyidae) on Litchi chinensis Sonnerat (Sapindaceae) in a commercial (Buieié) and in a non-commercial area (Campus of the Federal University of Viçosa), Viçosa, Minas Gerais, Brazil.

Suborder	**Family**	**Genus/Species**	**Commercial area**	**Non-commercial area**
Monogynaspida	Phytoseiidae	Amblyseius cf. pravus	7	0
		Amblyseius compositus	6	0
		Amblyseius herbicolus	21	44
		Amblyseius sp.	1	0
		Amblyseius sp. ♂	7	1
		Euseius alatus	2	0
		Euseius concordis	3	2
		Iphiseiodes zuluagai	72	26
		Phytoseiidae (immature)	118	353
		Phytoseius intermedius	137	598
		Phytoseius woodburyi	22	173
		Proprioseiopsis aff. dominigos	2	6
		Typhlodromalus manihoti	1	0
Prostigmata	Cunaxidae		1	15
	Eupodidae		13	59
	Tryophtydeidae	Tryophtydeus sp.1	39	5
		Tryophtydeus sp.2	3	25
	Tydeidae	Brachytydeus sp.	20	88
		Neolorryia sp.	3	1
	Iolinidae	Parapronematus acaceae	13	8
		Pausia sp.	32	71
		Pseudopronematulus sp.	90	103
	Xenocaligonellidae		0	1
	Stigmaeidae	Agistemus aff. brasiliensis	18	11
	Tetranychidae	Oligonychus aff. mangiferae	14	9
		Tetranychus aff. mexicanus	1	3
	Tenuipalpidae	Brevipalpus phoenicis	20	12
	Tuckerellidae	Tuckerella sp.	1	0
	Cheyletidae	Hemicheyletia sp.	0	70

		Mexecheles sp.	0	55
		Oudemansicheyla sp.	0	5
	Tarsonemidae	cf. Neotarsonemoides	4	0
		Tarsonemus sp. 1	138	47
		Tarsonemus sp. 2	1	0
Oribatida		Oribatida sp. 1	39	0
		Oribatida sp. 2	20	0
		Oribatida sp. 3	30	0
		Oribatida sp. 4	0	1
	Winterschmidtiidae	Czenspinskia sp.	85	67
		Oulenzia sp.	1	13
Total			985	1872

Table 2. Taxonomic classification of hexapods (Superclass Hexapoda) associated with galls of Aceria litchii (Keifer) (Acari: Eriophyidae) on Litchi chinensis Sonnerat (Sapindaceae) in a commercial (Buieié) and in a non-commercial area (Campus of the Federal University of Viçosa), Viçosa, Minas Gerais, Brazil.

Class	**Order**	**Commercial area**	**Non-commercial area**
Entognata	Collembola	86	31
Insecta	Blattodea	1	1
	Coleoptera	1	3
	Diptera	132	71
	Hemiptera	3	3
	Hymenoptera	0	1
	Psocoptera	12	45
	Thysanoptera	14	18
Total		249	173

CHAPTER II

First description of iolinid mites (Acari: Tydeoidea) from Brazil[1]

Guilherme Liberato da Silva[1*], Tairis Da-Costa[2], Célia Siqueira Ferraz[3], Angelo Pallini[3,4], Noeli Juarez Ferla[2,4]

[1]Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal do Rio Grande do Sul. Porto Alegre, RS, Brasil. E-mail: gibaliberato_148@hotmail.com

[2]Laboratório de Acarologia, Tecnovates, UNIVATES – Centro Universitário. Lajeado, RS, Brasil. E-mail: njferla@univates.br

[3]Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil

[4]CNPq Researcher

*Corresponding author: gibaliberato_148@hotmail.com

Abstract

The family Iolinidae is recorded from Brazil for the first time. Two new species: Pausia litchiae **n. sp.** and Pseudopronematulus nadirae **n. sp.** collected from leaves of Litchi chinensis Sonn. (Sapindaceae) at Viçosa County, Minas Gerais State are described and illustrated.

Key words: Litchi chinensis, Iolinidae, Pausia, Pseudopronematulus, taxonomy

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[1]Silva GL, Da-Costa T, Ferraz CS, Pallini A, Ferla NJ (2017) First description of iolinid mites (Acari: Tydeoidea) from Brazil. Published in Systematic & Applied Acarology 22(5): 694–701. http://doi.org/10.11158/saa.22.5.8

Introduction

The family Iolinidae comprises 131 species in 36 genera (Zhang et al. 2011; Theron et al. 2012; Sadeghi et al. 2012; Ripka et al. 2013). This family was redefined and enlarged by André and Fain (2000) to encompass the subfamilies Tydaeolinae, Pronematinae and Iolininae of Tydeidae based on a cladistic analysis. Their economic importance is poorly known, and seemingly lower in comparison with pests and predatory mites such as tetranychid and phytoseiid mites, respectively (McMurtry et al. 2013; Sadeghi et al. 2012). However, iolinids that live on foliage have an important role in providing alternative food for phytoseiid mites when other prey is unavailable (Flaherty and Hoy 1971; Calvert and Huffaker 1974) in biological control of other pest mites such as tenuipalpids and eriophyids. They can puncture and feed on spider mite eggs as well (Knop and Hoy 1983; Hessein and Perring 1986, 1988).

Three species are known in Pausia: P. taurica Kuznetzov 1972, P. magdalenae Baker & Delfinado (1976) and P. colonus Theron & Ueckermann 2012 and two species in Pseudopronematulus: P. acus Fan and Li, 1992 and P. augrabiensis Ueckermann & Grout 2007. Here, we describe and illustrate a new species for each genus, Pausia and Pseudopronematulus.

Material and Methods

The specimens were observed under a binocular microscope and mounted on glass slides in Hoyer's medium. Drawings were made using a camera lucida apparatus and the lines were highlighted using Corel Draw X5®.

Specimens were observed by means of a phase contrast microscope (Axio Scope.A1 - Zeiss). The nomenclatural terms and setal notations of the idiosoma and appendages follow that of Kaźmierski (1998) and Panou et al. (2000). All measurements are given in micrometers (µm). The classification system used is based on André (1980).

The holotype measurements are shown in bold type followed by the mean and range in parentheses.

Systematics

Family **Iolinidae** Pritchard, 1956

Subfamily **Pronematinae** André, 1980

Genus **Pausia** Kuznetzov, 1972: 1739

Type species: Pausia taurica Kuznetzov, 1972

Diagnosis. Pausia is defined as follows: tarsus I with ordinary empodium, lacking claws, opisthosoma with 10 pairs of setae (ps 1-2 included); chaetotaxy of leg segments: tarsi 8(ω)–6(ω)–6–5, tibiae 4(ω)–2–2–2, genua 3–3–2–1, femora 3–3–2–(1–1), trochanters 1–1–1–0, epimeral formula: 3–1–4–2; femur IV divided into basi and telofemur; palp chaetotaxy: 5–1–2.

Pausia litchiae n. sp. Silva, Da-Costa & Ferla (Figs 1-5)

Description. Female chaetotaxy of idiosoma and appendages typical for the genus (Panou et al., 2000, p: 322).

Dorsum - Idiosoma oval. Length **203** (228, 203-249), width **126** (129, 101-142). Dorsal side completely striated. Striation of prodorsum longitudinal as those between c1 and d1. Opisthosoma with U-shaped striation between d1 and e1 setae, transverse between e and between f1, reversely curved between h1. Eyes absent. Sejugal suture arched anterior to level setae la and ex. All dorsal setae slender, spindle-like, slightly serrate (including bo), not reaching bases of setae in next row with exception of bothridial setae (bo). Prodorsum with four pairs of setae and opisthosoma with 10 pairs of setae. Lyrifissures ia located between setae c1 and d1, lateral to line connecting bases of these setae; im situated close to e1, anterolaterally; ip located close to f2, anterolaterally. Dimensions: bo **20** (21, 18-24), ro **9** (9, 9-10), la **11** (12, 10-13), ex **16** (16, 14-18), c1 **9** (9, 8-10), c2 **12** (12, 10-13), d1 **10** (10, 8-11), e1 **9** (11, 8-12), f1 **9** (10, 9-10), f2 **19** (18, 16-20), h1 **9** (10, 9-10), h2 **17** (17, 17-18) and dorsally situated ps1 **9** (8, 7-9). Distance between setae: ro-ro **15** (15, 14-16), la-la **32** (31, 31-32), bo-bo **34** (33, 31-34), c1-c1 **28** (30, 27-33), d1-d1 **25** (28, 25-29), e1-e1 **40** (41, 39-42), f1-f1 **15** (15, 13-15). Ventrally situated ps2 **12** (12, 11-13), ps3 absent (Fig. 1).

