FREDY ALEXANDER RODRÍGUEZ CRUZ

BIOLOGICAL CONTROL OF BROAD MITES IN CHILI PEPPER AND PHYSIC NUT

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

VIÇOSA
MINAS GERAIS - BRASIL
2014

FREDY ALEXANDER RODRÍGUEZ CRUZ

BIOLOGICAL CONTROL OF BROAD MITES IN CHILI PEPPER AND PHYSIC NUT

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

APROVADA: 28 de Fevereiro de 2014.

Angelo Pallini Filho
(Coorientador)

Arnoldus Rudolf Maria Janssen
(Coorientador)

Joao Alfredo Marinho Ferreira

Cleide Maria Ferreira Pinto

Madelaine Venzon
(Orientadora)

Ficha catalográfica preparada pela Seção de Catalogação e Classificação da Biblioteca Central da UFV

T

R696b
2014

Rodríguez Cruz, Fredy Alexander, 1977-
Biological control of broad mites in chili pepper and physic nut / Fredy Alexander Rodríguez Cruz. - Viçosa, MG, 2014.
xii, 76f. : il. (algumas color.) ; 29 cm.

Orientador: Madelaine Venzon.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Ácaro no controle biológico de pragas. 2. Ácaro de plantas. 3. *Polyphagotarsonemus latus* - Controle biológico. I. Universidade Federal de Viçosa. Entomologia. Programa de Pós-graduação em Entomologia. II. Título.

CDD 22. ed. 632.96

À minha mãe Carmen Rosa

Aos meus avôs Manuel e Carmen

À minha família

À minha Orientadora, Madelaine Venzon

Ao professor Angelo Pallini Filho

Ao professor Arne Janssen

Ao Professor Augusto Ramirez

Aos amigos e colegas do laboratório de Entomologia da EPAMIG

Aos amigos e colegas do laboratório de Acarologia da UFV

À Paola Andrea

À vida

OFEREÇO

AGRADECIMENTOS

À Universidade Federal de Viçosa pela maravilhosa oportunidade de realização deste curso.

À Empresa de Pesquisa Agropecuária de Minas Gerais – EPAMIG – Unidade Regional Zona da Mata, pelas instalações cedidas para a realização dos trabalhos.

Ao CNPq pelo consentimento da bolsa de estudo para que esse trabalho fosse realizado.

Meus sinceros agradecimentos a minha orientadora Madelaine Venzon, pela sua paciência, infinita ajuda e fé, para que essa tese se tornasse realidade.

Ao Professor Arne Janssen, por todas e cada uma das suas críticas e sugestões. Duras as vezes, porém muito importantes e necessárias para o meu crescimento professional.

Ao Professor Angelo Pallini pela sua ajuda e dicas para chegar até aqui.

Ao Professor Augusto Ramirez, pelo seu apoio na distancia.

À Elaine da secretaria de entomologia pelas informações dos tramites e constante disposição em me ajudar.

Ao Marcus Vinicius pela ajuda e assistência nos experimentos de casa de vegetação.

Ao José Geraldo da EPAMIG, pelos papos, risadas e ajuda no trabalho de campo.

Ao Zé Joao e Aristeu, pelas areas cedidas e pelo seu apoio para a realização dos trabalhos de pimenta malagueta em campo.

Ao Luciano Jacob, pelo ajuda e dedicação no experimento de pinhão manso e pela informação climatológica coletada em Oratórios.

Aos amigos, André, Michela, Elaine, Eugenia, Dany, Pedro, Felipe, Rafael, Joao, Helder, Edmar, Paulo, Maíra e Juliana Maria. De todos vocês aprendi, aprendo e aprenderei muito e muitas coisas, embora as vezes não são muitos os papos.

À minha família pelo suporte nos momentos difíceis e pelo seu amor.

A Paola Andrea, pelo seu afeto e sua infinita paciência.

A todas as pessoas que dedicaram uma palavra de apoio no momento.

A meus avôs, Manuel e Carmen, pelas suas palavras cada vez que delas precisei.

E à minha mãe Carmen Rosa “Rosita”, o motor de minha vida. Escutar teu riso foi, é e será a mais maravilhosa terapia que eu tenho para ainda ter fé na vida. Eu te amo mãe, e por você eu teve a forca necessária para conseguir. OBRIGADO.

BIOGRAFIA

Fredy Alexander Rodríguez Cruz, filho de Carmen Rosa Cruz. Formado como Engenheiro Agrônomo pela Universidad Nacional de Colômbia no ano de 2007 sob a orientação do Professor Augusto Ramirez. No ano 2008 inicia o curso de mestrado na Universidade Federal de Viçosa sob a orientação da Dra. Madelaine Venzon. Sob a orientação dessa mesma professora foi realizado o curso de Doutorado, cuja tese é aqui apresentada.

ÍNDICE

RESUMO

RODRÍGUEZ CRUZ, Fredy Alexander, D. Sc., Universidade Federal de Viçosa, Fevereiro de 2014. **Controle biológico do ácaro-branco em pimenta malagueta e em pinhão manso.** Orientadora: Madelaine Venzon. Coorientadores: Arnoldus Rudolf Maria Janssen e Angelo Pallini Filho.

O ácaro-branco *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae) é uma praga chave de distribuição mundial que ataca várias espécies de plantas de alto valor econômico. No Brasil, este ácaro é considerado praga chave da cultura de pimenta malagueta e do pinhão manso, devido a sua frequente ocorrência em areas produtoras e aos danos causados. Na maioria das vezes seu controle é baseado na aplicação de produtos químicos, com todos os problemas derivados de seu uso abusivo. Uma alternativa ao controle químico é o uso do controle biológico. Os principais inimigos naturais dos ácaros fitófagos são ácaros da família Phytoseiidae. Vários inimigos naturais hão sido registrados em associação com o ácaro-branco no Brasil, os fitoseídeos (*Amblyseius herbicolus, Neoseiulus barkeri, Euseius concordis, Iphiseiodes zuluagai* and *Typhlodromus transvaalensis*) e uma espécie da família Blattisociidae (*Lasioseius floridensis*). Como um primeiro passo para a seleção de agentes de controle biológico para o ácaro-branco, foram avaliadas as taxas de predação e oviposição das espécies *A. herbicolus*, *N. barkeri* e *L. floridensis* em duas situações: uma mistura dos estádios do ácaro-branco e em todos os diferentes estádios da praga. Num segundo passo, foi avaliado em condições de casa de vegetação, a eficiência dos fitoseídeos, *A. herbicolus* e *N. barkeri*, no controle do ácaro branco em pimenta malagueta em diferentes relações predador: presa. Num segundo experimento, foi avaliado o controle em plantas de pimenta malagueta infestadas com o ácaro-branco, com e sem liberação de predadores e seu impacto na produção de frutos. Um terceiro passo, foi avaliado o controle do ácaro-branco em plantas de pinhão manso e pimenta malagueta infestadas artificialmente com a praga em condições de campo, com e sem liberação dos fitoseídeos e seu efeito na produção da pimenta malagueta. Nos experimentos de laboratório, os fitoseídeos predaram e ovipositaram quando se usou a mistura dos estádios do ácaro-branco e em cada um dos estádios. *Amblyseius herbicolus* apresentou uma maior taxa de predação e oviposição, nas duas situações avaliadas em comparação a

