CLEBER MACEDO DE OLIVEIRA

INTERACTIONS OF *Ricoseius loxocheles* (ACARI: PHYTOSEIIDAE) AND COFFEE LEAF RUST

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

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APROVADA: 12 de julho de 2012.

Marcos Antonio Matiello Fadini

Andre Luís Matioli

João Alfredo Marinho Ferreira
Coorientador

Madelaine Venzon
Coorientadora

Angelo Pallini Filho
(Orientador)

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BIOGRAFIA

Cleber Macedo de Oliveira, filho de Maria da Consolação Macedo Gomes de Oliveira e Dimas Miranda de Oliveira, nasceu em Ipatinga, Minas Gerais em 15 de julho de 1986.

Iniciou o curso de Agronomia no ano de 2005 na Faculdade Federal Integrada de Diamantina (FAFEID), atualmente Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) e em julho de 2007 transferiu-se para a Universidade Federal de Viçosa. Em julho de 2010 obteve o título de Engenheiro Agrônomo pela Universidade Federal de Viçosa. Durante a graduação foi monitor das disciplinas Hidráulica, Irrigação e Drenagem e Entomologia Agrícola. Foi bolsista de Iniciação Científica por dois anos sob orientação do Pesquisador Marcos Antonio Matiello Fadini no Laboratório de Entomologia na Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG). Após esse período foi estagiário voluntário no Laboratório de Acarologia da Universidade Federal de Viçosa sob orientação do pesquisar Angelo Pallini.

Em agosto de 2010 iniciou o curso de Mestrado em Entomologia pela Universidade Federal de Viçosa, também sob orientação do pesquisador Angelo Pallini e coorientação de João Alfredo Marinho Ferreira e Madelaine Venzon. Em julho de 2012 submeteu-se a defesa da dissertação tendo como membros da banca os pesquisadores já citados e Marcos Antonio Matiello Fadini e Andre Luis Matioli.

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SUMARY

ABSTRACT

OLIVEIRA, Cleber Macedo, M.Sc., Universidade Federal de Viçosa, july 2012. **INTERACTIONS OF *Ricoseius loxocheles* (ACARI: PHYTOSEIIDAE) AND COFFEE LEAF RUST**. Adviser: Angelo Pallini Filho. Co-advisers: João Alfredo Marinho Ferreira e Madelaine Venzon.

Coffee crops have economic losses due pests and diseases. Among them, the phytophagous mites deserve attention due to the reducing photosynthetic area caused on the leaves. Besides of mite damages, some pathogens also attack coffee plants, as the coffee rust fungus, *Hemileia vastatrix* (Uredinales). This pathogen cause yield losses around 30% in some varieties of *Coffea arabica*. Predatory mites from the Phytoseiidae family normally control phytophagous mites and can develop and reproduce using various alternative food sources than their primary prey, tetranychid mites. Within the Phytoseiidae family that is composed by carnivorous and pollen-feeding mites, we surveyed in the field the species *Ricoseius loxocheles* (De Leon) (Acari: Phytoseiidae) on the necrosis area caused by coffee rust during its reproductive phase. Searching in the literature showed that there is little information related to this mite development, biological parameters and feeding habits. So, this study was carried out to assess the taxonomy, development, survivorship and reproduction parameters of *R. loxocheles* feeding on coffee rust fungus, its predation capacity on *Oligonychus ilicis* (McGregor, 1971) and its reproductive success. Coffee rust supported the survival, development and reproduction of the phytoseiid mite and that it was not able to feed on different stages of *O. ilicis*. Survival and oviposition of *R. loxocheles* was only observed when this mite was in arenas with fungi. In arenas without fungi these parameters were equal as arenas without food. It is known that reproduction has a higher nutritional requirement than development. The fertility of *R. loxocheles* fed on coffee rust is highest that the other phytoseiid fed in others fungi or some mites or pollen. We suggest that this mite is a phytoseiid generalist and it is necessary more studies to measure the ability of this mite specie to feed on other food sources, such as herbivore mites pests of coffee crops. It is possible that *R. loxocheles* has a role in the control of coffee rust since it feeds on a large amount of rust uredospore.

RESUMO

OLIVEIRA, Cleber Macedo, M.Sc., Universidade Federal de Viçosa, julho de 2012. **INTERAÇÃO ENTRE *Ricoseius loxocheles* (ACARI: PHYTOSEIIDAE) E FERRUGEM-DO-CAFEEIRO**. Orientador: Angelo Pallini Filho. Coorientadores: João Alfredo Marinho Ferreira e Madelaine Venzon.

O cafeeiro apresenta perdas econômicas devido a pragas e doenças. Entre as pragas, os ácaros fitófagos merecem atenção devido à redução da área fotossintética. Além dos danos dos ácaros, alguns patógenos também atacam plantas de café como a ferrugem-do-cafeeiro, *Hemileia vastatrix* (Uredinales). Este patógeno causa perdas de produtividade de 30% em algumas variedades de *Coffea arabica*. Ácaros da família Phytoseiidae normalmente controlam populações de ácaros fitófagos e podem desenvolver-se e reproduzirem usando diversas fontes alimentares, além dos ácaros presa. Dentro da família Phytoseiidae, que é composto por ácaros carnívoros e os que se alimentam de pólen, em levantamentos foi encontrado a espécie *Ricoseius loxocheles* (De Leon) (Acari: Phytoseiidae) sobre áreas de necrose causada pela ferrugem do cafeeiro, durante sua fase reprodutiva. Existem poucas informações sobre o seu desenvolvimento, parâmetros biológicos e hábitos alimentares. Avaliou-se a taxonomia, desenvolvimento, parâmetros de sobrevivência e reprodução de *R. loxocheles* alimentando-se de urediosporos da ferrugem do cafeeiro e sua capacidade predatória e sucesso reprodutivo sobre *Oligonychus ilicis* (McGregor, 1971). Uredosporos da ferrugem-do-cafeeiro apoiaram a sobrevivência, desenvolvimento e reprodução do ácaro fitoseídeo estudado e este não foi capaz de predar *O. ilicis*. A sobrevivência e oviposição de *R. loxocheles* só foi observada quando este ácaro alimentou-se de ferrugem. Em arenas sem o fungo esses parâmetros foram iguais as arenas sem alimento. A reprodução tem a exigência nutricional mais elevada do que o desenvolvimento. *R. loxocheles* alimentados com ferrugem obteve parâmetros reprodutivos mais elevados do que outros fitoseídeos alimentados em outros fungos, alguns ácaros ou pólen. Conclui-se que este ácaro é um fitoseídeo generalista e são necessários mais estudos para medir a capacidade de alimentarem-se em outras fontes alimentares, tais como ácaros fitófagos pragas do cafeeiro. É possível que *R. loxocheles* tenha um papel no controle da ferrugem do cafeeiro, uma vez que se alimenta de uma grande quantidade de uredosporos da ferrugem.

CHAPTER 1

GENERAL INTRODUCTION

Among the pests that can feed on coffee plants, the herbivorous mites *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae), *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) and *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae) are the most important on coffee plants in Brazil (Reis and Zacarias 2007; Moraes and Flechtmann 2008).

Oligonychus ilicis is usually known as coffee-red-mite and usually feeds on the upper surface of the leaves. The area used for feeding becomes brown and covered with a delicate web (Moraes and Flechtmann 2008; Reis et al. 1997). Long periods without rain increase its population; its damage can cause defoliation and retard the development of new plants. They occur in small spots, in special around the area near places where there is more dust, and it can affect the entire crop in case of absence of control (Reis e Souza, 1986). Additionally, the use of some chemical pesticides (i.g. pyretroids) would increase the mite population because of the effect of this product on the natural enemies (Moraes e Flechtmann 2008).

Brevipalpus phoenicis is a polyphagous and cosmopolitan mite, which proliferates more during the drier seasons of the year (Oliveira 1986, 1995; Reis et al. 2000). *B. phoenicis* are usually known as ring-spot mite because this species is a vector of the coffee-ring-spot virus (CoRSV) (Chagas 1973; Chagas et al 2003). They can be found on both surfaces of the leaf and in lower densities than *O. ilicis*. The ringspot disease is present in the main coffee producing areas in Brazil. The symptoms are intense defoliation and fruit drop in consequence of the development of the disease (Chagas et al. 2003).

Polyphagotarsonemus latus is a polyphagous mite that is commonly found feeding on coffee plants and some other plant species. This mite species prefers to infest leaves on new branches. When the population is very high, the new leaves become curved and later on became dry. Until now, there is no study in the literature confirming this species as a severe pest on coffee plants. However, it is necessary to control this mite species when under high humidity conditions in nurseries, otherwise the plants cannot grow new leaves and there would be a delay on the development of the plant (Reis and Zacarias 2007).