Venter - Faintly striated. Striation longitudinal between metasternal setae (mt). Ventral setae similar in shape to dorsal setae, serrate but shorter and slenderer. Lyrifissures ih located posteroventrally. Four pairs of aggenital setae. Setal lengths: ag1 **7** (7, 6-7), ag2 **7** 7, ag3 **8** (8, 7-8), ag4 **8** (9, 8-10). No genital and eugenital setae, *mtβ*= *mtα* **8** (8, 8-9), 1b=3d **13** 13, 3b=4b **11** 11, 2a=3c **12** (12, 12-13), 1c=pt **10** 10 (Fig. 2).

Gnathosoma - Visible from above. Length **54** (53, 49-55), width **35** (35, 33-37). Palpal setation (5–1–2) (Fig. 3A). Palps terminate with straight eupathidium (p'ζ), not forked distally, **5** 5 long. Subcapitulum with transverse striation at setae Sc region, Sc1 **8** 8, Sc2 **8** 8 (Fig. 3B). Cheliceral stilettos **11** (11, 11-12) (Fig. 3C).

Legs - Apotele I with ordinary empodium, lacking claws; setation (tarsus to trochanter): I: 8+ω–3+κ+ω–3–3–1(Fig. 4A), II: 6+ω–2–3–3–1(Fig. 4B), III: 5–2–2–2–1 (Fig. 4C), IV: 5–2–2–1+1–0 (Fig. 4D). Epimeral formula: 3–1–4–2. Measurements: tarsus I length/width: 23/8, ft'ζ **9** (9, 8-9), ft"ζ **20** (21, 20-22), tc'ζ **17** (17, 17-18), tc"ζ **18** (17, 16-18), p'ζ **9** 9, p"ζ **9** 9, u' **6** 6, u''**6** 6, ωI **1** 1. Solenidion ωI minute, 1/8 width of tarsus I. Tibia I with "I" shaped famulus κ (1), solenidion ω (1). Tarsus II (Fig. 4C) with short, spherical, and visible solenidion ωII (5 μm).

Male: Not found.

Remarks - The new species (Fig. 5) can be distinguished from other species of Pausia by following characters: ps3 absent, tarsus III with five setae (vs. six in other species), tibia I with three setae (vs. four in other species). Setae bo, ex, h2 and ps2 much shorter than those of P. colonus. In addition, all dorsal setae of P. litchiae **n. sp.** are much shorter than that in P. taurica and P. magdalenae.

Type material - Holotype female, 10/XI/15 at Viçosa County, Minas Gerais State, Brazil, Collector: C. S. Ferraz, on Litchi chinensis Sonn. (Sapindaceae); deposited at Departamento de Entomologia e Acarologia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil. Paratypes: two females, 10/XI/15, two females, 26/X/15, and one female, 12/VI/15 at Viçosa, Minas Gerais State, Brazil, Collector: C. S. Ferraz, on Litchi chinensis Sonn. (Sapindaceae). Deposited at Museu de Ciências Naturais (ZAUMCN), UNIVATES – Centro Universitário, Lajeado, Rio Grande do Sul, Brazil.

Etymology - The new species is named after the host plant.

Genus **Pseudopronematulus** Fan and Li, 1992: 396

Type species. Pseudopronematulus acus Fan and Li, 1992.

Diagnosis. This genus can be defined as follows: setae ro posteromedial to bothridial setae (bo); setal formula of genua 3–3–2–1; opisthosoma with 10 pairs of setae; tarsus I without apotele and femur IV divided.

Pseudopronematulus nadirae n. sp. Silva, Da-Costa & Ferla (Figs 6-10)

Description. Female, chaetotaxy of idiosoma and appendages typical for the genus (Panou et al., 2000, p: 322).

Dorsum - Idiosoma oval. Length **198** (200, 193-222) and width **121** (124, 111-150). Dorsal side completely striated. Striation of prodorsum longitudinal as those between c1 and d1, transverse between d1 and setae h. Eyes absent. All dorsal setae slender, spindle-like, slightly serrate (including bo), setae ro, bo, f1, f2, h1, h2 reaching bases of setae in the next row. Prodorsum with four pairs of setae and opisthosoma with 10 pairs of setae. Lyrifissures ia located between setae c1 and d1, in line connecting bases of these setae; im situated close to e1; ip located close to f2, anterolaterally. Dimension: bo **29** (31, 29-32), ro **14** (16, 14-18), la **13** (14, 13-15), ex **18** (19, 18-20), c1 **14** (15, 14-17), c2 **14** (15, 14-17), d1 **15** (16, 15-16), e1 **17** (18, 17-19), f1 **17** (17, 16-19), f2 **25** (26, 24-28), h1 **14** (16, 15-17), h2 **21** (21, 19-21). Distance between setae: ro-ro **22** (21, 18-22), la-la **39** (40, 39-42), bo-bo **37** (36, 34-39), c1-c1 **38** (37, 33-43), d1-d1 **36** (34, 32-37), e1-e1 **36** (35, 33-36), f1-f1 **14** (13, 12-14). Ventrally situated ps1 **10** (11, 10-13), ps2 **4** 4, ps3 **7** (8, 7-9) (Fig. 6).

Venter - Faintly striated. Striation longitudinal between metasternals setae (mt). Ventral setae similar in shape to dorsal setae, serrate but shorter and slenderer. Lyrifissures ih located posteroventrally. Four pairs of aggenital setae. Setal lengths: ag1 **6** (6, 6-7), ag2 **6** (7, 6-7), ag3 **7** (7, 7-8), ag4 **8** (9, 7-10). No genital and eugenital setae, mtβ=mtα **7** 7, 1b **10** 10, 1c **11** (11, 11-12), pt **7** 7, 2a **14** (14, 14-15), 3b **17** (17, 16-18), 3c **14** 14, 3d **18** (17, 17-19), 4b **15** (15, 14-15) (Fig. 7).

Gnathosoma - Visible from above. Length **43** (48, 42-54), width **41** (44, 35-54). Palpal setation (5–1–2) (Fig. 8A). Palp tarsus terminate with T-shaped eupathidium (p'ζ) slightly broadened and forked distally, **4** 4 long. Subcapitulum with striae longitudinal behind setae Sc1 and transverse behind Sc2, Sc1 5, Sc2 11 (Fig. 8B). Cheliceral stilettos **8** 8 (Fig. 8C).

Legs – Setation (tarsus to trochanter): I: 8+ω–3+κ+ω–3–3–1 (Fig. 9A), II: 6+ω–2–3–3–1 (Fig. 9B), III: 5–2–2–2–1 (Fig. 9C), IV: 5–2–1–1/1–0 (Fig. 9D). Femur IV divided. Epimeral formula: 3–1–4–2. Tarsus apotele I absent. All leg setae are serrate. Tarsi II–IV each with two claws and a hairy empodium. Measurements: tarsus I length/width: 20/ 9, ft'ζ **13** (13, 12-14), ft"ζ **29** (28, 28-29), tc'ζ **17** (17, 16-17), tc"ζ **35** (34, 33-36), p'ζ **20** 20, p"ζ **27** (27, 26-29), u' **4** 4, u''**4** 4, ωI **6** 6. Solenidion ωI as long as half-width of tarsus. Tibia I with "I" shaped famulus κ **3** 3 as long as club-like solenidion ω **2** 2. Tarsus II (Fig. 9B) with minute, spherical, scarcely visible solenidion ωII **2** 2.

Male: Not found.

Remarks – Pseudopronematulus nadirae **n. sp.** (Fig. 10) differs from P. acus and P. augrabiensis by having two setae on tibia II (three in P. augrabiensis), tarsus IV with five setae (six other species), tarsus II with six setae (seven in other species), setae serrated on tarsus I (smooth in other species). Setae bo, f2, la and ex are much shorter than those in P. acus and P. augrabiensis.

Type material - Holotype female, 26/X/15, Viçosa County, Minas Gerais State, Brazil, Collector: C. S. Ferraz, on Litchi chinensis Sonn. (Sapindaceae); deposited at Departamento de Entomologia e Acarologia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil. Paratypes: two females, 09/V/15, one female, 12/VI/15, one female, 25/VII/15, one female, 21/V/15 at Viçosa County, Minas Gerais State, Brazil, Collector: C. S. Ferraz, on Litchi chinensis Sonn.; deposited at Museu de Ciências Naturais (ZAUMCN), UNIVATES – Centro Universitário, Lajeado, Rio Grande do Sul, Brazil.