N. barkeri. Entretanto, *L. floridensis* apresentou taxas de predação e oviposição baixas ou nulas nas duas situações avaliadas. Em casa de vegetação, *A. herbicolus* e *N. barkeri* controlaram as populações do ácaro-branco nas diferentes relações predador:presa; as plantas controle mostraram sintomas de um ataque severo sete dias após a infestação, incluindo a queda de folhas. No segundo experimento, os fitoseídeos mantiveram baixas as populações de ácaro-branco através do tempo. Assim mesmo, as plantas de pimenta malagueta com presença dos predadores apresentaram um maior número de frutos com maior peso do que as plantas controle. As plantas controle exibiram danos severos, incluindo queda de folhas. Em condições de campo, plantas de pinhão manso sem predadores exibiram altíssimas populações do ácaro-branco, sintomas severos, queda de folhas e altos valores na escala de notas de dano. Entretanto, plantas com predadores mostraram baixas populações da praga ao longo do tempo e não manifestaram sintomas severos. Em pimenta malagueta, as plantas sem predadores apresentaram maior número de ácaros-branco, curvamento e bronzeamento das folhas, porém a queda de folhas foi muito menor que registrada no experimento de casa de vegetação. Plantas de pimenta malagueta com presença de predadores exibiram baixo número de ácaros-branco e não apresentaram bronzeamento nem queda de folhas. Não houve diferença estatística no número e peso de frutos entre plantas de pimenta malagueta com e sem predadores, mas as plantas controle apresentaram frutos mais pequenos. Os predadores *A. herbicolus* e *N. barkeri*, foram efetivos no controle de populações do ácaro-branco nos diferentes passos avaliados neste estudo. As duas espécies predaram e ovipositaram ao se alimentar da praga. Em condições de casa de vegetação as plantas de pimenta malagueta foram beneficiadas pela presença dos predadores apresentando baixas populações da praga através do tempo, resultando na produção de frutos maiores e mais pesados. Em campo, os dois fitoseídeos tiveram a capacidade de manter em baixas densidades as populações do ácaro-branco no tempo, tanto em pinhão manso quanto em pimenta malagueta evitando o aparecimento de sintomas severos como os registrados nas plantas controle. *Amblyseius herbicolus* e *N. barkeri* podem ser considerados bons agentes de controle biológico do ácaro-branco. As duas espécies controlaram populações da praga em diferentes relações predador:presa, em condições de cultivo protegido e no campo. Os predadores conseguiram-se manter e aumentar em número no tempo, tanto em casa de vegetação quanto no campo, confirmando os resultados de

laboratório. Adicionalmente, os predadores conseguiram aumentar seu número em baixas densidades de ácaro-branco, indicando que eles podem fazer uso de recursos alternativos como o pólen ou néctar das flores de pimenta malagueta. O potencial de controle destes fitoseídeos pode ser aproveitado em outras culturas susceptíveis ao ataque do ácaro-branco, como papaia, feijão, batata ou gérbera, tanto em casa de vegetação quanto em campo aberto.

ABSTRACT

RODRÍGUEZ CRUZ, Fredy Alexander, D. Sc., Universidade Federal de Viçosa, February, 2014. **Biological control of broad mites in chili pepper and physic nut.** Adviser: Madelaine Venzon. Coadvisers: Arnoldus Rudolf Maria Janssen and Angelo Pallini Filho.

The broad mite *Polyphagotarsonemus latus* (Banks 1904) is an important worldwide pest, with economic impact of several crops. In Brazil, this mite is considered a key pest of chili pepper and physic nut, due to their frequent occurrence in planting areas and damage caused to plant hosts. Its control is based on application of agrotoxics with several problems derived from misuse. An alternative to chemical control is biological control. The main natural enemies of phytophagous mites are predatory mites from the phytoseiidae family. Several natural enemies have been recorded in association with broad mites in Brazil, including the phytoseiids (*Amblyseius herbicolus, Neoseiulus barkeri, Euseius concordis, Iphiseiodes zuluagai* and *Typhlodromus transvaalensis*) and one blattisociid mite species (*Lasioseius floridensis*). As a first step to select biological control agents for broad mites, we evaluated the predation and oviposition rates of predatory mite of species *A. herbicolus, N. barkeri* and *L. floridensis* on a mixture of broad mite stages and on all different stages of the pest. As a second step, we evaluated under greenhouse conditions the phytoseiids *A. herbicolus* and *N. barkeri* on chili pepper with different predator:prey ratios. In a second experiment, we evaluated the control on chili pepper plants infested with broad mites, with and without predators and their impact on fruit production. In a third step, we assessed the control of broad mites on physic nut and chili pepper plants, artificially infested with the pest, under field conditions with and without phytoseiids and their effect on the chili pepper production. In laboratory experiments, the phytoseiids preyed and oviposited when offered a mix of broad mite stages or on each stage separately. *Amblyseius herbicolus* showed higher predation and oviposition rates on the mix of broad mite stages and on each stage separately compared with *N. barkeri* rates. Meanwhile, *L. floridensis* showed oviposition and predation rates low or zero on the mix of broad mite stages and on each stage separately. In the greenhouse, *A. herbicolus* and *N. barkeri* controlled broad mite population in the different predator:prey ratios; control plants showed symptoms of a severe attack seven days after infestation, including foliar abscission. In a second experiment, the phytoseiids maintained the broad mite populations at low density over time. Chili pepper plants with predators had a higher number of fruits with greater weight that control plants. Control plants showed higher values on scale notes of injury with severe damage, including foliar abscission. Under field conditions, physic nuts and chili peppers without predators showed a very high population of broad mites with higher values on scale notes of injury. These plants showed severe symptoms and foliar abscission. However, plants with predators showed a low population of pest through time with low values on scale notes of injury without presence of severe symptoms. In chili pepper, plants without predators had higher number of broad mite, curling and bronzing of leaves, but leaf fall was much lower than recorded in the greenhouse experiments. Chili pepper plants with predators showed low number of broad mites and showed no symptoms. There was no statistical difference in the number and weight of fruits from chili pepper plants with and without predators, but control plants had smaller fruits. The predators *A. herbicolus* and the Brazilian strain of *N. barkeri* showed effectivess in controlling broad mite populations on the different steps evaluated in this study. Both predators preyed and oviposited when feeding on the pest. Under greenhouse conditions, chili pepper plants were benefited by presence of predators, showing low populations of broad mites through time, resulting in the production of larger fruits with higher weight. In field, both phytoseiids had the ability to maintain broad mite populations on low density through time on physic nut and chili pepper plants, preventing the development of severe symptoms in the plants. *Amblyseius herbicolus* and *N. barkeri* can be considered good biological control agents of the broad mite. Both species controled pest populations with different predator:prey ratios in protected cultivation and in the field. Predators were able to maintained and increased on number through time when fed on broad mite, confirming the laboratory results. The potential of control of *A. herbicolus* and *N. barkeri* can be exploited in other crops susceptible to broad mite attack as bean, papaya, potato or gerbera, both on the greenhouse and open field conditions.