Predatory mites that belong to the Phytoseiidae family are the most important natural enemies of the herbivore mites (Moraes 1991). Among them, *Iphiseiodes zuluagai* Denmark & Muma and *Amblyseius herbicolus* (Chant) (Acari: Phytoseiidae: Amblyseiinae) are the most common species of predatory mites found on coffee plants (Reis and Zacarias 2007; Carvalho et al. 2012; Franco et al. 2008). These species are associated to the natural control of *O. ilicis* and *B. phoenicis* in the south of Minas Gerais State (Pallini Filho et al. 1992).

Coffee plants can host of different pathogens, such as fungi, bacteria and nematodes, both in nursery and in the field. The incidence of pathogens in coffee crops is a factor that can reduce the productivity and the quality of the coffee beverage while increasing the production costs (Godoy et al. 1997).

The coffee rust *Hemileia vastatrix* Berk and Br. has been the main problem of coffee crops everywhere in the world. There are 32 different races of the coffee rust fungus (I, II, III and XV races are the most important ones) and they are well established in 12 American countries: Brazil, Paraguay, Argentina, Bolivia, Peru, Ecuador, Colombia, Nicaragua, El Salvador, Honduras, Guatemala and Mexico (Godoy et al. 1997).

Coffee rust can lead to production losses as high as 30% on favourable conditions to the disease. Losses can reach more than 50% in *Coffee arabica* (Zambolim et al. 2005;

Zambolim et al. 1997; Shiomi et al. 2006). The main damage caused by coffee rust is the premature fall of leaves and desiccation of branches, causing reduction in plant productivity and the productive life of the crop and its control is accomplished with protectant fungicides or systemic pesticides, often not selective to insects (Zambolim et al. 1997). This method of control has increased the cost of production, degradation of natural resource, problems of poisoning by pesticide applicators, increased risks of residues, and the emergence of resistant races of the fungus. These reasons have led to a growing demand for disease management practices and to fungicides of lower cost and toxicity (Zambolim and Vale 1999).

Several studies reported the biological control of fungi by the use of Tydeidae mites (English-Loeb et al. 1999; Norton et al. 2000; Norton et al. 2001; English-Loeb et al. 2005). However, the most studied case refers to the tri-trophic interaction (mite-fungus-plant) with vine plants infected by the fungus *Uncinula necator* (Schwein.). This pathogen causes low quality of the fruit and high economic losses but at the same time is a food source of a Tydeidae mite [(*Orthotydeus lambi*) (Baker) (Acari: Tydeidae)] (English-Loeb et al. 1999; Norton et al. 2000; English-Loeb et al. 2005). Norton et al. (2001) suggested that the host plant could mediate this tri-trophic interaction in order to control the pathogen because vine plants have domatia on their leaves that are inhabited by Tydeidae mites (Norton et al. 2000). These domatia can contribute to maintain the fungivorous mite population on the plant (Norton et al. 2001).

The Phytoseiidae mite *Ricoseius loxocheles*is De Leon (Axari: Phytoseiidae) is commonly found in coffee crops infested by *H. vastatrix* in the Zona da Mata Mineira (personal observation). It is the only species belonging to the genus *Ricoseius* Ribaga in the subfamily Amblyseiinae Muma (Figure 1). According to Fletchman (1976) this mite species can feed on uredospores of coffee rust, suggesting that this species may have importance in the dissemination of the pathogen. However, none study was carried out to certify whether this mite species can play as a vector of the coffee rust. The aim of this thesis was to study the tri-trophic interaction in coffee crops in order to evaluate the biological role of the predatory mite *R. loxocheles* in the *C. arabica*-*H. vastatrix*-phytophagous mites system. There are few studies about the interaction mites-pathogen-plant and none published study about this mite species so far.

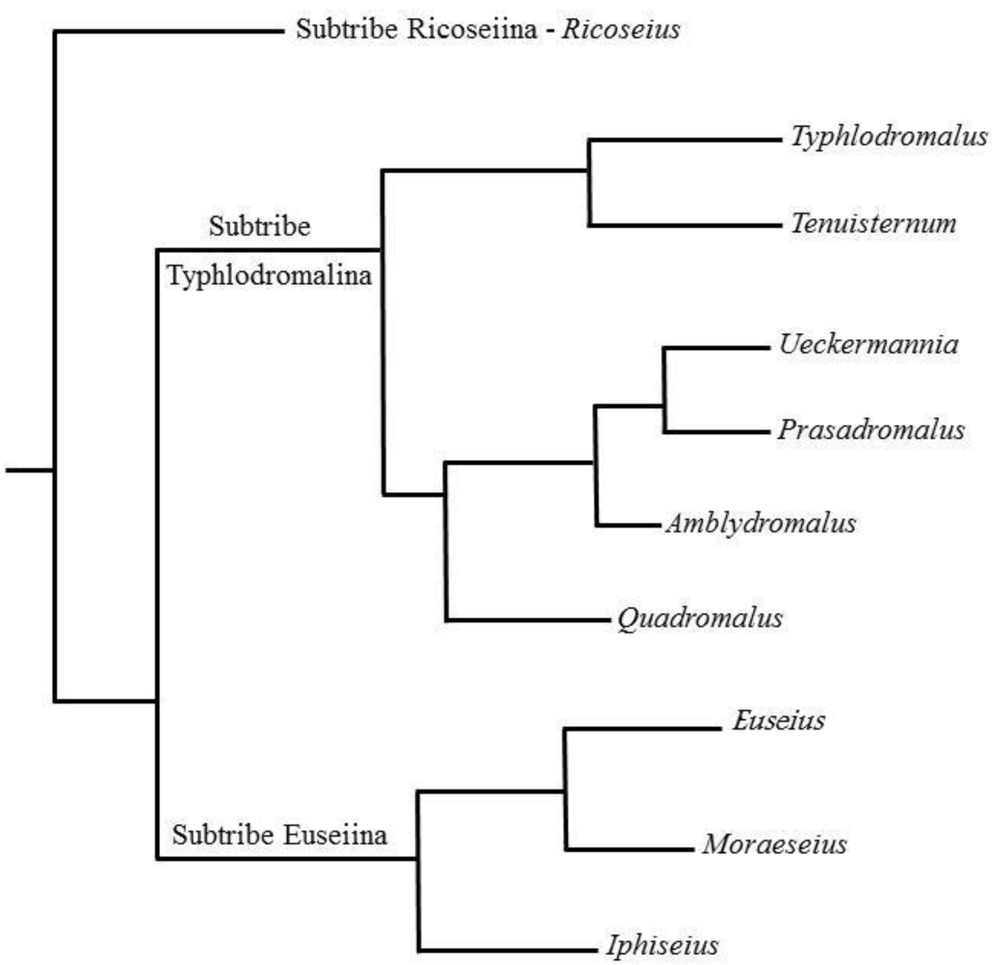

Figure 1. Proposed cladogram of the Euseiini tribe, adapted from Chant and McMurtry (2005).

In the Chapter 2, I studied the taxonomy and biological parameters of *R. loxocheles* feeding on coffee rust, estimating the life and fertility table parameters and the intrinsic population grow rate..

In the Chapter 3, I studied the predation rate of *R. loxocheles* on *O. ilicis* and its influence in regulating a population of the coffee-red-mite.

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CHAPTER 2

Taxonomy, biological parameters and life table of the phytoseiid mite *Ricoseius loxocheles* De Leon (Acari: Phytoseiidae) feeding on coffee rust (*Hemileia vastatrix*)

Abstract

The leaf surface of coffee is inhabited by an extraordinary diversity of mites. Many of these mites are understudied and our knowledge about their biology and food. Habits is poor Plant pathologists have investigated various microorganisms as potential biological control agents of microbial plant pathogens. Some pathogens also attack coffee plants, especially the coffee rust *Hemileia vastatrix* (Uredinales). This pathogen can cause yield losses around 30% in some varieties of *Coffea arabica*. Predatory mites of the Phytoseiidae family normally control herbivorous mites but can also develop and reproduce on various diets. The family is composed basically by carnivorous and pollen-feeding mites. We found the species *Ricoseius loxocheles* (De Leon) (Acari: Phytoseiidae) on the necrotic areas caused by coffee rust during the reproductive phase of the fungus. A literature search showed that there is little information about the development and biological parameters of this species. We assessed the taxonomy, development, survivorship and reproduction parameters of *R. loxocheles* feeding on coffee rust fungus. The mite can survive, develop and reproduce successfully on a diet of coffee rust. It is known that reproduction has a higher nutritional requirement than development. The fertility of *R. loxocheles* fed on coffee rust is higher than other phytoseiids fed in other fungi, some mites or pollen. Additional studies are necessary to investigate the use of other food sources by this predatory species, such as herbivorous mites that are pests on coffee.