Etymology - The species name is given in honour of Nadir Silva da Costa (in memoriam), mother of the second author, cruelly murdered in her home.

Acknowledgments

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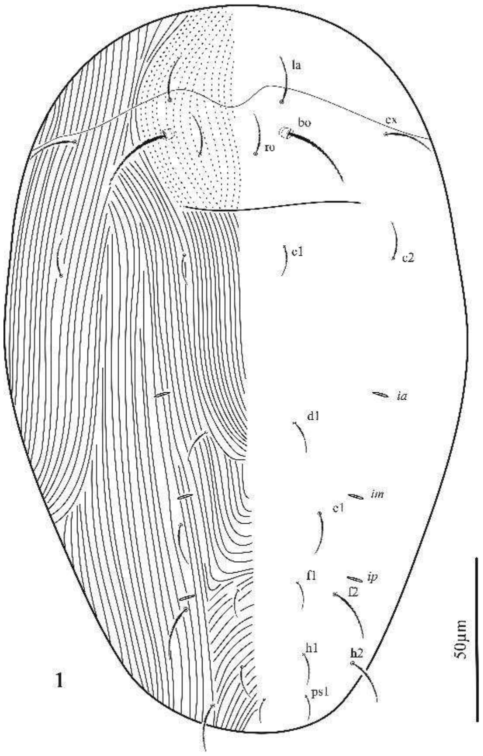

Figure 1. Pausia litchiae **n. sp.**, Female (holotype), dorsal view.

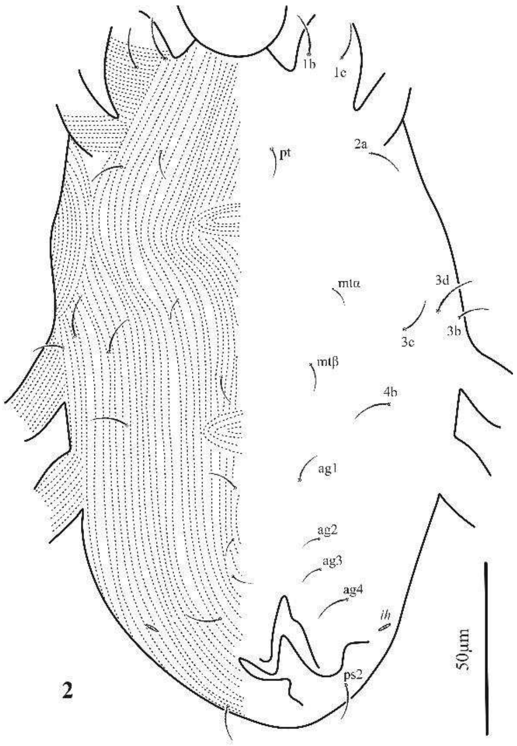

Figure 2. Pausia litchiae **n. sp.**, Female (holotype), ventral view.

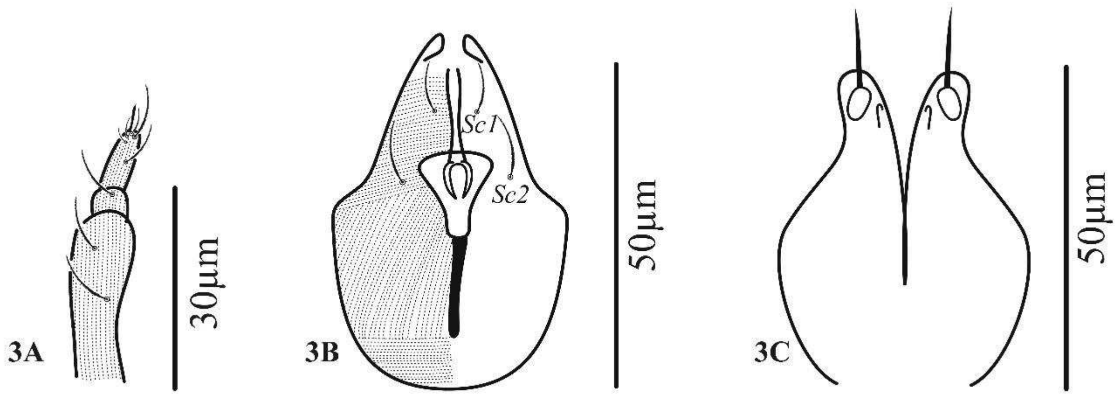

Figure 3. *Pausia litchiae* **n. sp.**, Female (holotype), gnathosoma: [A] pedipalp, [B] subcapitulum, [C] chelicera.

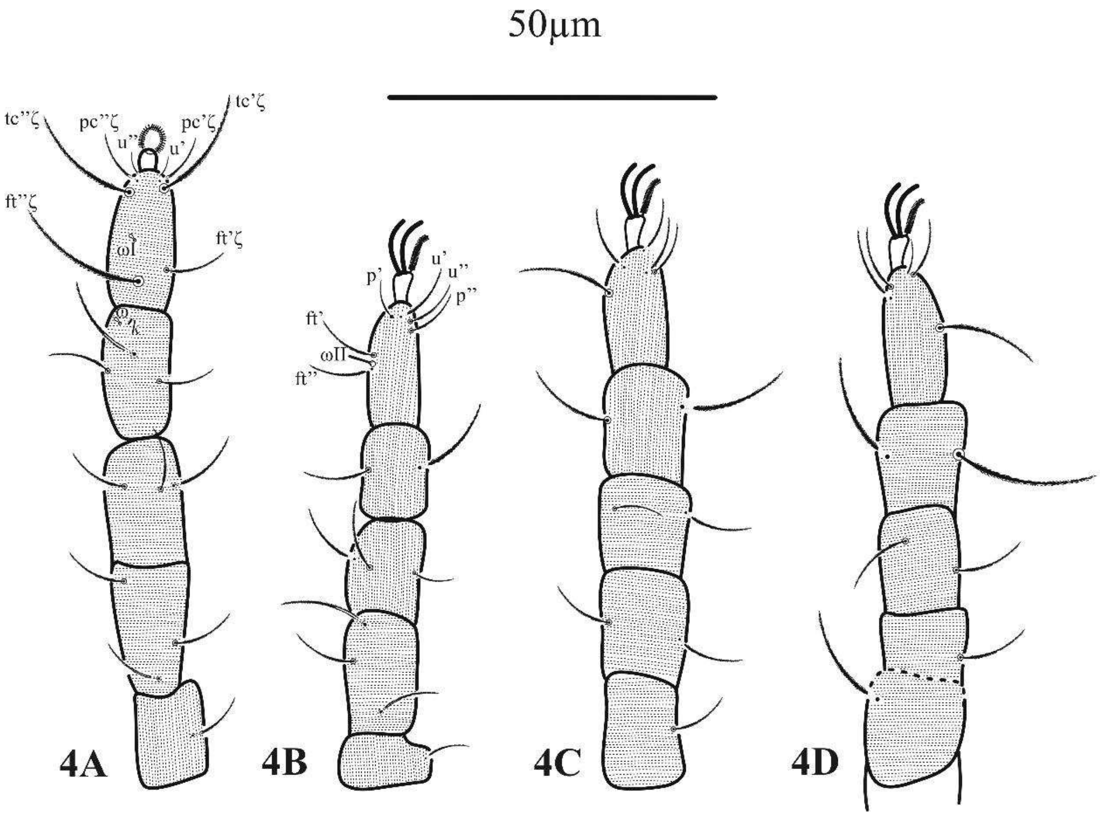

Figure 4. Pausia litchiae **n. sp.**, Female (holotype), [A] leg I, [B] leg II, [C] leg III, [D] leg IV.