INTRODUCTION AND GENERAL DISCUSSION

The biological control is an essential tool in the strategies of integrated pest management, aiming at the reduction of the use of agrotoxics (Vantornhout 2006). Biological control is generally defined as the use of parasitoid, predator, antagonist or competitor to supress or reduce a pest populations to a level at which they are not harmful (Van Driesche and Bellows 1996). Biological control is based on the concepts of population equilibria and population regulation exercised by natural enemies in pest populations (Bellows and Hassel 1999) and requires a fundamental understanding of many aspects of the population ecology.

Several species of mites are considered key pest of several crops. Among these highlight the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). This species is a polyphagous pest, capable of attacking more than 60 botanical families, infesting protected and open-field crops across the world (Gerson 1992, Gerson and Weintraub 2012). The broad mite has a short life cycle, one generation in aproximately five days (25°C to 30°C), and warm and wet conditions favour the pest (Jones and Brown 1983, Gerson and Weintraub 2012). It can survive and reproduce on several non-crop plants (Gerson and Weintraub 2012, Rodríguez-Cruz unpublished data). Broad mite dispersion occurs through several abiotic and biotic means: wind, infested plants, phoresy with whiteflies and other greenhouse pests or by males who carry the pupae (Palevsky et al. 2001, Gerson and Weintraub 2012).

In Brazil, the broad mite is a key pest of several important crops (Moraes and Fletchmann 2008). Among these are chili pepper (*Capsicum frutescens* L.) and physic nut (*Jatropha curcas* L.) (Lopes 2009, Venzon et al. 2006, 2013, Sarmento et al. 2011). Chili pepper is cultivated mainly by family farmers in small areas and the production is destined for industrial process and *in natura* market. Physic nut is cultivated for biodiesel industry and is planted in small and large areas in Brazil (Sarmento et al. 2011).

There are no acarides officially registered in Brazil for broad mite control on any of these crops (Agrofit 2014). Nevertheless, some farmers apply agrotoxics, mainly with abamectin as active ingredient, in an attempt to control the pest.

However, most of time this control is unsucessful, because of delayed application or incorrect concentrations of the products (Venzon et al. 2006).

The use of biological control arise as alternative to chemical control. Phytoseiidae family is well know as natural enemies of pest mites (McMurtry et al. 2013). Several phytoseiids have been evaluated as biological control agents of the broad mite in world with promising results (Weintraub et al. 2003, Jovicich et al.2006, La et al. 2009, Van Maamen et al. 2010). In Brazil, five phytoseiids and one blattisociid mite species have been registered in association with broad mites in different crops in the greenhouse and in open-fields (Matos 2006, Venzon et al. 2006, Brito et al. 2011, Sarmento et al. 2011, Rodríguez-Cruz unpublished data).

In this thesis, I studied the potential of the phytoseiids *Amblyseius herbicolus* (Chant), a Brazilian strain of *Neoseiulus barkeri* Hughes and the blattisociid mite *Lasioseius floridensis* Berlese as potential agents for broad mite control in chili pepper and physic nut. As a first step to select biological control agents for broad mites. In chapter 1, it was evaluated the predation and oviposition rates of predatory mite species on two different situations: predators feeding on each prey stages individually and predators feeding on a mix of pest stages. *Amblyseius herbicolus* and *N. barkeri* preyed and oviposited on each broad mite stages and on the mix of stages. Meanwhile, *L. floridensis* showed a very low predation and oviposition rate on each broad mite stages and on mix of broad mite stages.

In chapter 2, I studied broad mite control in chili pepper plants with *A. herbicolus* and *N. barkeri* under greenhouse conditions. Broad mite control was evaluated through two experiments. In the first experiment, chili peppers plants were infested with 20 or 40 broad mite females, followed by independent releases of two females of *A. herbicolus* or *N. barkeri*. Four predator:prey ratios for each predator species were tested. Seven days after infestation and predator release, the broad mite number in chili pepper plants was recorded. In a second experiment, chili pepper plants were infested with 20 broad mite females, followed by independent release of two females of *A. herbicolus* or *N. barkeri*. The broad mite number on the experimental plants was recorded five, ten and fifteen days after infestation and predators release. Two months after infestation, the chili pepper production was evaluated. In both experiments, the phytoseiid species controlled broad mite populations. Plants with predators showed notes of injury low without development the characteristic symptoms of the pest attack. Additionally, chili pepper production of plants with predators was significantly higher than of plants without predators. Plants with predators had a greater number of fruits per plant, which also had a higher weight, than plants without predators.

In chapter 3, broad mite control with *A. herbicolus* and *N. barkeri* was evaluated under field conditions. Physic nut and chili pepper seedlings were transplanted to different areas and artificially infested with broad mites. After seven days of the infestation, was evaluated the success of it on both crops, evaluated as presence of different broad mite stages on the plants. A week after of confirmation of success of the infestation, the predators were released (predator:prey ratio of 1:4.5 and 1:2.5 for physic nut and chilli pepper, respectively) on both crops. Broad mite infested plants without predators served as control. Broad mite number on physic nut and chili pepper plants was recorded during eight and six weeks after the predator release, respectively. Broad mite populations were higher in control plants than on plants with release of predators on both crops. On chili pepper and physic nut, control plants showed higher values on scale notes of injury with severe damage, including foliar abscission. Physic nut control plants showed higher defoliation that chili pepper control plants. Physic nut and chili pepper plants with predators had lower broad mite populations over time. Additionally, on these plants showed low values on the scale notes of injury and severe symptom or damages were not recorded. Other fact recorded in the field experiment was less control of pest populations when predators were released in combination compared to control by predators when they were released independently. Showing that a negative relationship occurs between the two predators. *Neoseiulus barkeri* was more affected by the presence of *A. herbicolus,* showing a lower number of individuals through time when the two predatory species were released in combination.