Key words: Prey stages; Phytophagous mites; Life-history parameters

Introduction

Biological control with natural enemies is a viable alternative to chemical control (Van Driesche et al. 2008; Hajek, 2004; Colfer et al. 2003; Onzo et al. 2012). The use of natural enemies prevents environmental risks associated with chemical pesticides, such as pest resistance, environmental pollution, and worker health impacts. Several predatory mite species in the family Phytoseiidae have been used for t biological control of phytophagous mites (Colfer et al. 2003; Hanna et al. 2005; McMurtry and Croft 1997).

Phytoseiidae mite species have been classified as specialists or generalists according to their feeding habits (McMurtry and Croft 1997; Schausberger and Croft 2000a; b). Some of them are specialists adapted to feed on some mites that produce quite dense webs such as Tetranychidae. Generalists can use alternative food when prey are absent such as pollen, nectar (Schausberger and Croft 2000a) and fungi (Zemek and Prenerova 1997).

Besides the damage caused by phytophagous on coffee crops, some pathogens also attack the crop, especially the coffee rust, *Hemileia vastatrix* Berk and Br. This fungus can lead to yield losses as high as 30% on favourable conditions to the disease (Zambolim et al. 2005; Zambolim et al. 1997; Shiomi et al. 2006). The main damage caused by the coffee rust is the premature fall of leaves and desiccation of branches, causing reduction in plant yield and in the productive crop life (Zambolim et al. 1997).

Rust control is based on spraying of protectant fungicides or systemic pesticides, often not selective to beneficial insects and this method of control has increased the cost of production, degradation of natural resources, causes poisoning on applicators, increased risks of residues on the final products and the emergence of resistant races of the fungus. These reasons have led to a growing demand for disease management practices more sustainable and fungicides with lower cost and toxicity (Zambolim and Vale 1999).

There is a high diversity of mites in the coffee plants (Mineiro et al. 2001; 2006a; 2006b; Mineiro et al. 2008; Spongoski et al. 2005), but the studies about mite diversity on coffee have target mainly the phytophagous mites (Mineiro et al. 2001). Studies about predatory, fungivorous and mites with other food habits on this crop are few (Pallini Filho et al. 1992). The Phytoseiidae family is very abundant on coffee (Spongoski et al. 2005). There is not a close relationship between phytoseiid mites with fungal spores (McMurtry and Croft, 1997), but *Ricoseius loxocheles* De Leon mite belongs the Phytoseiidae family was found feeding on coffee rust (*Hemileia vastatrix*) but their interaction is unknown. It is known very little about the feeding ecology of these mites or if they provide any measurable benefit to their host plants.

Flechtmann (1976) commented that *R. loxocheles* can probably feed on uredospore of coffee rust and possibly spread the disease among plants due to its behaviour of moving actively through the leaves. Whether this species should be considered a possible agent of control or of spreading coffee rust is unknown.

To partly answer this question, we assess the taxonomy, development, survivorship and reproduction parameters of *R. loxocheles* feeding on coffee rust. The knowledge of the biology of these phytoseiid is important to understand their role in the coffee crops.

Materials and methods

Stock culture of *R. loxocheles*

Ricoseius loxocheles were collected from coffee plants infected by rust (*H. vastatrix*) in July 2010 in Viçosa (20° 45' 14" S 42° 52' 55" W), State of Minas Gerais, Brazil. The colony was reared on arenas that consist on underside coffee leaves infected by rust, placed underside on top of a moist sponge. The arenas were placed inside trays (250 x

150 x 50 mm) (Figure 2). The edges of the leaves were surrounded by moistened cotton to prevent mites from escaping. The arenas were replaced when its turgidity was severely reduced. One small piece of plastic was used with a shelter to mites. Rearing and experiment were kept inside a climate chamber at 25 ± 1°C, 60 ± 10% RH and at 12:12 light: dark cycle. Arenas were examined daily. When over-population of the mites was observed, predators were transferred to new arenas infected with rust.

Figure 2. Rearing arenas of *R. loxocheles* on coffee leaves infected by coffee rust.

Taxonomy

For the taxonomy studies, the measurements are given in micrometers (μm) and nomenclature setae is based on Chant and McMurtry(1994).

Immature development

To determine the development and survival of immature mites, eggs with the same age were used in the experiment and they were obtained from egg-waves. The egg-wave consisted in several adult females allowed to lay eggs on detached rust coffee infected leaves on wet cotton wool, placed inside a tray. The adults were removed after 24 h and the eggs were carefully transferred to experimental units. Each experimental unit consisted of coffee leaf disk (0.8 cm in diameter) infected by rust, placed underside up in a disk with agar (0.8 cm in diameter) inside a Petri dish (2cm Ø, 1cm high). Water was added in the Petri dish to prevent the escape of mites and to keep the disk turgid. When the leaf disk was deteriorating, it was taken from the experimental unit and placed it on the top of a new arena to allow the mites to move on to it. The duration and survivorship of each stage was determined by observations that were carried out at 7 am, 12 am and 6 pm. At the end of the immature development, the sex ratio was determined. All developmental stages of the mite were photographed with a digital camera (AxioCam ERc5s) coupled to a stereomicroscope (Zeiss 2000 - C) with increase of 3.2 x or 5.0 x.

Reproduction and life table parameters

Newly mated females of *R. loxocheles* (9 days old) were taken from the egg-wave. They were transferred to each experimental unit as described above, with 3.2 cm Ø and the Petri dish used had 3.5 cm Ø and 4.0 cm high. The mites were observed at 12h-intervals to record the first oviposition date. After the first oviposition the mites were observed at a 24-h interval. The number of eggs laid was recorded daily until the female died.

The life table was built with the experimental data obtained above. The net reproduction rate (R_o), the mean generation time (T), the doubling time (D_t), and the finite rate of increase (λ) were calculated using the method recommend by Birch (1948):

$$R_0 = \Sigma\,(l_x m_x)$$

$$\mathrm{T} = \Sigma\,(m_x l_x x)/(m_x l_x)$$

$$\mathrm{Dt} = Ln\,(2)/r_m$$

$$\lambda = e^{r_m}$$

The intrinsic rate of increase (r_m) was calculated by Lotka equation (Carey, 1993):

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

The m_x is the number of female offspring produced per female at age x; l_x is the proportion of females alive (survival) from birth to age x; $m_x l_x$ is the total number of females produced by female during the time interval. The finite rate of increase (λ) represents the number of individuals added to the population/time/female that will result on females. The Jackknife procedure was used to estimate a standard error for the r values (Meyer et al. 1986).

Results

Taxonomy

Subtribe Ricoseina Chant and McMurtry (Figure 3, 4 and 5)

Ricoseiina Chant and Mc Murtry, 2005: 192.

Type genus: *Amblyseius (Ricoseius)* De Leon, 1965a: 128

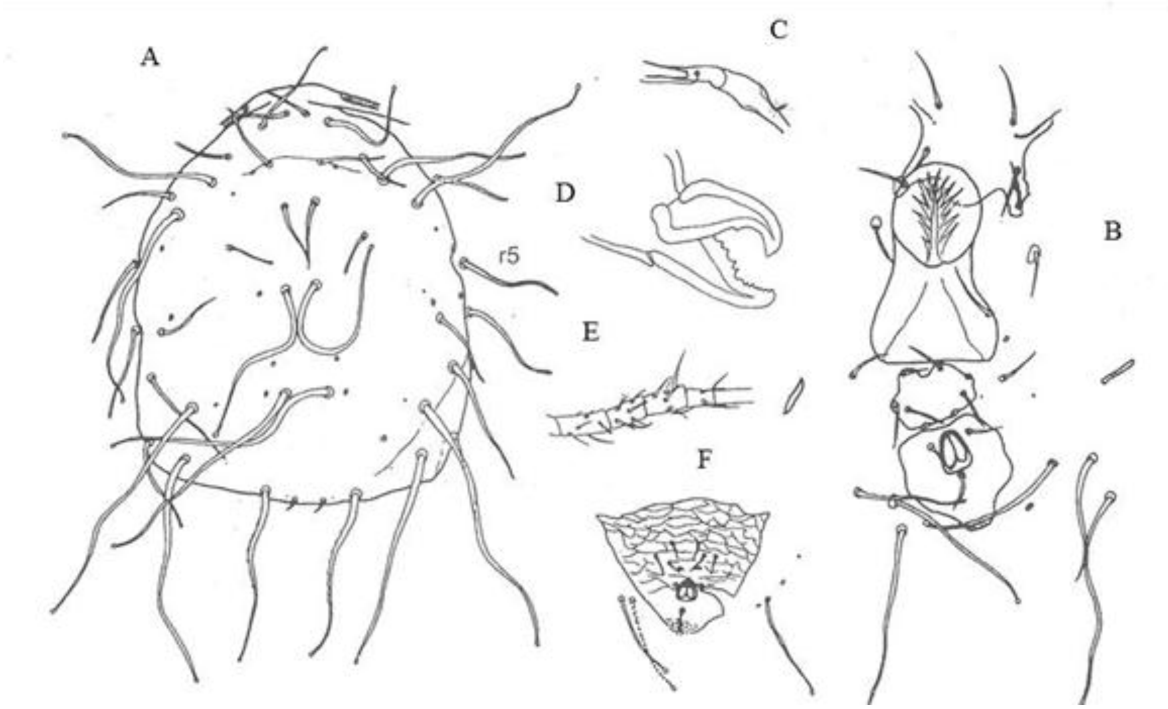

Figure 3. *Ricoseius loxocheles* (De Leon), 1965 – A. dorsal shield; B. female ventral surface; C. spermatheca; D. chelicera; E. leg IV; F. male ventrianal shield, adapted from De Moraes et al. (2004).