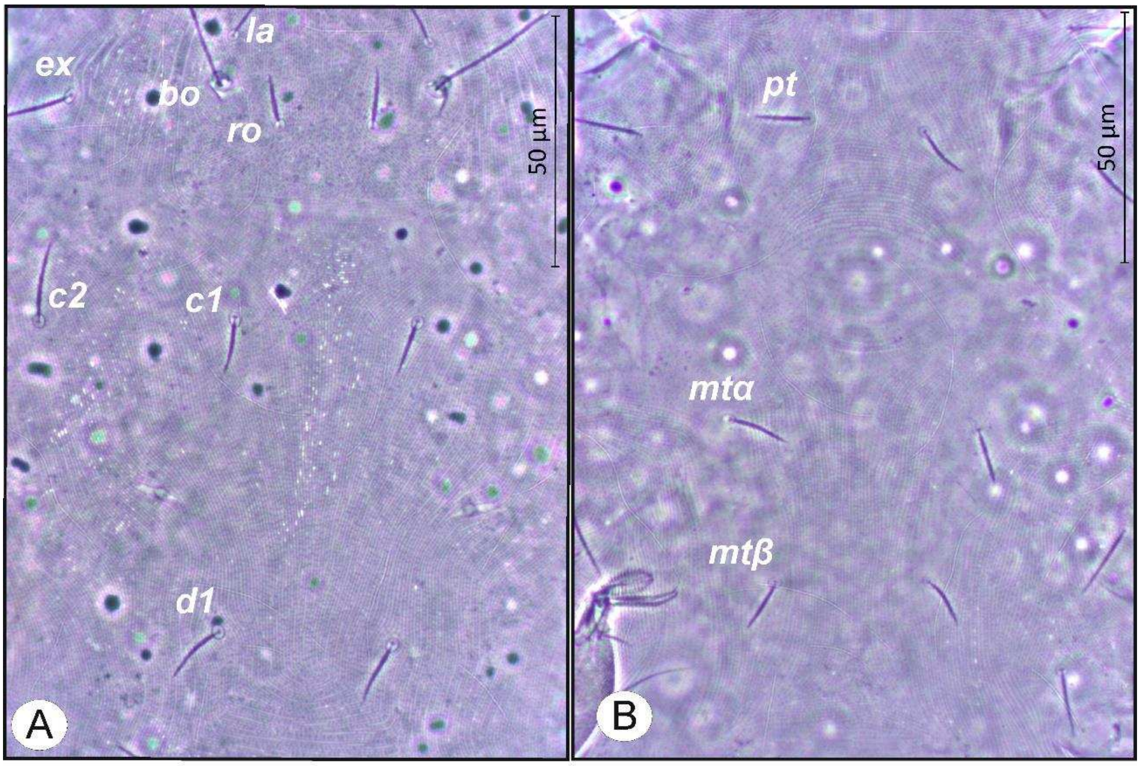

Figure 5. Dorsal view of *Pausia litchiae* **n. sp.** [A] and ventral [B]. Female (paratype).

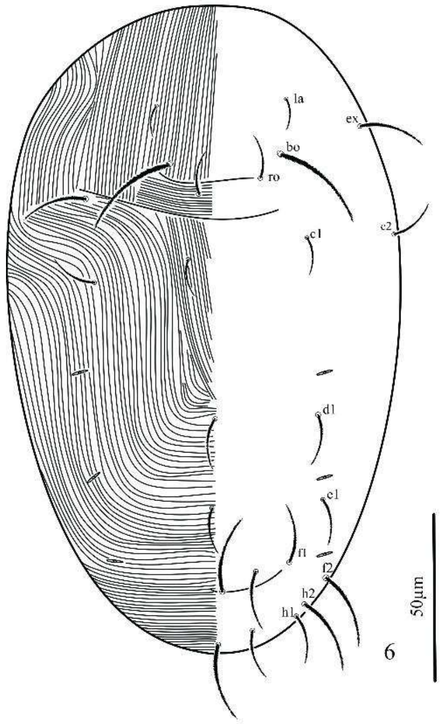

Figure 6. Pseudopronematulus nadirae **n. sp.**, Female (holotype), dorsal view.

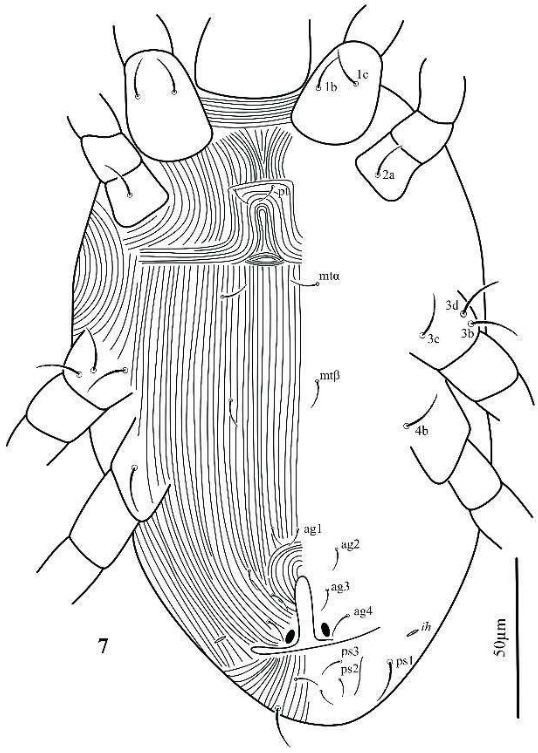

Figure 7. Pseudopronematulus nadirae **n. sp.**, Female (holotype), ventral view.

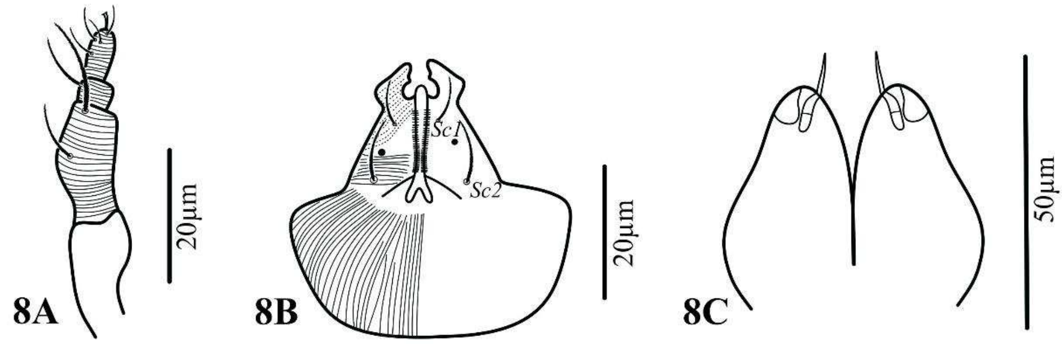

Figure 8. Pseudopronematulus nadirae **n. sp.**, Female (holotype), gnathosoma: [A] pedipalp, [B] subcapitulum, [C] chelicera.

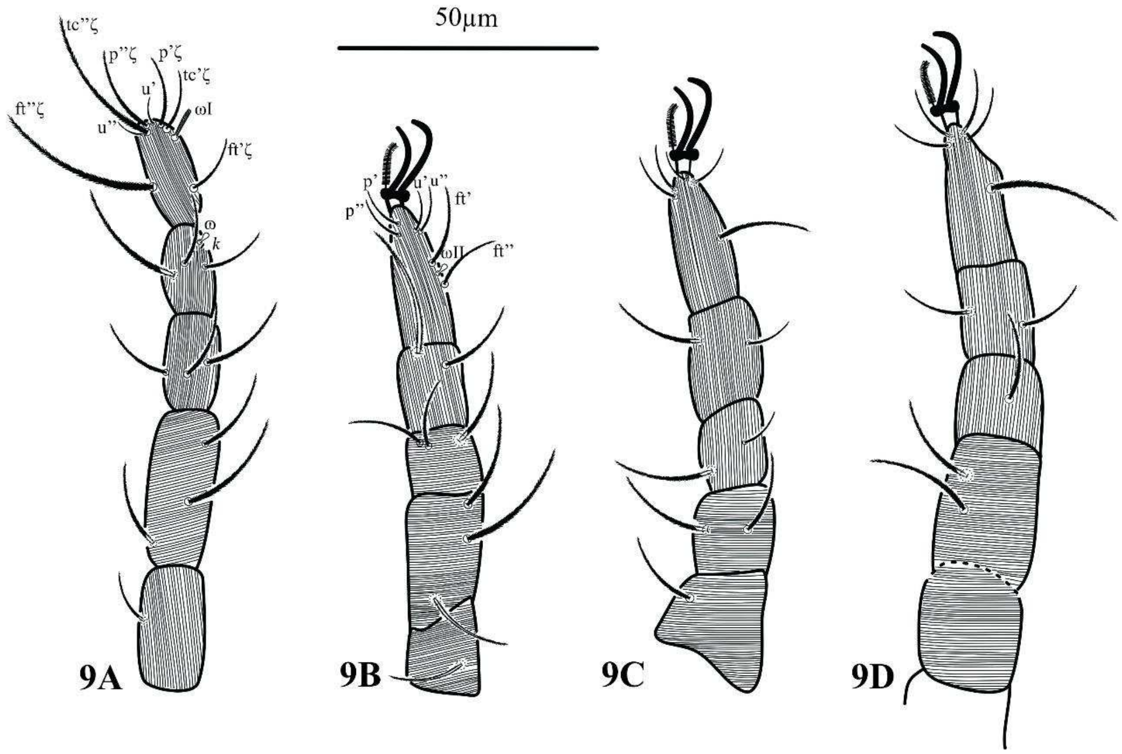

Figure 9. Pseudopronematulus nadirae **n. sp.**, Female (holotype), [A] leg I, [B] leg II, [C] leg III, [D] leg IV.