Concluding, the phytoseiid species *A. herbicolus* and *N. barkeri* are able to control broad mites. Predation and oviposition rates recorded here for these species are similar or better that those recorded for other phytoseiids species considered promising agents for broad mite control on the world, such as *Amblyseius swirskii*, *Amblyseius largoensis* or *Amblyseius californicus* (Castagnoli and Falchini 1993, Van Maanen et al. 2010, Rodríguez-Morell et al. 2010). For Brazil, *A. herbicolus* and *N. barkeri* showed predation and oviposition rates superior than recorded for *Iphiseiodes zuluagai* and *Euseius concordis*, species listed as promising biological control agents of broad mite in this country (Sarmento et al. 2011). The predatory capacity was confirmed under controlled conditions (greenhouse), where the two predators are able to control the pest populations on different predator:prey ratios and through time. The predators showed a performance similar under greenhouse conditions. In open-field, the predators controlled broad mite populations on both crops. However, *A. herbicolus* showed a better performance that *N. barkeri* probably because greater adaptability, as this specie was recorded in the region where the experiments were conducted (Matos 1996).

Releasing these predatory mite species may be one an important tool of integrated management programs aiming to control this important pest on other susceptible crops both in protected ambient as on open field on Brazil and the world.

References

Britto EPJ, Lindquist EE, de Gilberto GJ (2011). Redescription of *Lasioseius floridensis* Berlese, 1919 (Acari: Mesostigmata: Blattisociidae), with notes on closely related species. Zootaxa 2905: 1-15.

Castagnoli M, Falchini L (1993). Suitability of *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) as prey for *Amblyseius californicus* (McGregor) (Acari: Phytoseiidae). Redia 2: 273-279.

Gerson U (1992). Biology and control of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae*).* Experimental and Applied Acarology 13: 163-178.

Gerson U, Weintraub PG (2012). Mites (Acari) as factor in greenhouse management. Annual Review of Entomology 57: 229-247.

Jones VP, Brown RD (1983). Reproductive responses of the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae), to constant Temperature-Humidity regimes. Annals of the Entomological Society of America 76: 466-469.

Jovicich E, Cantliffe DJ, Stoffella PJ, Osborne LS (2008). Predatory mites released on transplants can protect from early broad mite infestations. Acta Horticulturae 782: 229-233.

La SY, Paik CH, Lee GH, Kim, DH (2009). Biological control of *Polyphagotarsonemus latus* (Acari: Tarsonemidae) by *Amblyseius cucumeris*. Korean Journal of Entomology 48: 347-354.

Matos CHC (2006). Mecanismos de defesa constitutiva em espécies de pimenta *Capsicum* e sua importância no manejo do ácaro branco *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae). Dissertation, Federal University of Viçosa.

McMurtry J, De Moraes G, Sourassou NF (2013). Revision of the lifestyle of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematic and Applied Acarology 18(4): 297-320.

Ministerio de Agricultura, Pecuaria e Abastecimento, Agrofit (2014) http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed 2 February 2014.

Moraes GJ, Fletchmann CHW (2008). Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil, Holos, Ribeirão Preto, Brazil.

Palevsky E, Soroker V, Weintraub P, Mansour F, Abu-Moach F (2001). How species-specific is the phoretic relationship between the broad mite, P*olyphagotarsonemus latus* (Acari: Tarsonemidae), and its insect hosts? Experimental and Applied Acarology 25: 217-224.

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

Vantornhout I (2006). Biology and ecology of the predatory mite *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae). PhD Thesis, Ghent University.

Van Driesche RG, Bellows TS (1996). Biological Control. Chapman and Hall, New York. 539 pp.

Van Maanen R, Vila E, Sabelis SW, Janssen, A (2010). Biological control of broad mite (*Polyphagotarsonemus latus*) with the generalist predator *Amblyseius swirskii*. Experimental and Applied Acarology 52: 29-34.

Venzon M, Oliveira RM, Perez AL, Rodríguez-Cruz FA, Filho SM (2013). Lime sulfur toxicity to broad mite, to its host plants and to natural enemies. Pest Management Science 69: 738-743.

Venzon M, Matos CHC, Rosado MC, Pallini A, Santos IC (2006). Pragas associadas à cultura de pimenta malagueta e estratégias de manejo. Cultivo da Pimenta, Informe Agropecuário 27: 75-86. EPAMIG, Belo Horizonte, Brazil.

Sarmento RA, Rodriguez DM, Faraji F, Erasmo FAL, Lemos F, Teodoro AV, Kikuchi WT, dos Santos GR, Pallini A (2011). Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on Jatropha curcas plants in Brazil. Experimental and Applied Acarology 53: 203-214.

Weintraub P, Kleitman S, Mori R, Shapira N, Palevsky E (2003). Control of the broad mite (*Polyphagotarsonemus latus* (Banks) on organic greenhouse sweet peppers (*Capsicum annuum* L.) with the predatory mite, *Neoseiulus cucumeris* (Oudemans). Biological Control 27: 300–309.

CHAPTER 1: EVALUATION OF PREDATORY MITES AS POTENTIAL AGENTS FOR BROAD MITE CONTROL

Abstract

The broad mite *Polyphagotarsonemus latus* (Banks) is considered a key pest of various crops worldwide. In Brazil, it is a major pest of chili peppers. Two phytoseiids (*Amblyseius herbicolus* and *Neoseiulus barkeri*) and a blattisociid mite species (*Lasioseius floridensis*) have been recorded in association with *P. latus* on gerbera and on chili pepper in Brazil. As a first step to select biological control agents for this pest, here we evaluated the predation and the oviposition rates of these predatory mite species in two situations: each stage of *P. latus* offered individually to the predators and a mix of the stages of pest. The phytoseiids *A. herbicolus* and *N. barkeri* preyed and oviposited on each stage of the pest and on the mix of broad mite stages. The predation and oviposition rates of *A. herbicolus* were higher on mix of broad mite stages and on each stage individually than that recorded for *N. barkeri*, except for oviposition rate when fed on broad mite pupae stage. Meanwhile, *L. floridensis* showed low or zero predation and oviposition rates on mix of broad mite stages and on each stage separately. These results show that *A. herbicolus* and *N. barkeri* are potential biological agents to control broad mites.

Key-words: *Amblyseius herbicolus, Neoseiulus barkeri, Lasioseius floridensis Polyphagotarsonemus latus, Capsicum frutensces,* Biological control

1 – **Introduction**

The broad mite *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae) is a widely distributed pest, feeding on more than 60 botanical families in the world (Gerson 1992). It feeds and causes damage on many economically important crops such as cotton, beans, papaya, lemon, grapefruit, cucumber and several solanaceous plant species (Vieira and Chiavegato 1998, Haji et al. 2001, Basset 1981, Collier et al. 2004, Venzon et al. 2008). In Brazil, *P. latus* is a key pest of chili pepper (*Capsicum frutensces* L.) (Venzon et al. 2013). Because of their small size (0.1 – 0.2 mm long), they go unnoticed at the beginning of the infestation, and their presence is evident only when the first symptoms appear on the plants (Venzon et al. 2008). Damage occurs on chili pepper apices, resulting in bronzing and curling of leave and foliar abscission after severe attacks (Venzon et al. 2011).