The adult female of this monotypic tribe has female idiosomal setal pattern 11A:9B/JV-3:ZV, with 34 pairs of setae, with seta r5 present. Some specimens have the female ventrianal shield divided into separate ventral and anal plates, while other specimens apparently do not have this feature.

Setae r5 retained on 3 other species in the family Phytoseiidae, all in the subfamily Amblyseiinae: *Archeosetus rackae*, *Evansoseius macfarlanei* and *Macroseius biscutatus*.

There is one monotypic genus in the tribe Ricoseiina.

Ricoseius De Leon (Figure 3).

Amblyseius (Ricoseius) De Leon, 1965a: 128.

Ricoseius, Muma and Denmark, 1968: 233.

Type species: *Amblyseius (Ricoseius) loxocheles* De Leon, 1965a: 128.

The single species in this genus has the dorsal shield smooth ovoid with the posterior margin squared (only adults) and with a marginal notch at the level of seta r5. Seta r5 present in addition to setae r3 and R1. Most dorsal setae elongate, extraordinarily sinuous on mounted specimens with the tips minutely knobbed. Setae J2 and J4 inserted somewhat behind their usual positions. Setae S4 and Z5 extremely elongate, the latter longer than setae Z5, which is usually the longest seta on the dorsal shield. Ratio seta s4:Z1 = 3.8:1.0, the result of an extreme lengthening of seta s4. Sternal shield poorly sclerotized with posterior projection, with ST1-3 inserted on the shield. Female ventrialnal shield reduced, divided into separate ventral and a pair of prominent crescentic pores. There has been some forward migration of setae JV2 and ZV2. Setae JV4, JV5 and ZV3 elongate with minute terminal knobs. Peritreme short extending only to level of setae j3, peritremal shield narrow, fused anteriorly with dorsal shield. Chelicerae well developed, fixed digit with 11-12 prominent teeth evenly spaced along the digit (Figure 3D). Deutosternal groove 4-6 µm wide, somewhat narrower than with the subtribe Euseiina and within the range for the subtribe Typhlodromalina. All legs with somedorsal setae distally knobbed but none greatly elongate. The chetotaxy on leg IV on femur, genu and tibia is 5-7-6 to male, female and deutonymphs and 4-5-6 to protonymphs. Measurements of body length and body width are female (595±25.6 µm; 297±16.7 µm), male (420±22.2 µm; 231±16.1 µm), deutonymph (511±90.2 µm; 255±35.4 µm) and protonymph (373±17.7 µm; 221±30.1 µm). To difference adult female from deutonymph, the opistossomal setae are tinner and spermatheca is not present.

R. loxocheles is known only from plant material collected in Brazil (Minas Gerais, Goias and São Paulo States), Puerto Rico, Cuba and Florida (De Leon and Tennesse 1965;

Denmark HA and Muma MH 1973; Flechtmann, 1976; Ramos & Rodríguez, 2006; Montes et al. 2010; Verona, 2010; Rezende & Lofego, 2011).

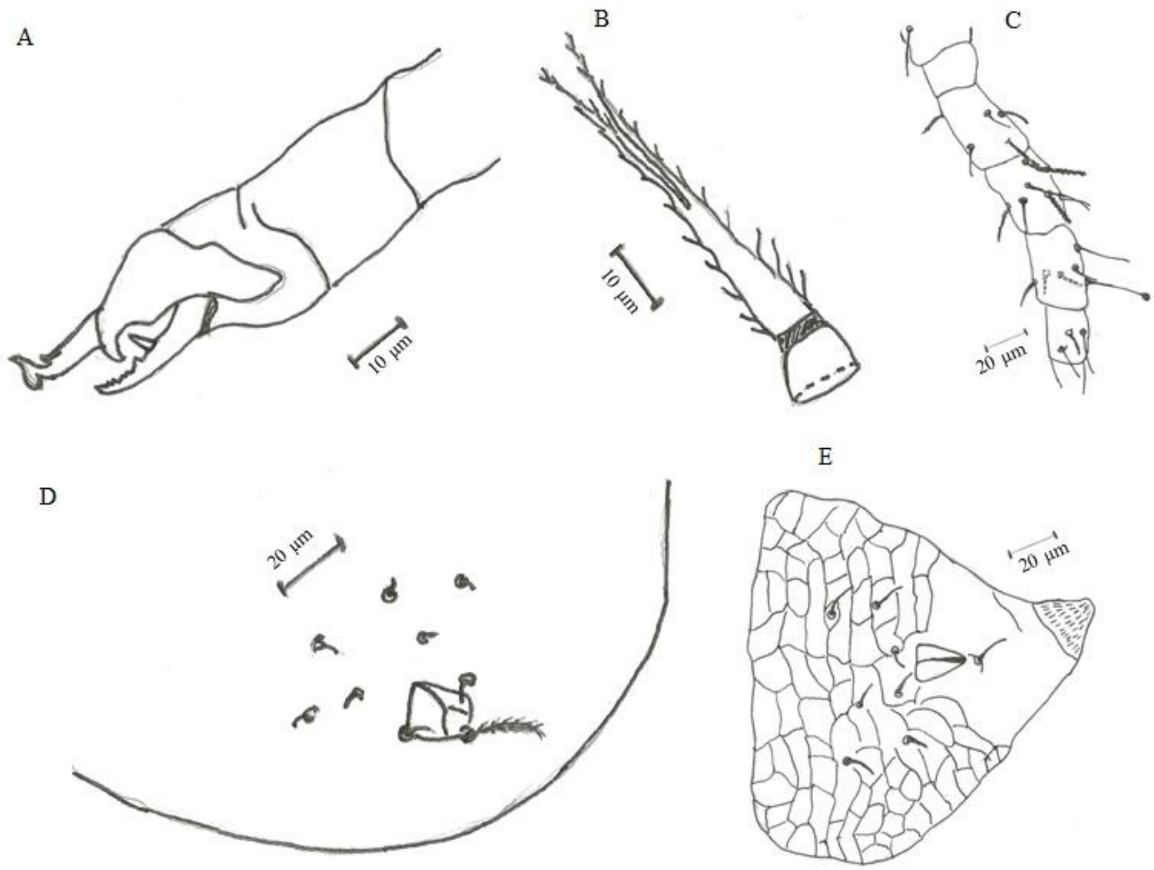

Figure 4. *Ricoseius loxocheles* (De Leon), 1965 A - Espermadactilum ♂; B – Tritosternum ♀; C - Leg IV ♂; D – Opsitonotum Protonymph; E – Ventrianal shield ♂.

Immature development

The eggs are translucent and have an elliptical shape, characteristic of the phytoseiids. These mites usually oviposit near the vein among the uredospores rust, and the eggs are covered with coffee rust uredospore. The egg stage was the longest phase of the immature development (Table 1).

The larvae have three pairs of legs, whereas the other mobile stages have four pairs of legs. The shortest stage of development was the deutonymph (Table 1). The adult body has the shape of water drop, with the podosoma being narrower than opisthosoma, typical for many species of phytoseiids (Figure 3).