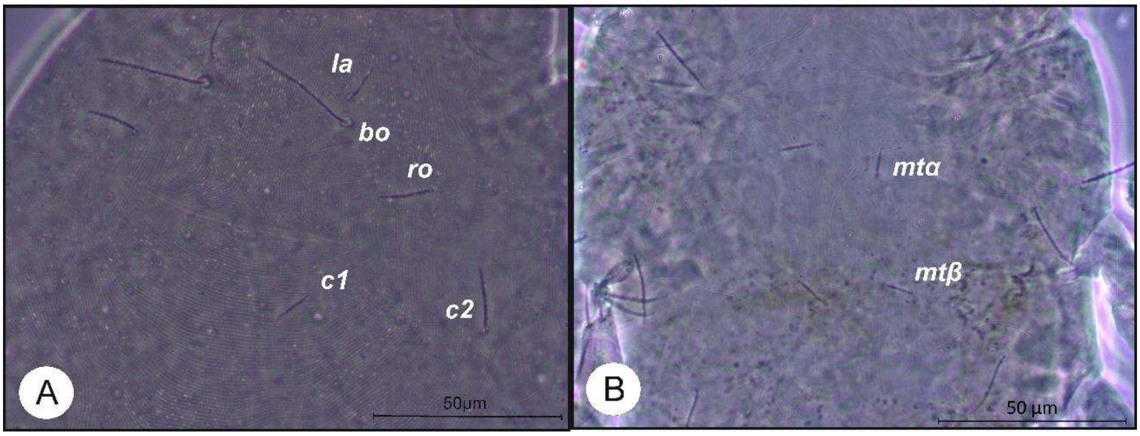

Figure 10. Dorsal view of Pseudopronematulus nadirae **n. sp.** [A] and ventral [B]. Female (paratype).

CHAPTER III

The unsuitability of the predatory mites Phytoseius intermedius and Amblyseius herbicolus to control Aceria litchii under field conditions

Abstract

Several predatory mites have been found in association with Aceria litchii (Keifer) (Acari: Eriophyidae), which is considered one of the main pest of the litchi crop through the world. However, few studies for the management of this mite using biological control have been carried out so far. Here, it was investigated the capacity of the predatory mites Phytoseius intermedius Evans & MacFarlane and Amblyseius herbicolus (Chant) (Acari: Phytoseiidae) to control A. litchii on litchi plants under field conditions. Additionally, the released population of A. herbicolus was supplemented with alternative food (cattail pollen). To evaluate the control, the dynamic of erinea on plants that received these predatory mites was assessed for three months. The number of predatory mites on the plants was counted at the end of this period. It was observed that the proportion of leaflets infested with A. litchii significantly increased throughout the time in all treatments. However, no significant difference was observed in the proportion of infested leaflets among treatments. A total of 2,097 mites, belonging to 13 species were collected along with the two species that were released. Nevertheless, no significant difference was observed in the number of predatory mites among treatments, suggesting that neither the predator numbers nor the species composition prevented the increase of the number of erinea along the experiment. In the same way, the pollen addition did not provide greater population growth of A. herbicolus on the litchi plants.

Key words: alternative food, biological control, litchi erinose mite, gall, Phytoseiidae.

Introduction

The litchi erinose mite Aceria litchii (Keifer) (Acari: Eriophyidae) is considered one of the main pest of the litchi crop throughout the world. It induces the formation of a gall called erineum (pl. erinea) on leaves, inflorescences, and fruits, which can negatively affect the yield of crop (Waite and Gerson 1994; Sabelis and Bruin 1996; Westphal and Manson 1996; Mitra 2002; Waite and Hwang 2002; De Azevedo et al. 2014). The erineum patch occurs on the abaxial surface of leaves and sometimes may cover almost the entire leaf surface. In the early stages of development, the trichomes are whitish and later become reddish brown, looking like a patch of felt (Nishida and Holdaway 1955; Jeppson et al. 1975; Keifer et al. 1982; Westphal and Manson 1996; Waite and Hwang 2002).

The complex environment within an erineum provides shelter and protection for A. litchii against the application of pesticides, which is one of the main forms used for its control, in addition to pruning (Jeppson et al. 1975; Westphal and Manson 1996; Waite and Hwang 2002; Srivastava et al. 2016). Thus, the biological control, which consists of the use of natural enemies to reduce the population density of pest species, appears as a management alternative for A. litchii control as a practice more sustainable in the long term (DeBach 1964; Bale et al. 2008).

Among the strategies of biological control, one is the inoculative biological control, which consists of the intentional release of natural enemies with the expectation that they will multiply and control de pest for an extended period (Eilenberg et al. 2001). The inoculative release can also be combined with the provision of alternative or supplementary food for the natural enemies. Being some predators omnivorous, it gives an advantage for biological control because those mites could persist on plants longer than do the strictly carnivorous species, by feeding on alternative foods when prey is absent or scarce (Symondson et al. 2002; Duarte et al. 2015; Janssen and Sabelis 2015).

Efforts to find natural enemies for control of A. litchii have resulted in a list of several predatory mites from the Phytoseiidae family as candidates (Waite and Gerson 1994; Picoli et al. 2010; De Azevedo et al. 2014). Phytoseiid mites are important biological control agents of phytophagous mites and they have been extensively used in biological control programs (Helle and Sabelis 1985, Gerson et al. 2003; McMurtry et al. 2013). Among the many species reported in association with A. litchii on litchi trees, Phytoseius intermedius Evans & MacFarlane and Amblyseius herbicolus (Chant) (Acari: Phytoseiidae) receive special attention due their frequency and co-occurrence with this pest (Picoli et al. 2010; De Azevedo et al. 2014; Chapter 1). Moreover, laboratory studies have shown that A. litchii is a suitable prey for both P. intermedius and A. herbicolus (Azevedo et al. 2016; personal observation).

Some other traits indicate that these predatory mites could be good candidates for biological control of the litchi erinose mite. For instance, P. intermedius was also reported on plant species that present high densities of trichomes over their leaves (Demite et al. 2008). Some predatory mite species within the Phytoseiinae subfamily are smaller than other phytoseiid mites. Furthermore, they have loss of soma setae, and some of the dorsal shield setae are stout and usually serrate. So, some authors suggest that such traits could improve the movement of these mites between the leaf trichomes (McMurtry et al. 2013; Tixier and Kreiter 2014). Regarding A. herbicolus, some studies have shown that it can reproduce and develop properly when fed either on mites or different pollens (Reis et al. 2007; Rodríguez-Cruz et al. 2013). Duarte et al. (2015) showed that the addition of pollen to chilli pepper plants, Capsicum frutescens Linnaeus (Solanaceae), allowed A. herbicolus to establish before the infestation of pest mites, which resulted in effective control.

Here we investigated the capacity of the predatory mites P. intermedius and A. herbicolus to control of A. litchii on litchi plants under field conditions. We accessed the dynamic of leaves with erinose on plants that received an inoculative release of these predatory mites. Additionally, we supplemented the population of A. herbicolus with pollen with the aim of increasing the population of this predator before the period of A. litchii increase, what could prevent it from attaining damaging levels.

Materials and Methods

Plants and mites rearing

Seedlings of Litchi chinensis Sonnerat (Sapindaceae) var. Bengal (with approximately 0.8 m height) were obtained from a nursery located at Cataguases, Minas Gerais, Brazil. The plants were transplanted to plastic pots (10 L) containing a mixture of soil and bovine manure (3:1) and were fertilised every 15 days with 5 g of N-P-K (10-10-10) and irrigated when necessary. The plants were kept in an area at the Campus of the Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil. The area was surrounded by an edifice on one side and a steep slope with fragments of native vegetation on the others. The climate of the region is type Cwa, characterised by altitude tropical climate with rainy summer and dry winter, according to the Köppen-Geiger classification.