Broad mites are controlled with synthetic acaricides (Peña 1988, Gerson 1992, Venzon et al., 2006). The misuse of these pesticides and the continuous use of certain active ingredients have led to serious problems, such as pest resistance, the reduction or elimination of beneficial species, toxicity to the applicators and the presence of residues on fruits (Peña 1990, Peña and Osborne 1996, Pinto et al. 2012). An alternative for chemical pest control is the use of biological control. Biological control is the use of predators, parasitoids or competitors for supress or reduce the pest populations (Van Driesche and Bellows 1996).

Several families of predatory mites are known as natural enemies of pest mites. (McMurtry et al. 2013). Among these, some phytoseiid species have been shown promising for control of broad mite on the world, such as *Neoseiulus californicus*, *N. barkeri*, *Amblyseius swirskii* and *A. largoensis* (Fan and Petitt 1994, Peña and Osborne 1996, Weintraub et al. 2003, La et al. 2009, Rodríguez-Morell et al. 2010, van Maanen et al. 2010). In Brazil, four phytoseiids and one species of blattisociid mite have been recorded associated with broad mites (Venzon et al. 2006, Britto et al. 2011, Sarmento et al. 2011, Rodríguez-Cruz, unpublished data). The phytoseiid *Amblyseius herbicolus* (Chant) was found in association with broad mites on chili pepper plants in the state of Minas Gerais (Brazil) (Matos 1996, Venzon et al. 2006). This phytoseiid species completes its life cycle on exclusive diet of broad mite (Rodríguez-Cruz et al. 2013). However, its predatory capacity and oviposition rate on broad mite stages is unknown. *Neoseiulus barkeri* Hughes was collected from a commercial plantation of gerbera (*Gerbera* sp.) in Mogi das Cruzes (state of Sao Paulo, Brazil). This species has been tested previously as natural enemy of broad mites in the United States (Fan and Petit 1994, Peña and Osborne 1996). Despite the known association of *N. barkeri* with this important pest, the potential of this Brazilian strain for broad mite control has not been yet evaluated. The blattisociid mite species *Lasioseius floridensis* Berlese was also recorded preying on broad mites on commercial crops of gerbera (Britto et al. 2011). These authors studied its biology on different diets, including a mixture of broad mite stages. However, its potential as biological control agent for each broad mite stages was not yet evaluated.

Here, it was evaluated the predatory capacity of these three species of predatory mites on two situations: on each stage individually and a mix of broad mite stages and the oviposition rate when them fed on these prey stages, as a first step for evaluation of potential agents of control on this important pest.

2 - Materials and methods

2.1 Rearing methods

Chili pepper plants were obtained by sowing seeds in a commercial substrate (Tropstrato®, HT hortaliças, Brazil) in polystyrene trays (67 x 34 x 5.5 cm and 128 cells). Seedlings with two pairs of true leaves were transplanted to plastic pots (l L) containing a mixture of soil and organic manure (3:1). Potted plants were kept inside wooden frame cages (0.70 x 0.70 x 0.70 cm) covered with fine-mesh gauze (90 µm) in a greenhouse. Plants were irrigated twice a week.

Polyphagotarsonemus latus was collected from infested chili pepper plants in the county of Oratórios in the experimental area of the Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG) (Minas Gerais, Brazil, 20° 24' 0'' S, 42° 48' 0'' W). They were reared on potted chili pepper plants described above. When plant quality decreased because of broad mite infestation, new plants were introduced into the cages.

Amblyseius herbicolus was collected from infested chili pepper plants as described above and from *P. latus*-infested chili pepper plants maintained in a greenhouse in Viçosa (Minas Gerais, Brazil, 20°45´14´ S, 42°52´54´´ W). *Amblyseius herbicolus* was reared on arenas consisting of a PVC sheet (20 x 12 cm) placed on top of a foam pad (28 x 15 x 3 cm), surrounded by moistened cotton wool, which served both as water source and as barrier to prevent predators from escaping. Castor bean pollen (*Ricinus communis* L.) was used as food; it was provided on a small PVC sheet (4 x 2 cm), which was placed on the arena. Another small sheet PVC sheet (4 x 2 cm) with cotton yarns (1 cm) under it was provided on the arena as shelter and as oviposition site.

Neoseiulus barkeri was supplied by PROMIP® (Limeira, Brazil) and *Lasioseius floridensis* was supplied by ESALQ (Piracicaba, Brazil), and were reared on arenas as above. A mixture of mould mite stages (*Tyrophagus putrescientiae* (Shrank) (Acari: Acaridae) was used as food source for both mite species. Mould mites were reared on similar arena as the predators, receiving crumbs of crackers as food source (Marilan, Brasil®), which were supplied every 15 days. The predator arenas were kept in a climate room (25 ± 1°C, 60 ± 10% RH and 14 hours photophase).

2.2 Predation and oviposition

Predation and oviposition rates of three predator species were measured during three days on each stage of broad mite individually and a mix of pest stages. For obtain eggs of *P. latus*, petioles of leaves from clean plants (c. 90 days old) were inserted individually into plastic tubes (4 x 1.5 cm) with moistened cotton wool. On each leaf, eighty adult *P. latus* females were transferred and allowed to oviposit for 24 hours. After this period, females were removed and leaves with more than 100 eggs were selected for experiments. Thus, the arena for eggs evaluation consisted of young chili pepper leaves (± 7 cm^2). For the stages of larvae, pupae and adults of *P. latus* and mix of stages, the arenas consisted of chili pepper leaf discs (diam. 30 mm). The arenas (leaves and leaf discs) were placed individually in Petri dishes (diam. 45 mm) on an agar layer with their abaxial side up, because *P. latus* preferably inhabits this side of the leaves. The agar layer was cut around the leaves or leaf discs, the excess of agar was removed and water was added to the Petri disc to avoid leaf dehydration and predator escape. Hence, the arenas were located on top of an agar island surrounded by water. The arenas of each broad mite stage evaluate individually received 120 larvae, 90 pupae or 70 adults for *A. herbicolus* and 100 larvae, 70 pupae or 50 adults for *N. barkeri and L. floridensis*. The arenas of the mix of broad mite stages received 30 adults, 25 larvae and 25 pupae for *A. herbicolus* and *N. barkeri* or 20 adults, 20 larvae and 20 pupae for *L. floridensis*. Pilot experiments confirmed that these densities would not result in prey depletion during the experiments.