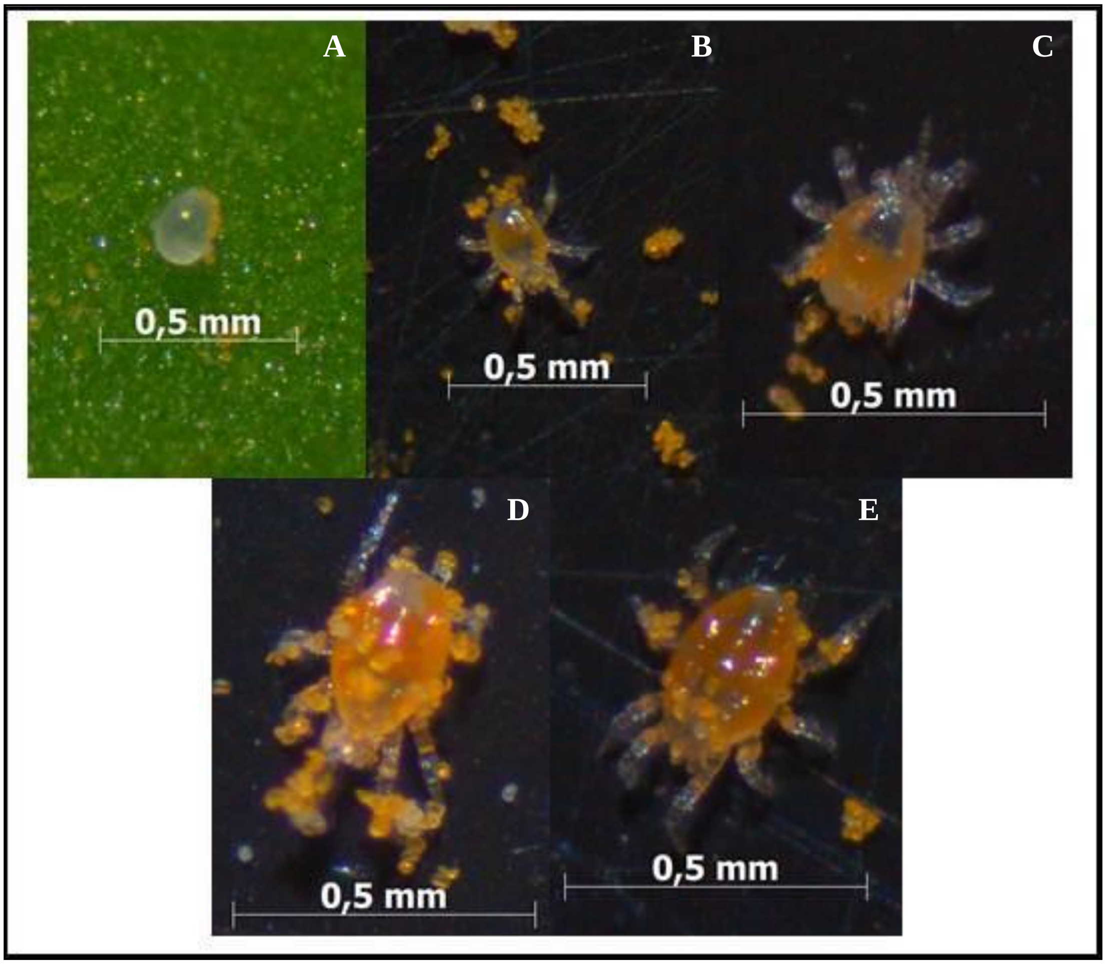

Figure 6. Development stages of *Ricoseius loxocheles*. A) Egg; B) Larvae; C) Protonymph; D) Deutonymph; E) Adult.

Survival varied with development stage. The higher survival was the larva stage. The highest mortality was observed with protonymphs (Table 1).

Table 1. Duration (days ±S.E.) and survival of immature of *Ricoseius loxocheles* feeding on uredospore of coffee rust, *Hemileia vastatrix*.

Stage	Duration	Survival (%)
Egg	2.96 ± 0.25	88.89
Larva	1.96 ± 0.14	95.83
Protonymph	2.09 ± 0.22	86.96
Deutonymph	1.80 ± 0.21	95.00
Egg to adult	8.88 ± 0.29	70.37

Reproduction and life table parameters

The daily oviposition rate and the total egg production by females were low (Table 2). The sex ratio observed was the 85.9%.

Table 2. Duration (days ± S.E.) of reproductive parameters of the adult phase, longevity and oviposition rates of *Ricoseius loxocheles* feeding on uredospore of coffee rust, *Hemileia vastatrix*.

Parameter	
Pre-oviposition in days	3.59 ± 0.60
Oviposition in days	12.95 ± 3.05
Post-oviposition in days	3.05 ± 0.59
Longevity in days	19.59 ± 0.84
No. of eggs/female/day	0.76 ± 0.07
No. of eggs/female	9.47 ± 0.91

The intrinsic rate of increase (r_m) was 0.09 (0.09086545 - 0.09217413) females/female/day and the finite growth rate (λ) equal to 1.09. The average duration of each generation (T) was 18.90 days. The number of offspring produced per female (m_x) reached a peak on day 13 of adulthood (1.06) and decreased from this time to reach zero at

23^th^ days (Figure 4). The population has the potential to double every 7.63 days.

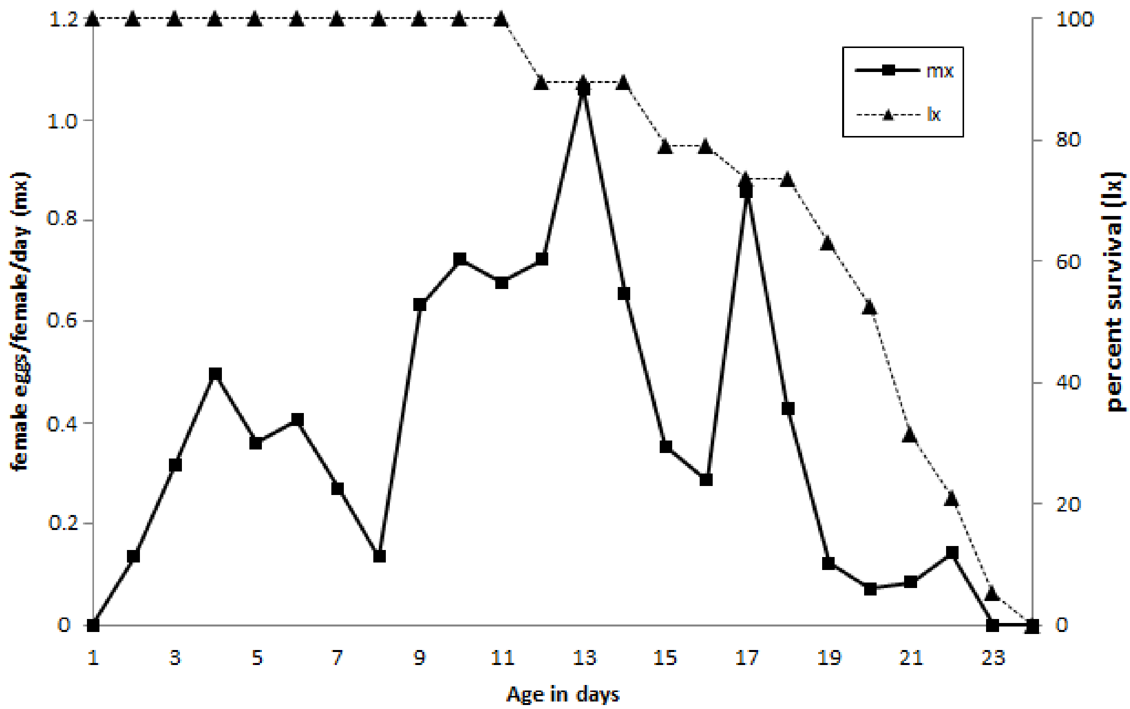

Figure 7. Age-specific survivorship (l_x) and age-specific birth rate (m_x) of *R. loxocheles* fed on coffee rust.

Discussion

This is the first study that involves *R. loxocheles*. We compared our data with species belonging to another genus, but from the same tribe (Euseiini) in which *R. Loxocheles* belongs

Ricoseius loxocheles can develop and reproduce successfully when feeding exclusively on coffee rust. According the definition of Overmeer (1985) uredospores can be considered an alternative food for *R. loxocheles*, because mites can not only survive and develop, but also reproduce when feeding on it.

The results are consistent with previous studies on other fungi-phytoseiid relationships which also demonstrate the ability of other species of phytoseiid mites to develop and reproduce successfully when feeding on other species of fungi (Bakker and Klein 1992; Zemek and Prenerova 1997; Pozzebon and Duso 2008). For example, grape leaves infected by *Plasmopara viticola* were a suitable substrate for the development and reproduction of *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Pozzebon and Duso 2008). The former mite can develop sucessfully when feeding on *Erysiphe orontii* conidia (Zemek and Prenerova 1997). Cassava mildew, *Oidium manihotis* Henn., was suggested to be an adequate food for the development and reproduction of *Typhlodromalus limonicus* (German and McGregor) but it supported the development and not the reproduction of *Typhlodromalus aripo* DeLeon (Bakker and Klein 1992).

The survival of *R. loxocheles* when fed on uredospore of coffee rust is higher than the observed for *Typhlodromus pyri* fed on *P. viticola* and *Uncinula necator* (Pozzebon and Duso 2008; Pozzebon et al. 2009) and is similar the survival observed to *T. pyri* fed on *Erysiphe orontii* (Zemek and Prenerova 1997). The phytoseiid had the highest value of the intrinsic rate of increase than the other three phytoseiid mentioned above when fed on other fungi. With the higher survival and the highest intrinsic rate of increase we can suggest that uredospore of coffee rust have high nutritional value. The family Phytoseiidae has great economic importance because many species are natural enemies of phytophagous mites and small insects (McMurtry and Croft, 1997). Another possible study is the role of this mite in control of coffee rust. English-Loeb et al. (2007) demonstrated the efficacy of a tydeid mite, *Orthotydeus lambi*, in controlling grape powdery mildew on mature vines. The study showed the importance of mites to biological control of fungi. *Ricoseius loxocheles* feeds on a large amount of uredospore of coffee rust and also have the behaviour of carrying on the back a large amount of rust uredospore. Further studies are being conducted to evaluate the ability of this phytoseiid to disperse or control coffee rust.