The rearing of the predatory mites P. intermedius and A. herbicolus started in 2015 with individuals collected from litchi plants infested by A. litchii at the campus of the Federal University of Viçosa (20º 45' 32.2" S; 42º 52' 05.9" W; 624.5 m altitude) and in Buieié Site (20º 42' 22.3" S; 42º 50' 55.5" W; 703.8 m altitude), in Viçosa, Minas Gerais, Brazil. These mites were kept in arenas, which consisted of plastic trays (14.8 x 4.0 x 24.0 cm) filled with foam (2.0 cm height) kept constantly soaked with distilled water and with black PVC sheets (20.0 x 10.0 cm) on top of it. Hydrophilic cotton wool was used to cover the edges of the PVC sheets, avoiding the escaping of the mites. Some cotton threads covered by a small tent-shaped piece of PVC sheet were placed in the arena to serve as shelter and oviposition site (McMurtry and Scriven 1965; Van Rijn and

Tanigoshi 1999; Duarte et al. 2015). These rearings were kept inside larger plastic trays filled with detergent and water (approximately 1:100, v/v) to prevent the contamination among the mite populations.

Both predators were reared in similar arenas but were fed with different food sources. We used detached leaves of tomato plants infested by Aculops lycopersici (Tryon) (Acari: Eriophyidae) plus bee honey (Santa Bárbara®) diluted in distilled water (1:10) to feed P. intermedius. The population of A. lycopersici was granted by Professor Dr. Manoel Guedes C. Gondim Junior from the Federal Rural University of Pernambuco. The eriophyid mites were reared on tomato plants (Solanum lycopersicum var. Santa Clara I-5300). The colonies of A. herbicolus were fed with pollen from Typha sp. (Typhaceae) (McMurtry and Scriven 1965; Van Rijn and Tanigoshi 1999; Duarte et al. 2015), which was collected from plants found in the rural area of Viçosa, Minas Gerais, Brazil. All mite rearings were maintained under controlled conditions (25 ± 3 ºC, 70 ± 10 % RH and 12 h light) in climate rooms at the laboratory. Predatory mites were fed twice a week.

Aceria litchii control

To investigate the capacity of the predatory mites P. intermedius and A. herbicolus to control A. litchii, we accessed the dynamic of erinea on litchi plants instead of directly access the population of A. litchii. This was done for many reasons such as: (i) the microscopic size of A. litchii; (ii) the life habit of A. litchii, which lives hidden inside the erineum induced on leaves; (iii) the direct counting necessarily would demand a destructive sampling and consequently a much large number of plants to perform the experiment.

Due to the difficulty in finding litchi seedlings without A. litchii, the experiment was adapted to such condition. Thus, before starting the experiment, we counted the number of leaflets with and without erinea of each plant to evaluate the infestation level.

We then separated these plants into nine groups, each group containing four plants with the same infestation level (proportion of leaflets with erinea). Later, we distributed the treatments evenly between each group, resulting in an equal infestation level across all them. The plants were arranged in the experimental area on a grid of 12 x 3, so that each plant had as neighbouring the plants of other treatments, as much as possible.

Each litchi plant was inoculated with 20 adult females A. herbicolus or P. intermedius. The predatory mites from the rearing arenas were sucked into micropipette tips (1,000 µl) connected to a plastic hose. The largest opening of the micropipette tips was sealed with a thin mesh tissue, while the smallest end, the side in which the mites were sucked, was sealed with Parafilm®. The tips were attached to the upper branch of each plant with a cotton thread, and the parafilm was removed from the opening of the micropipette tip to allow the mites to leave. The treatments were: Ctr = no predator (control); Ah = Amblyseius herbicolus; Ah+P = Amblyseius herbicolus + Typha sp. pollen; Pi = Phytoseius intermedius. Each treatment had a total of nine replicates (plants). On treatment Ah+P, 0.15 g of pollen was added on two leaflets of each plant three times a week.

The evaluation of leaflets infested by A. litchii was done every 15 days, for 90 days, evaluating the presence of erinea on each leaflet of each plant. At the end of the experiment (90 days), the population of predatory mites was accessed on each plant. To do this, all the leaflets were detached from the plants and were put in a tagged paper bag and kept in a cooler with ice packs and transported to the laboratory. The leaflets were stored in a refrigerator at 10 ± 3° C until being evaluated under stereo microscope (Zeiss® Stemi 2000-c). Each leaflet was inspected, and the predatory mites were collected with a thin hair brush (Condor® #000) and transferred to a plastic vial (2.0 mL) with alcohol 70%. Later, the specimens were mounted on microscope slides using Hoyer’s medium and examined under a microscope (Zeiss® Axioskop 40) for identification. The specimens were deposited at the Laboratory of Acarology (Department of Entomology, Federal University of Viçosa).

Statistical analyses

The proportion of infested leaflets were analysed among treatments using a linear mixed-effects model (LME). Treatment and time were used as fixed factors and plant identity as a random factor to correct for repeated measures. Non-significant interactions and factors were removed from the model until a minimal adequate model was reached (Crawley 2012). The number of predatory mites of each species was compared per treatment with a multivariate analysis of variance (MANOVA). All the statistical analyses were done using the computer software R version 3.1.0 (R Development Core Team 2014).

Results

The litchi plants did not present a continuous shoot emission during the experiment, as noted in Figure 1. They presented a short period of shoot elongation, followed by a period of leaf maturation, before the next period of shoot emission. Moreover, it is during this peak of the flushing that occurs the induction of new galls by A. litchii (Figure 1).

Thus, we observe that the proportion of leaflets infested with A. litchii significantly increased along the time on all treatments (LME, likelihood ratio = 42.38; d.f. = 6; $p < 0.0001$; Figure 2). In overall, the plants had 20 ± 7% of leaflets with erinea at the beginning of the experiment, and 90 days later they presented 30 ± 8% of their leaflets with erinea. However, no significant difference was observed on the proportion of infested leaflets among treatments (LME, likelihood ratio = 1.60; d.f. = 4; $p = 0.66$), as well as there was no significance in the interaction between the treatment and time (LME, likelihood ratio = 5.37; d.f. = 7; $p = 0.15$).

No significant difference was observed on the number of predatory mites of each species among treatments (MANOVA; Pillai's trace = 0.51; $F_{3,84} = 0.83$; p = 0.68; Figure 3). A total of 2,097 adult mite specimens of the Phytoseiidae family were collected, identified, and assigned to 15 species (Table 1). The most abundant species were A. herbicolus, Euseius alatus De Leon, Euseius ho (De Leon), Iphiseiodes zuluagai Denmark & Muma, Leonseius regularis De Leon, and P. intermedius (Table 1). The less abundant (less than 20 individuals per species) were all gathered in the group called 'Others', as shown in Figure 3.

Discussion

Three months after the beginning of the experiment, we observed that A. herbicolus succeeded to establish in nearly 1/3 of the plants where it was released while P. intermedius established only on one out of nine plants. Additionally, other species of native predatory mites infested the litchi plants. However, the incidence of erinea on plants increased throughout the experiment on all treatments. As the litchi plant grew and produced new branches, the proportion of infested leaflets increased as well. This continuous increase of A. litchii population on infested plants with predatory mites indicates that these species may be not suitable candidates for the biological control of the erinose mite.

Eriophyid mites are small individuals that live in refuges or structures produced on their host plants (galls). Thus, they become exposed to predators and adverse climatic conditions just during their migration (Sabelis and Bruin 1996). The litchi erinose mite induces the production of a dense covering of trichomes on the abaxial leaf surface, where it could hide from their natural enemies. This mite has 110 to 135 µm in length, which allows it to move in the small spaces inside the erineum (Jeppson et al. 1975; Keifer et al. 1982; Sabelis 1996; personal observation). As just it has occurred in other systems that studied the relationship between eriophyids living in protected habitat and their predators (Lesna et al. 2004; Aratchige et al. 2007; Lima et al. 2012; Silva et al. 2016), the predators P. intermedius and A. herbicolus may not have succeeded preying on A. litchii because they were not able to enter on the erineum, due to their size. Phytoseius intermedius presents a dorsal shield with approximately 305 µm in length and 153 µm in width (Evans and Macfarlane 1961) while A. herbicolus presents dorsal length 343 µm and width 235 µm (Chant 1959).