Amblyseius herbicolus reproduces by parthenogenesis telytoky (Moraes and Mesa 1988). Thus, an adult female of *A. herbicolus* (1-2 days old) was added directly to each arena (disc or leaf). For *N. barkeri* and *L. floridensis,* sexual reproduction, pairs of females and males were isolated for two days, allowing them to mate. Subsequently, a newly mated female was added to each arena (disc or leaf). Twenty replicates were carried out for each *P. latus* stage and each predator species. Ten replicates were carried out for the mix of broad mite stages. Leaves with *P. latus* eggs were replaced daily. The densities of other prey stages were kept constant by adding new individuals to the discs every day. The arenas were kept in a climate chamber (25 ± 1°C, 60 ± 10% RH and 14 hours photophase).

The number of individuals that preyed on each broad mite stage and on the mix of broad mite, based on the presence of prey remains, as well as the number of predator eggs, was counted daily with a stereoscopic microscope (Nikon® SMZ 645). Because the oviposition rate of predatory mites on the first day is affected by previous diet (Sabelis 1990), we did not include these data in the analysis.

2.3 Statistics

Predation and oviposition were analysed using generalized linear models (GLM) with a quasi-Poisson error distribution for correction of overdispersion (Crawley 2007). Additionally, we compared the oviposition rate recorded for *A. herbicolus* and *N. barkeri* on each situations evaluated and independently for each predator through of generalized linear models (GLM) with a quasi-Poisson error distribution. Differences between the means were obtained through the Wald test provided by package “contrast” (Kuhn et al. 2008). The analyses were performed using the statistical software R 2.15 (R Development Core Team 2012).

3 – Results

All predator species fed on a mixture of broad mite stages. However, the number of individuals that preyed on the mix of broad mite stages differed significantly among predator species (Dev= 123.42, *df*=2, p<0.001). The predation was much higher for the two species of phytoseiid that for *L. floridensis* (Fig. 1). In each broad mite stages offered individually, the number of individuals preyed on each stage differed significantly with predator species (Deviance= 15026.3, *df* = 2, p < 0.001) (Fig. 2). The phytoseiids *A. herbicolus* and *N. barkeri* fed on all stages. Predation by *L. floridensis* was close to zero, especially on larvae and on eggs of broad mites (Fig. 2). Of the three species evaluated *A. herbicolus* showed the highest predation rate on the different broad mite stages. Its consumption rate of broad mite pupae was highest, followed by broad mite larvae and eggs. Broad mite adults were preyed the least (Fig. 2). *Neoseiulus barkeri* consumed most broad mite adults, followed by larvae and pupae. A lowest consumption was recorded on broad mite eggs (Fig. 2).

The oviposition rate differed significantly between predator species on mix of broad mite stages (Dev= 29.70, *df*= 2, p<0.001) (Fig. 3), *A. herbicolus* showed a higher oviposition that *N. barkeri* and *L. floridensis*. The oviposition rate differed significantly when the broad mite stages were evaluated individually (Dev= 11.89, *df*= 3, p <0.001) (Fig. 4) for *A. herbicolus* and (Dev= 31.99, *df*= 3, p < 0.001) (Fig. 4) for *N. barkeri*. *Lasioseius floridensis* showed no significant difference in oviposition rate when it fed on different broad mite stages (Dev = 0.68, *df*= 3, p = 0.71) and the oviposition was low.

Amblyseius herbicolus showed the highest oviposition rate when it fed on broad mite adults, followed by broad mite larvae. The lowest oviposition rate of this species was registered on broad mite pupae (Fig. 4). For *N. barkeri*, the highest oviposition rate was recorded when it fed on broad mite adults, followed by broad mite pupae; the lowest oviposition rate was recorded when the predator fed on broad mite eggs (Fig. 4). The oviposition rate of *L. floridensis* was generally low (Fig. 4).

There are significant difference between oviposition rates between mix of stages and each stage evaluated individually on each phytoseiid species (Dev=96.15, *df*=4, p <0.001; Dev = 97.57, *df*=4, p <0.001 for *A. herbicolus* and *N. barkeri*, respectively).

4 – Discussion

As a first step in the evaluation of the potential agents for control of broad mites, we evaluated the predation and oviposition rates of three predatory mite species in two differents situations: 1) when they fed on each broad mite stages individually and 2) when they fed on a mixture of the different stages of the pest. The phytoseiids *A. herbicolus* and *N. barkeri* preyed and oviposited in both cases. Few studies have evaluated the predation capacity of the phytoseiid *A. herbicolus*. This predator has the ability to complete its life cycle on a diet consisting exclusively of broad mites, a mixture of larvae, female adults and probably the eggs deposited by the female (Rodríguez-Cruz et al. 2013).

Amblyseius herbicolus presented a greater consumption of preys in both cases evaluated. This species had a higher consumption on the broad mite pupae and larvae when fed independently on each stage. This higher consumption may be related to the fact that pupae are quiescent without any protective mechanism or antipredator behaviour. Additionally, an only attack is necessary to penetrate and suck the pupae and larvae contents (personal observation). When the predator attack the broad mite adult, the effort is greater because to cuticle of this stage, and demand a greater manipulation of the prey (personal observation). Meanwhile, on the arena of the mixture of broad mite stages, the consumption observed was similar between adults, larvae and pupae of broad mite (evidenced by shrivelled corps). This fact is important because it indicates that the predator does not prefer a particular broad mite stage as food, not depending of particular stage to feed and reproduce. Thus, *A. herbicolus* may reduce the broad mite populations consuming the stages responsible for the dispersion to other locations on the plant (larvae, pupae and adults) and the generation of new individuals (adult females).

The oviposition rate recorded for *A. herbicolus* on a mixture of broad mite stages was higher that recorded when the predator feed on each stage individually. This fact may indicate that a variety of stages is more nutritious than consumption of each stage individually. The oviposition rate of *A. herbicolus* was similar or superior to that recorded for other phytoseiids considered as potential biological control agents of broad mites on the world (Castagnoli and Falcini 1993, Rodríguez-Morel et al. 2010, van Maanen et al. 2010). This rate was superior than recorded on *Iphiseiodes zuluagai* and *Euseius concordis*, phytoseiid species evaluated as biological control agents of broad mite in Brazil (Sarmento et al. 2011).

Neoseiulus barkeri has been shown a promising biological control agent of broad mites before (Fan and Petitt 1994). The Brazilian strain of *N. barkeri* showed the ability to prey on all broad mite stages. This species do not show a preference for specific pest stages in mixture of broad mite stages. Similarly to *A. herbicolus*, the oviposition rate was superior on the mix of broad mite stages than it recorded on each stage individually. The control of broad mites by this predator can be done by the reasons cited for the *A. herbicolus*. In addition, this predator is able to feed on the whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) (Nomikou et al. 2001). The consumption of whitefly can lead to broad mites control, because the phoretic relationship between the two arthropods (van Maanen et al. 2010).