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CHAPTER 3

Predation and reproduction of phytoseiid mite *Ricoseius loxocheles* (De Leon, 1965) (Acari: Phytoseiidae) on *Oligonychus ilicis* (McGregor, 1917) (Acari: Tetranychidae)

Abstract

Phytophagous mites are among the pest problems of the coffee plant and are responsible for large losses. The most common species is the coffee-red-mite, *Oligonychus ilicis* McGregor (Acari: Tetranychidae). In Brazil, chemical control is the main method of combating pest mites. Biological control with natural enemies is a viable alternative to chemical control. Predacious mites, in particular those belonging to the Phytoseiidae and Stigmaeidae families, have been widely used for biological control of pest mites in fruits and other crops worldwide. The Phytoseiidae are very abundant in coffee, and among them *Ricoseius loxocheles* (Acari: Phytoseiidae) is commonly found in coffee plants infested by coffee rust (*Hemileia vastatrix*) in the Zona da Mata Mineira. We measured predation and oviposition of *R. loxocheles* having coffee-red-mite as food. *Ricoseius loxocheles* was not able to feed on *O. ilicis*. Survival and oviposition with only prey were the same as without food. This phytoseiid does not really use *O. ilicis* as food. It is suggested that *R. loxocheles* is one phytoseiid that uses fungi as a main food source.

Key words: Prey stages; Phytophagous mites; Predator-prey interactions

Introduction

The most common species of phytophagous mites on the coffee plant is the coffee-red-mite, *Oligonychus ilicis* McGregor (Acari: Tetranychidae). The mite was considered the second pest on *Coffea canephora* Pierre in the State of Espirito Santo (IBC, 1985). Coffee-red-mite is considered an important coffee pest in many producing countries and it is found on the upper leaf surface causing reduction of photosynthesis rate and premature leaf drop as a consequence of the infestation (Reis, 2005).

Long periods without rain and with low humidity are the best conditions for the development of the mite. It can cause defoliation of the plants, and on young crops it can delay its development (Reis and Souza, 1986).

In Brazil, the chemical control is the main method for controlling pest mite populations. The use of non-selective pesticides for controlling pests has favoured a pest upwelling and outbreaks, including phytophagous mites. Additionally, this method of control can reduce or exterminate the beneficial species of predatory mites (Hill and Foster 1998; Vidal and Kreiter 1995).

Predacious mites, in particular those belonging to the Phytoseiidae and Stigmaeidae families, have been widely used for biological control of pest mites on fruits and other crops worldwide (McMurtry 1983; Childers 1994; Wood et al. 1994; McMurtry and Croft 1997; Opit et al. 2004; Cakmak et al. 2009; Arthurs et al. 2009;).

Phytoseiid mites have an ecologically diverse group of species, including oligophagous specialist predators of *Tetranychus* spider mites and broadly polyphagous generalists that feed on mites, insects, fungi and pollen (e.g. McMurtry and Rodriguez 1987; McMurtry and Croft 1997; Pratt et al. 1999). They have been classified as either specialists or generalists according to diet breadth (McMurtry and Rodriguez 1987; Schausberger and Croft 2000a,b).

Ricoseius loxocheles (Acari: Phytoseiidae) is commonly found in coffee plants infested by coffee rust (*Hemileia vastatrix*) in the Zona da Mata Mineira (personal observation). According to the chapter 1, this mite species can develop with uredospore of coffee rust as a food. However, we suspect that it can feed on phytophagous mites because we found that they co-occurs on coffee plants.. In this chapter we studied the predation and reproduction capacity of *R. Loxocheles* havingthe coffee-red-mite *O. Ilicis* as prey.

Materials and methods

Ricoseius loxocheles rearing

Ricoseius loxocheles were collected from coffee plants infected by rust (*H. vastatrix*) in July 2010 in Viçosa (20° 45' 14" S 42° 52' 55" W), State of Minas Gerais, Brazil. The colony was reared on arenas that consist on underside coffee leaves infected by rust, which were put on top of a moistened sponge placed inside a tray(250 x 150 x 50 mm) (Figure 1). The edges of the leaves were surrounded by moistened cotton to prevent mite from escaping. The arenas were replaced when its turgidity was severely reduced. Reading and experiment were kept inside a climate room at 25 ± 1°C, 60 ± 10% RH and at 12:12 light: dark cycle. Arenas were examined daily. When over-population of the mites was observed, predators were transferred to new arena.

One additional rearing of *R. loxocheles* was kept in the same condition as above, but the phytoseiids have with food source both uredospore rust and all stages of the prey *O. ilicis*. This culture was used because the conditioning of an animal to its regular food has been demonstrated for other arthropods by Peacock et al. (2003).

Figure 1. Rearing of *R. loxocheles* on coffee leaves infected by coffee rust.

Oligonychus ilicis rearing

Oligonychus ilcis were collected from coffee plants without the application of pesticides in Viçosa (20° 45' 14" S 42° 52' 55" W), State of Minas Gerais, Brazil. The colony was reared on arenas consisting of upper coffee leaves, which were put on top of a moistened sponge placed inside plastic trays (250 x 150 x 50 mm) (Figure 2). The leaves were surrounded by hydrophobic cotton, which served as a barrier to avoid the escape of mites. Each rearing unit (cotton + leaf + sponge) was placed in a plastic tray (70 x 170 x 260 mm) with sufficient water to saturate the wet sponge and hold the cotton. The coffee leaves were replaced when they begin to show signs of deterioration or when the mite population was very high. Cultures and experiment were kept inside a climate room at 25 ± 1°C, 60 ± 10% RH and at 12:12 light:dark cycle. Arenas were examined daily.

Figure 2. Rearing of *O. ilicis* on upper coffee leaves.

Predation capacity of *R. loxocheles* on *O. ilicis*

The experimental units consisted of coffee leaf disks (2.8 cm Ø) placed inside plastic cups (68 mm Ø and 50 mm depth). An entomological pin was attached in the center of the cup and the coffee leaf disk were fixed by this pin and kept floating on the water inside and in the center of the cup, thus preventing it to touch the edge of the cup and escapes of the mites (Figure 3).

An experiment was set to assess the ability and preference of the phytoseiid species to feed on all stages of *O. ilicis*: eggs, juveniles (started only with larva but without discrimination of larva, protonymph and deutonymph during the experiment) and adult females. We tested 11 treatments, with different combinations of food sources, such as: RlHv= uredospore coffee rust; RlHvAOi = uredospore coffee rust + adults *O. ilicis*; RlAOi= adults *O. ilicis*; RlHvJOi = uredospore coffee rust + juveniles *O. ilicis*; RlJOi= juveniles *O. ilicis*; RlHvEOi= uredospore coffee rust + eggs *O. ilicis*; RlEOi= eggs *O. ilicis*; Rl= without food, with a randomized design. We used more three treatment with prey separately to assess the natural prey mortality (AOi = Adults *O. ilicis* + uredospore coffee rust; JOi = Juveniles *O. ilicis* + uredospore coffee rust; EOi= Eggs *O. ilicis* + uredospore coffee rust).

Each experimental unit (replicate) with either adult females, 25 juveniles or 90 eggs of coffee-red-mite according to the treatment was carried-out. Twenty-four hours after transferring the prey, the predators were released individually on each experimental unit. A single gravid female of *R. loxocheles* (18 days old) was obtained from an egg-wave, as described in chapter 1. The experiment units were kept under laboratory conditions at 25 ± 1°C, relative humidity of 60 ± 10% and at 12:12 light: dark cycle.

The predation rate was assessed 24, 48, 72 and 96 hours after the introduction of *R. loxocheles* on each replicate through quantifying the amount of prey on the disk. It was performed 12 replicates per treatment. We also evaluated the survival and oviposition of the phytoseiid in the different treatments.

Predation and oviposition rates of *R. loxocheles* were compared with Generalized Linear Models (GLM) with Poisson error distribution (Crawley 2007). The phytoseiid survival was submitted to survival analysis with Weibull distribution and with variable response to the proportion of alive mites and the time as an explanatory variable for death of mites (days). All analyses were performed using the statistical software R (R Development Core Team 2006).