The predatory mite P. intermedius is considered to have some morphological traits, compared to other predators, which gives it some advantage to overcome the erinea and prey upon A. litchii (McMurtry et al. 2013; Tixier and Kreiter 2014). Its small size, as well as the stout and serrate dorsal setae on P. intermedius, suggest that this predator could move more easily among the trichomes of the erineum (McMurtry et al. 2013; Tixier and Kreiter 2014). However, in our experiment, we observed an establishment of P. intermedius on only one out of the nine plants that were inoculated with this predatory mite (46 individuals were recollected in this plant). This indicates that in practice P. intermedius were not successful in exploiting A. litchii as prey within the erineum and appeared to be unable to prevent its outbreaks. The increase in the population of P. intermedius on this individual plant could be explained by the availability of eriophyid mites outside of the erinea, where these mites may become more vulnerable to predators (Sabelis 1996). Indeed, this individual plant harboured the highest number of leaflets with erinea among the plants that were infested with P. intermedius, what may indicate that a higher number of A. litchii was moving outside from old erinea to infest new leaflets with plant growth along the experiment.

Nevertheless, A. litchii may not be protected from their natural enemies all the time. As new shoots are produced on plants, the mites must leave an old erinea and walk to new leaflets aiming to colonize them. The erineum growth can vary on time since its initiation, taking hours up to few days, and within this time the mites are vulnerable to both abiotic (rain, solar radiation, dehydration, etc) and biotic factors (predators, fungi, etc) (Sabelis 1996). Melo et al. (2014) demonstrated in their study that Aceria guerreronis Keifer (Acari: Eriophyidae), an eriophyid mite that lives under the perianth of fruits of the coconut palm, survived on average for 11 h at 27 °C and 75 % RH, and could move linearly for 0.4 m. Furthermore, these authors also showed that ambulatory movement of A. guerreronis could take them to colonize other coconuts within the same bunch and perhaps also to other bunches on the same coconut palm. Thus, if a plant harbours a large population of predatory mites, as we were proposing on this study, A. litchii could face a frequent (usually fatal) encounter rate with these predators at the moment it would be moving on the litchi plant before to infest a new leaflet and induce the erineum.

Duarte et al. (2015) noted that A. herbicolus presented greater population growth when there was pollen available for it on the plant. Moreover, releasing this predator along with the addition of pollen, before the infestation by Polyphagotarsonemus latus Banks (Acari: Tarsonemidae) in chilli pepper plants, provided the establishment of predators and resulted in low P. latus numbers and the absence of symptoms of its damage on the plants. However, in our experiment, the population of A. herbicolus persisted only on one-third of the plants where they were released. On the other two-thirds, the predators were observed on insignificantly number or were extinct. A shortage of food could explain this low establishment of A. herbicolus. Along the evaluation of the experiment, we observed bees foraging on the plants and stealing part of the pollen that we added on them. These bees may have limited both the access of the predators to the pollen and its availability, therefore limiting the population growth of A. herbicolus.

Throughout our study, we also observed on litchi plants the occurrence of at least 13 phytoseiid species different from those that we released at the beginning of the experiment. That is not surprise as our experiment was carried out in an open field and the area was surrounded by natural vegetation, which probably harbours an astonishing diversity of arthropods. Consequently, a possible emigration of the predatory mites A. herbicolus and P. intermedius from litchi plants to the natural vegetation is not discarded as well. Among the predatory mite species that invaded the litchi plants, Euseius Wainstein and Iphyseiodes DeLeon genus have generalist feeding habit including in their diet insects, mites, and pollen (McMurtry et al. 2013). Euseis alatus, E. ho, I. zuluagai, and L. regularis are common phytoseiid mites in Brazil (Demite et al. 2017), since E. alatus and I. zuluagai have already been found in association with A. litchii in field surveys (Mineiro and Raga 2003; Picoli et al 2010; De Azevedo et al. 2014; Chapter 1).

These mites could possibly, then, compete for resources with the predators A. herbicolus and P. intermedius, that were the focus of our study, as well prey on each other. Traditional biological control usually consists of simple tritrophic food web involving one or two species of natural enemies (Janssen et al. 1998). So, the predatory mites invasion on our experimental plants may have led to more complex interactions (Janssen et al. 1998) among the predatory mites and them with the A. litchii and the plant. One of these interactions is the intraguild predation, which consists of the combination of exploitative competition and predation among potential competitors that use similar resources (Polis et al. 1989).

Interactions among natural enemy species can take different directions regarding their impact on the pest species. In some cases, the interaction between species of natural enemies can increase, decrease or not change the pest population (Janssen et al. 1998). Thus, these predator species often do not only attack the target pest but may also consume other natural enemies. The intraguild predation is considered widespread phenomenon among arthropod food webs and in many communities of biological control agents (Polis et al. 1989; Rosenheim et al. 1995; Janssen et al. 1998). However, other studies have shown that there is not much evidence in the literature for such negative effects of intraguild predation on biological control (Janssen et al. 2006, Rosenheim and Harmon 2006). In the case of A. litchii on litchi plants, we still do not know yet which the impact of these interactions on the dynamics of prey, predators, and biological control.

The gall induced by A. litchii creates a complex environment, which could decrease the encounter rates among predators and favour the coexistence of these diverse species on the plants. Furthermore, the environment created by the erinea might increase the availability of food resources in addition to providing shelter and protection against adverse environmental conditions and natural enemies influencing the distribution and abundance of these mites (Price et al. 1980; Jones et al. 1994, 1997; Fukui 2001; Crawford et al. 2007; Janssen et al. 2007; Cornelissen et al. 2016).

In conclusion, our study showed that neither the predator number nor the different species present on the plant did prevent the erinea increase throughout the experiment. Even the pollen addition did not provide greater population growth of A. herbicolus on the litchi plants. Despite the common occurrence in the field surveys in association with A. litchii, P. intermedius and A. herbicolus were unable to establish themselves consistently and control new infestations of the litchi erinose mite. Limitations for accessing A. litchii within the erineum may have influenced the performance of these predatory mites, which requires more detailed investigations to better understand their role as control agents of A. litchii. As well, the search for effective natural enemies to control A. litchii could also select other candidates for studies, which are smaller and can effectively enter the erinea and access A. litchii more easily.

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Table 1. Species of the Phytoseiidae family collected on litchi seedlings without predator (Ctr), with Amblyseius herbicolus (Ah), with Amblyseius herbicolus + Typha sp. pollen (Ah + P), and with Phytoseius intermedius (Pi) release.

Phytoseiidae Species	**Treatments**			
	Ctr	**Ah**	**Ah + P**	**Pi**
Amblyseius herbicolus	3	71	84	13
Amblyseius sp. 1	26	14	23	29
Amblyseius sp. 2	0	13	0	1
Euseius alatus	9	63	88	39
Euseius ho	83	86	94	106
Iphiseiodes zuluagai	176	65	139	231
Immatures	132	309	222	179
Leonseius regularis	63	112	36	0
Paraphytoseius sp.	0	0	0	1
Phytoseiulus macropilis	1	0	0	0
Phytoseius intermedius	1	184	77	61
Phytoseius woodburyi	0	1	1	3
Proprioseiopsis dominigos	17	23	32	14
Typhlodromalus manihoti	2	0	4	2
Typhlodromalus peregrinus	0	1	1	0
Typhlodromina sp.	0	0	1	3
Total	513	942	802	682