The blattisociid *L. floridensis* showed very low predation and oviposition rates, in the two cases evaluated. Our results differ from those obtained by Britto et al. (2012) who recorded oviposition when this predator fed on a mixture of all broad mite stages on disc of physic nut or bean. These authors reported significant difference among oviposition rates. In our experiment discs of chili pepper leaves were used. Buitenhuis et al. (2014) reported significant difference in the performance of *Amblyseius swirskii* (Acari: Phytoseiidae), according to the plant species in which the predator was released. Additionally, the relative humidity used in the study by Britto et al. (2012) was higher than in the current experiment. According to the authors, the genus *Lasioseius* in Brazil is found on natural vegetation, but in areas with high relative humidity.

Here, it was shown that both phytoseiid species were capable of feeding and ovipositing on the different broad mite stages offered individually or in a mix. The evaluation of biological control agents encompasses a set of criteria, without depending of the better characteristic of the natural enemy (Waage 1989). The systems with use of phytoseiids illustrate this scenario very well. Their success as biological control agents depend of several factors, among them the population grow rate relative to their prey (Sabelis and Van der Meer 1986). This rate should not necessarily be superior to the one presented by the prey, especially in predators classified as generalists like it case of the genus *Amblyseius* and *Neoseiulus* (McMurtry et al. 2013). The predator populations can be persist due to the use of alternative food as pollen or nectar (Ramakers 1990, van Rijn and Sabelis 1990, Sabelis and van Rijn 1997, van Rijn and van Houten 1991). Pollen can auxiliary on reproduction and maintenance of the juvenile stages of the predators. (Van Rijn and van Houten 1991). Additionally, pollen promotes the persistence of the predators in the field even when their prey is scarce (Van Rijn and Sabelis 1990, Nomikou et al. 2001). *Amblyseius herbicolus* and *N. barkeri* are able to use pollen of different plant species and *A. herbicolus* can complete its life cycle exclusively on a pollen diet (van Rijn and van Houten 1991, Nomikou et al. 2001, Rodriguez-Cruz et al. 2013).

In spite of the higher consumption of broad mites and oviposition showed by *A. herbicolus*. We considered that *A. herbicolus* and *N. barkeri* are promising potential biological control agents of the broad mite due to the values of predation and oviposition rates and the characteristic of use of alternative food described on the literature. Additionally, in the Brazilian scenario these species show better predation and oviposition rates that natural enemies previously evaluated for broad mite control, such as *I. zuluagai* and *E. concordis* (Sarmento et al. 2010). We propose experiment in greenhouse conditions as the next step in the evaluation of these predatory mites.

References

Basset P (1981). Observations of broad mite (*Polyphagotarsonemus latus*) (Acari: Tarsonemidae) attacking cucumber. Crop Protection 1: 99-103.

Blackwood JS, Schausberger P, Croft BA (2001). Prey-stage preference in generalist and specialist phytoseiid mites (Acari: Phytoseiidae) when offered *Tetranychus urticae* (Acari: Tetranychidae) eggs and larvae. Environmental Entomology 30: 1103-1111.

Britto EPJ, Lindquist EE, de Gilberto GJ (2011). Redescription of *Lasioseius floridensis* Berlese, 1919 (Acari: Mesostigmata: Blattisociidae), with notes on closely related species. Zootaxa 2905: 1-15.

Britto EPJ, Gago E, de Moraes GJ (2012). How promising is *Laseoseius floridensis* as a control agent of *Polyphagotarsonemus latus*? Experimental and Applied Acarology 56: 221-231.

Buitenhuis R, Shipp L, Scott-Dupree C, Brommit A, Lee W (2014). Host plant effects on the behavior and performance of *Amblyseius swirskii* (Acari: Phytoseiidae). Experimental and Applied Acarology 62: 171-180.

Castagnoli M, Falchini L (1993). Suitability of *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) as prey for *Amblyseius californicus* (McGregor) (Acari: Phytoseiidae). Redia 2: 273-279.

Collier KFS, Lima JOG, Albuquerque GS (2004). Predacious mites in papaya (*Carica papaya* L.) orchards: in search of a biological control agent of phytophagous mite pests. Neotropical Entomology 33: 799-803.

Crawley MJ (2007). The R book. Wiley, West Sussex.

Fan Y, Petitt FL (1994). Biological control of broad mite, *Polyphagotarsonemus latus* (Banks) by *Neoseiulus barkeri* Hughes on pepper. Biological Control 4: 390-395.

Gerson U (1992). Biology and control of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae*)*. Experimental and Applied Acarology 13: 163-178.

Haji FNP, Moreira AN, Lopes PRC, Ferreira RCF, Alencar JA, Barbosa FR (2001). Monitoramento e determinação do nível de ação do ácaro-branco na cultura da uva. EMBRAPA Semi-Árido, Circular Técnica 68.

La SY, Paik CH, Lee GH, Kim, DH (2009). Biological control of *Polyphagotarsonemus latus* (Acari: Tarsonemidae) by *Amblyseius cucumeris*. Korean Journal of Entomology 48: 347-354.

McMurtry J, De Moraes G, Sourassou N. F (2013). Revision of the lifestyle of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematic and Applied Acarology 18(4): 297-320.
Moraes GJ, Fletchmann CHW (2008). Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil, Holos, Ribeirão Preto, Brazil.

Peña JE (1988). Chemical control of broad mite (Acarina: Tarsonemidae) in limes (*Citrus latifolia*). Processing of Florida State of Horticultural Society 101: 247-249.

Peña JE (1990). Relationships of broad mite (Acari: Tarsonemidae) density to lime damage. Journal of Economic Entomology 83: 2008-2015.

Peña JE, Osborne L (1996). Biological control of *Polyphagotarsonemus latus* (Acarina: Tarsonemidae) in greenhouses and field trials using introductions of predacious mites. Entomophaga 41: 276-285.

Pinto CMF, Pinto CLO, Donzeles SML (2012). Boas práticas agrícolas na produção de pimentas. Pimentas: do produtor ao consumidor Informe Agropecuário 33: 21-31. EPAMIG, Belo Horizonte, Brazil.

R Development Core Team (2010). R foundation for statistical computing. Vienna, Austria.

Ramakers, PMJ (1990). Manipulation of phytoseiid thrips predators in the absence of thrips. Bull. SROP/WPRS 13: 169-172.

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

Van Driesche RG, Bellows TS (1996). Biological Control. Chapman and Hall, New York. 539 pp.

van Maanen R, Vila E, Sabelis M, Janssen A (2010). Biological control of broad mite (*Polyphagotarsonemus latus*) with the generalist predator *Amblyseius swirskii*. Experimental and Applied Acarology 52: 29–34.

van Rijn PCJ and Sabelis MW (1990). Pollen availability and its effects on the maintenance on populations of *Amblyseius cucumeris*, a predator of thrips. Med. Fac. Landbouw. Rijksuniv. Gent 55: 335-341.

van Rijn PCJ, van Houten YM (1991). Life history of *Amblyseius cucumeris* and *Amblyseius barkeri* (Acarina: Phytoseiidae) on diet of pollen. In: Modern Acarology Vol 2, Dusbabek F and Bukva V (Eds), pp 647-654. Academia, Prague and SPB Academic Publishing BV, Hague.