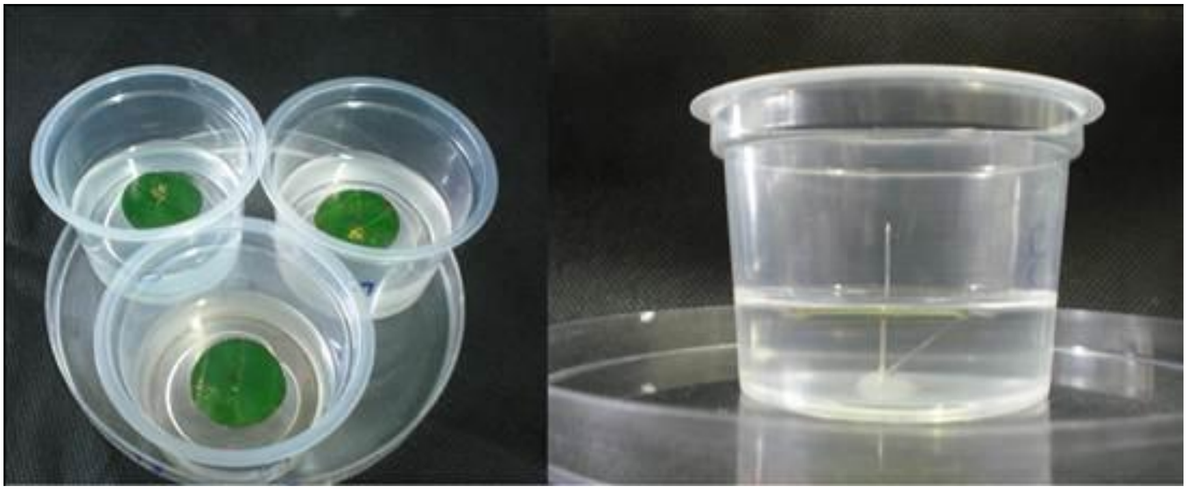

Figure 3. Coffee leaf disk (0.8 cm Ø) used in predation experiments, fixed by a pin and floating in water with food sources in accordance to the treatments.

Results

Ricoseius loxocheles was not able to feed on any stages of *O. ilicis* (Figure 4). The phytoseiid species did not prey adults of *O. ilicis* (χ^2= 7.7028, *df* = 3, *p* = 0.05257). The decrease in the amount of survival adults of the prey was similar between the treatments with and without the phytoseiid, so we can suggest that this decrease was due the natural mortality.

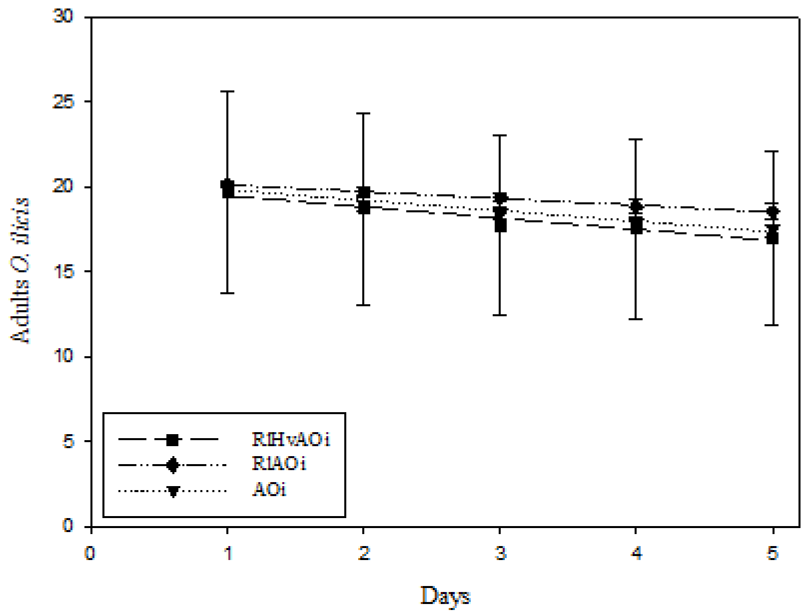

Figure 4. The dynamics of *O. ilicis* adults on coffee leaf disk with uredospore coffee rust and *R. loxocheles* (RLHVAOI), with *R. loxocheles* (RLAOI) or only *O. ilicis* adults (AOI) (Densities ±s.e.).

We found the same trend in the treatment with juvenile as food source, with no predation recorded. Even we found no statistic variation between the initial and final amount of juveniles, this difference was not significant ($\chi^2 = 0.0466$, $df = 2$, $p = 0.977$) (Figure 5).

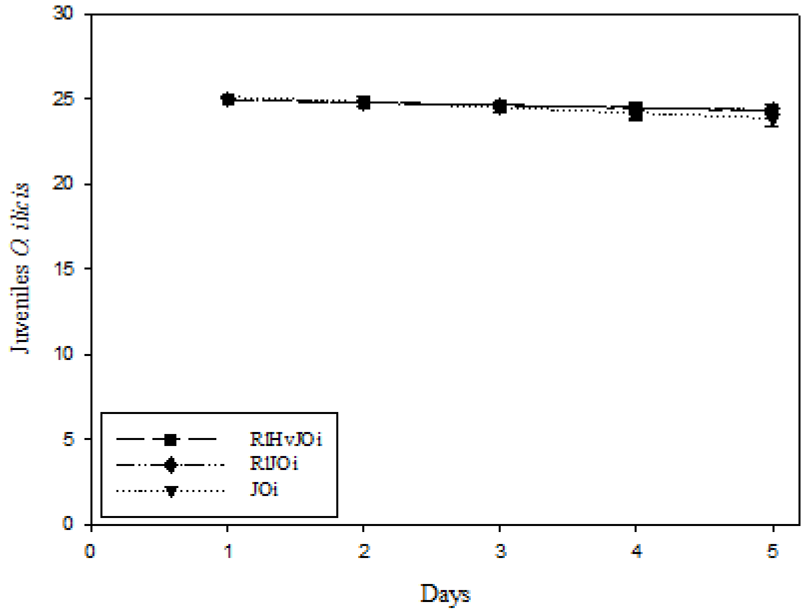

Figure 5. The dynamics of *O. ilicis* juveniles on coffee leaf disk with uredospore coffee rust and *R. loxocheles* (RlHvJOi), with *R. loxocheles* (RlJOi) or only juveniles of *O. ilicis* (JOi) (Densities ± s.e.).

We also evaluated the potential of predation of *R. loxocheles* on eggs of the prey *O. ilicis* and it was not significant the variation on eggs ($\chi^2 = 1.0542$, $df = 2$, $p = 0.5903$) (Figure 6).

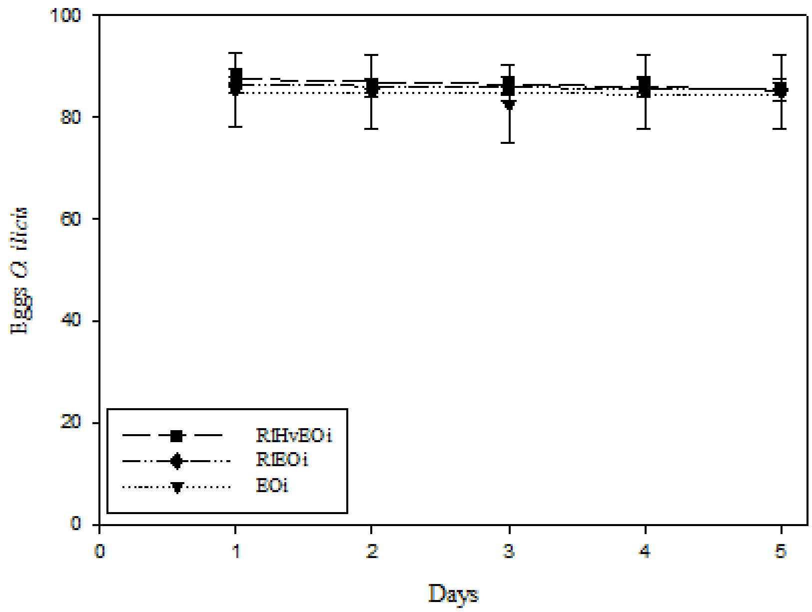

Figure 6. The dynamics of *O. ilicis* eggs on coffee leaf disk with uredospore coffee rust and *R. loxocheles* (Treatment 8), with *R. loxocheles* (Treatment 9) or only eggs of *O. ilicis* (Treatment 10) (Densities ± s.e.).

Ricoseius loxocheles oviposited when fed on uredospore of coffee rust or on a mixed of prey and uredospore. Oviposition rate ranged according to the treatment (χ^2= 111.84, *df* = 2, *p* = < 0.001), but there were no difference between the treatment with (χ^2= 1.3601, *df* = 3, *p* = 0.7149) or without O. ilicis (χ^2= 3.322e-08, *df* = 3, *p* = 1) (Figure 7).