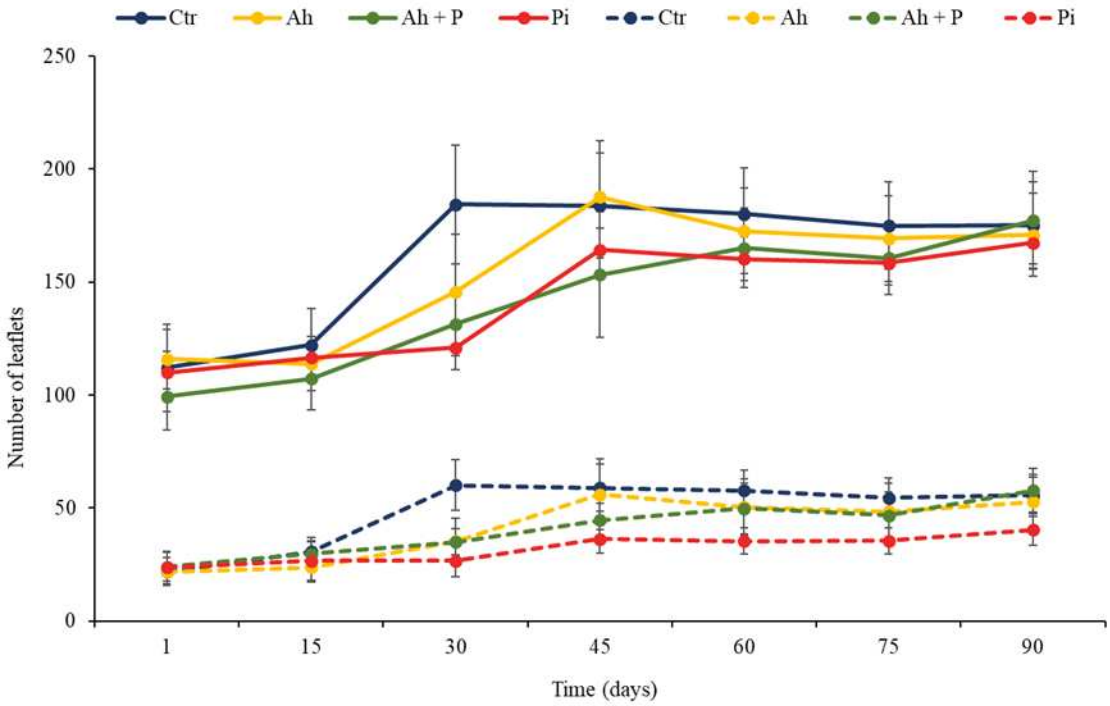

Figure 1. Total number of leaflets (±SE) (drawn lines) and number of leaflets with erineum (±SE) (dashed lines) on litchi seedlings without predator (Ctr), with Amblyseius herbicolus (Ah), with Amblyseius herbicolus + Typha sp. pollen (Ah + P), and with Phytoseius intermedius (Pi) release.

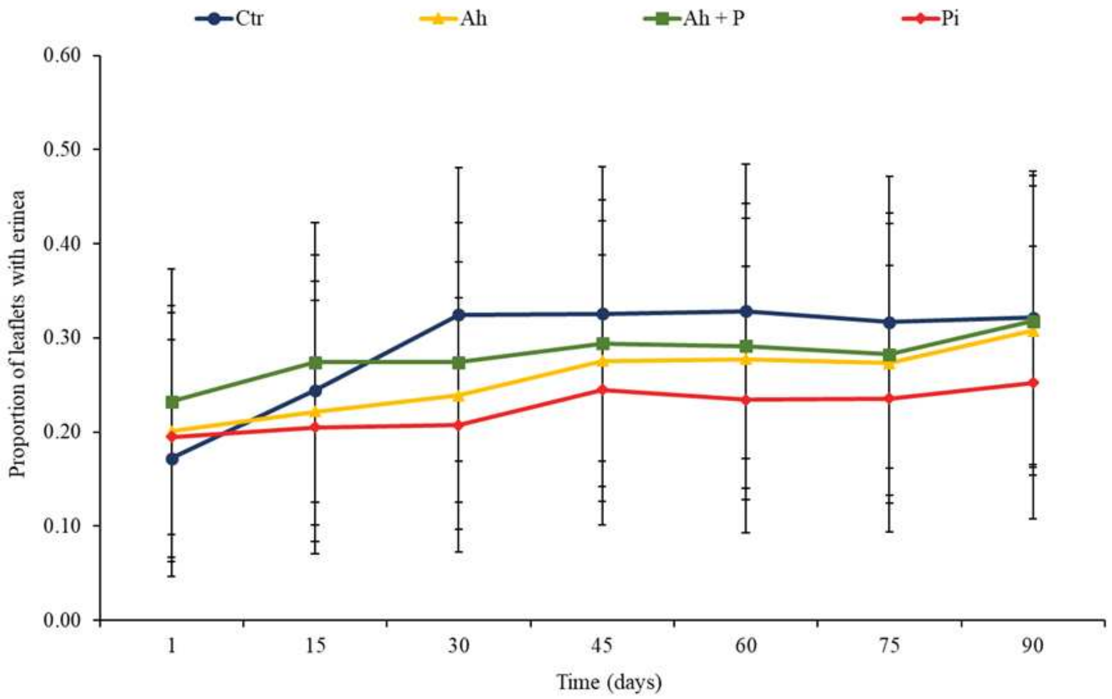

Figure 2. Proportion of leaflets with erinea (±SE) on litchi seedlings without predator (Ctr), with Amblyseius herbicolus (Ah), with Amblyseius herbicolus + Typha sp. pollen (Ah + P), and with Phytoseius intermedius (Pi) release.

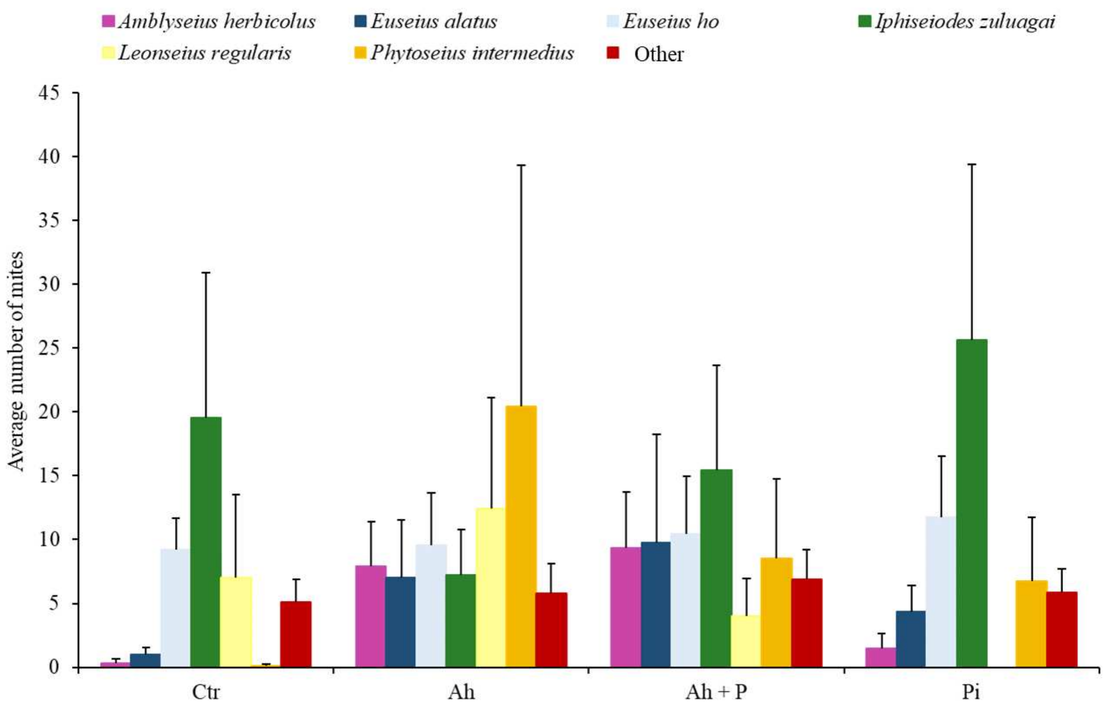

Figure 3. Average number of mites of the Phytoseiidae family on litchi seedlings without predator (Ctr), with Amblyseius herbicolus (Ah), with Amblyseius herbicolus + Typha sp. pollen (Ah + P), and with Phytoseius intermedius (Pi) release.

FINAL CONSIDERATIONS

Aceria litchii emergence is relatively recent in Brazil, which could indicate us that the process of colonization by the natural enemy species associated to this mite might be still in progress and the search for these organisms associated with it in the field is still necessary. Our study showed that the arthropods community associated to erinea induced by A. litchii is quite diverse and that the ecological interactions involving galls can be complex. Furthermore, among the species found, many of them were of predatory mites, being a greater number than the previous studies carried out in litchi in Brazil.

Two new species of the family Iolinidae, Pausia litchiae **n. sp.** e Pseudopronematulus nadirae **n. sp.** were also described and illustrated. The mites of this family are not always considered in field surveys on agricultural crops, but they should be more studied to assess their potential as predators for eriophyid mites, such as A. litchii, since they are smaller mites, they could more easily enter the erineum and prey on A. litchii.

The biological control of eriophyid mites consists primarily on the use of predatory mite of the Phytoseiidae family. However, in our study, the species P. intermedius and A. herbicolus were unable to establish themselves consistently and prevent the erinea to increase throughout the experiment, under the field conditions studied. Even the pollen addition did not provide greater population growth of A. herbicolus on the litchi plants.

So, limitations for accessing A. litchii within the erineum may have influenced the performance of these predatory mites, which requires more detailed investigations to better understand their role as control agents of A. litchii. Thus, the complex interactions among the predatory mites and among them with the A. litchii and the litchi plant must be further elucidated.