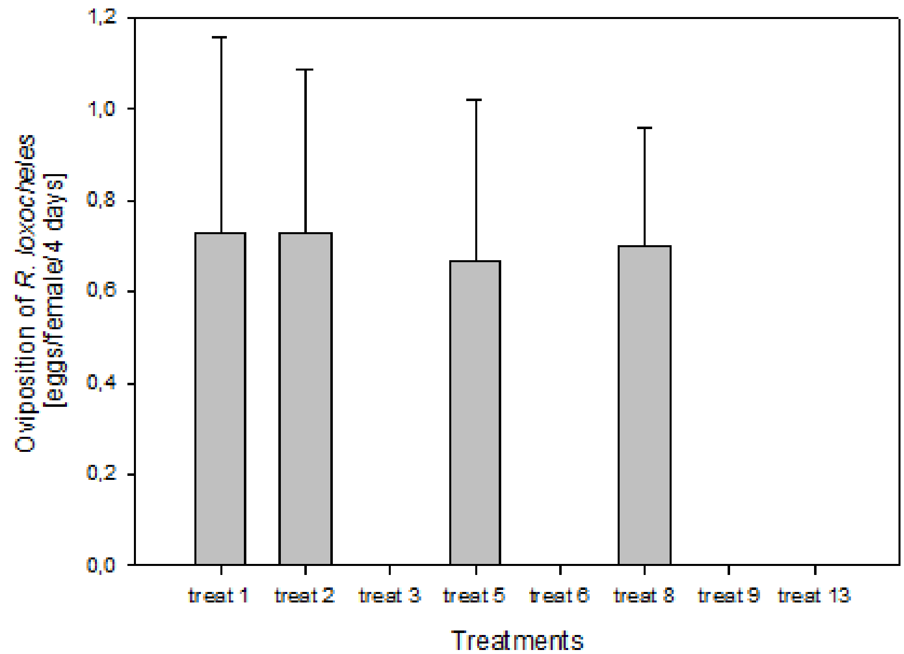

Figure 7. Number of eggs produced per *R. loxocheles* in 4 days for those treatments (RlHv= uredospore coffee rust; RlHvAOi = uredospore coffee rust + adults *O. ilicis*; RlAOi= adults *O. ilicis*; RlHvJOi = uredospore coffee rust + juveniles *O. ilicis*; RlJOi= juveniles *O. ilicis*; RlHvEOi= uredospore coffee rust + eggs *O. ilicis*; RlEOi= eggs *O. ilicis*; Rl= without food).

The survival of *R. loxocheles* was different among the treatments (χ^2= 63.5, *df* = 1, *p* = < 0.001) but there was no difference between the treatment with (*df* = 84, *p* = 0.6504) or without uredospore (*df* = 84, *p* = 0.4830) (Figure 8).

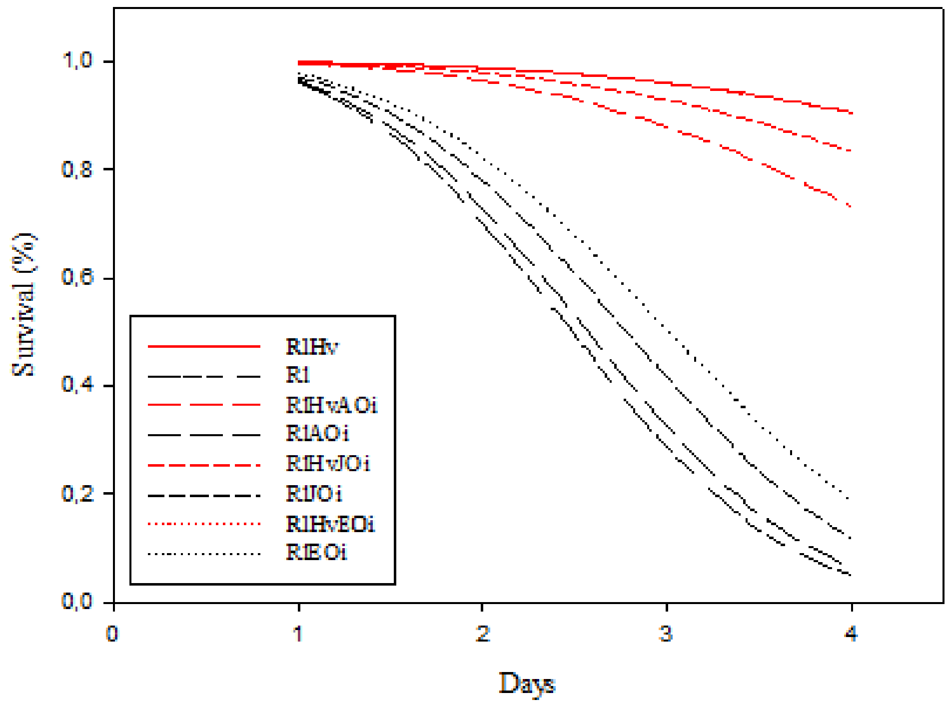

Figure 8. Survival of *R. loxocheles* feeding on different food sources at 25 ± 1°C, 60 ± 10% RH and at 12:12 light: dark cycle. Red and black lines are arenas with and without coffee rust, respectively. Treatments (RlHv= uredospore coffee rust; RlHvAOi = uredospore coffee rust + adults *O. ilicis*; RlAOi= adults *O. ilicis*; RlHvJOi = uredospore coffee rust + juveniles *O. ilicis*; RlJOi = juveniles *O. ilicis*; RlHvEOi = uredospore coffee rust + eggs *O. ilicis*; RlEOi = eggs *O. ilicis*; Rl= without food).

Discussion

Adult females of the phytoseiid mite *R. loxocheles* are not able to feed on any of the stages of the coffee-red-mite, *O. ilicis*. These observations are contrary to our expectations because many others Phytoseiidae species can develop and reproduce or only survive feeding on prey. This species does not use the prey neither as alternative food nor as supplementary food because it cannot survive eating only this prey. Spider mites are not necessarily the main food source or the preferred food for phytoseiids (Overmeer, 1985). In the current study we proved that this phytoseiid is specialized to feed on fungi and the coffe-red-mite is not an adequate food for it, however more studies using others phytophagous mites and other food sources need bedone to confirm the mite feeding habit

Ricoseius loxocheles could survive and oviposit only on arenas with uredospore of coffee rust or with uredospore coffee rust and prey. Survival and oviposition on arenas with only prey were the same as arenas without food, so coffee-red-mite was not really used as food.

Ramos & Rodríguez (2006) hypothesized that this phytoseiid mite can feed on Tetranychidae species on *Citrus* spp. However, they did not test this hypothesis at all. So, new investigations should be conducted to test the predation capacity of its phytoseiid mite and its reproductive success on different prey than *O. ilicis*. One possible prey for this phytoseiid mite is the vector of the coffee ring spot virus, *Brevipalpus phoenicis* (Geijskes, 1939) (Acari: Tenuipalpidae).

Although mainly eriophyoid mites and pollen have been considered as an alternative food (Overmeer, 1985; McMurtry and Rodriguez, 1987; Sabelis and van Rijn, 1996), other food sources such as nectar, honeydew and even plant tissue can play an important role in the nutrition of at least some phytoseiid species (Bakker and Klein, 1992; Tanigoshi et al., 1993; van Rijn and Tanigoshi, 1999; Magalhaes and Bakker, 2002; Nomikou et al., 2003a; b). There is some evidence that several phytoseiid species can utilize conidia of various mildew species as alternative food sources (Zemek, 2005). *Typhlodromus pyri* e *Amblyseius andersoni* can develop feeding on *Plasmopora viticola* (Pozzebon & Duso, 2008); *T. pyri* can also develop feeding on *Erysiphe orontii* (Zemek and Prenerova 1997) and some other Phytoseiidae can develop feeding on fungi (Chant, 1959; Kropczynska-Linkiewicz, 1973; Daftari, 1979, Bakker and Klein, 1992; Bakker, 1993). However, all of these phytoseiids used fungi with another non-prey alternative food source and *R. loxocheles* used fungi as a main food souce and we do not kown or recognized another food source for them yet.

Ricoseius loxocheles was found feeding on another rust, such as *Uredo coccolobae* on sea grapes (Denmark & Muma, 1973) and on unknown fungi on *Jatropha curcas* (Verona, 2010). However more studies need to be done to find out which source food *R. loxocheles* can use and what it is its role in the system which it is found.

As occurs in the tritrophic system fungus-vine-mite studied in which the mite is responsible for controlling the fungi *Uncinula necator* (English-Loeb et al,. 2007), it is possible that *R. loxocheles* has a role in the control of coffee rust since it feeds on a large amount of rust uredospore (chapter 1). Further investigation is needed to shed more light on the subject.

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General Conclusions

The uredospore of coffee rust (*Hemileia vastatrir)* could be characterized as a suitable food source for development and reproduction of *R. loxocheles*. This phytoseiid mite can be classified as a generalist species. It has a high reproductive potential when feeding on coffee rust.

Adult females of the phytoseiid mite *R. loxocheles* are not able to feed on the red-spider-mite, *O. ilicis*. These observations are contrary to ours expectations and that may occur with others Phytoseiidae species that can develop and reproduce or only survive feeding on prey.