Van Rijn P, Sabelis M (1993). Does alternative food always enhance biological control? The effect of pollen on the interaction between flower thrips and its predator. IOBC/WPRS Bulletin 16: 123-125.

Venzon M, Matos CHC, Rosado MC, Pallini A, Santos IC (2006). Pragas associadas à cultura de pimenta malagueta e estratégias de manejo. Cultivo da Pimenta, Informe Agropecuário 27: 75-86. EPAMIG, Belo Horizonte, Brazil.

Venzon M, Rosado MC, Molina-Rugama AJ, Duarte V, Dias R, Pallini A (2008). Acaricidal efficacy of neem against *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). Crop Protection 27: 869–872.

Venzon M, Amaral DSSL, Perez AL, Rodríguez-Cruz FA, Togni PHB, Oliveira RFO (2011). Identificação e manejo ecológico de pragas da pimenta da cultura da pimenta. EPAMIG, Viçosa, Brazil.

Venzon M, Oliveira RM, Perez AL, Rodríguez-Cruz FA, Filho SM (2013). Lime súlfur toxicity to broad mite, to its host plants and to natural enemies. Pest Management Science 69: 738-743.

Vieira M, Chiavegato L (1998). Biologia de *Polyphagotarsonemus latus* (Banks, 1904) (Acarina: Tarsonemidae) em algodoeiro. Pesquisa Agropecuária Brasileira 33: 1437-1442.

Sabelis MW (1981). Biological control of two spotted spider mites using phytoseiid predators. Part. I Molleding the predatory-prey interaction at the individual level. Agricultural Research Reports. Wageningen: PUDOC.

Sabelis MW and van der Meer J (1986). Local dynamics of the interaction between predatory mites and two-spotted spider mites. In: Dynamics of physiological structures populations, Metz JAJ and Diekmann (Eds), pp 1-24. Lecture notes in Biomathematics, Springer-Verlag.

Sabelis MW and van Rijn PCJ (1997). Predation by insects and mites. In: Thrips as crop pests, Lewis T (Ed), pp 259-354. CABI, London.

Sabelis MW (1990). How analyze prey preference when prey density varies- a new method to discriminate between effects of gut fullness and prey type composition. Oecologia 82: 279-285.

Waage J (1989). Ecological theory and the selection of biological control agents. In: Critical issues in biological control, Mackauer M, Ehler E and Roland J (Eds) pp 135-157. Intercept, Andover.

Weintraub P, Kleitman S, Mori R, Shapira N, Palevsky E (2003). Control of the broad mite (*Polyphagotarsonemus latus* (Banks) on organic greenhouse sweet peppers (*Capsicum annuum* L.) with the predatory mite, *Neoseiulus cucumeris* (Oudemans). Biological Control 27: 300–309.

Figures

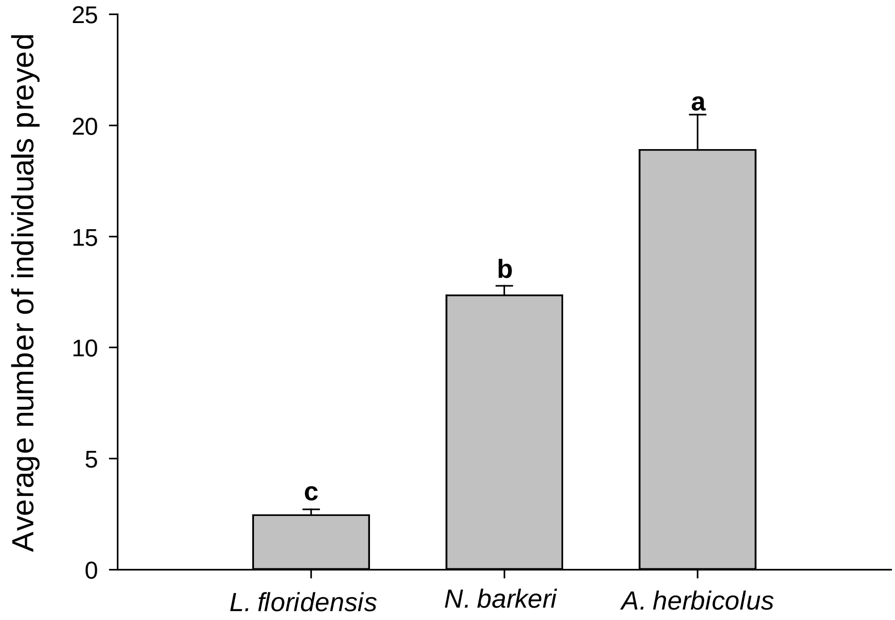

Figure 1. Average number of *P. latus* preyed (+SE) by the predatory mite *Amblyseius herbicolus, Neoseiulus barkeri* and *Lasioseius floridensis* on mix of *P. latus* stages. Different letters above the bars denote significant differences in the number of the individuals preyed by each predator species per broad mite stage (Wald test).

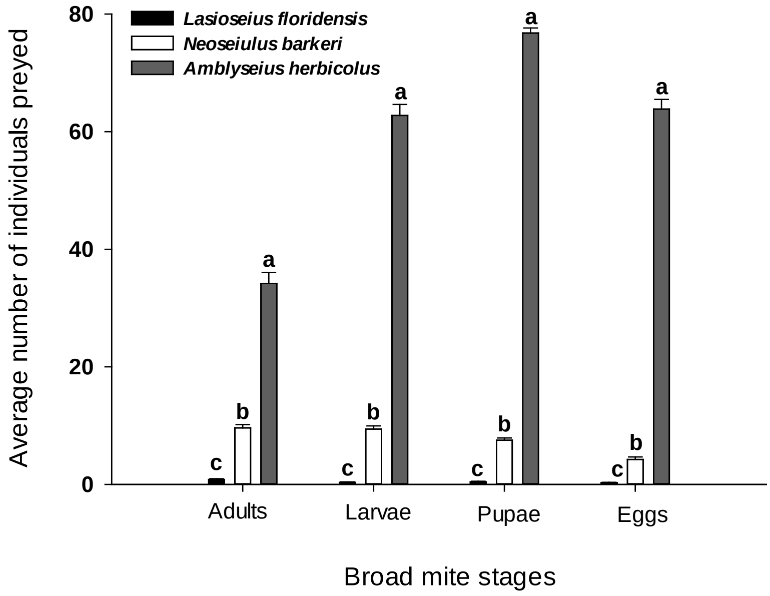

Figure 2. Average numbers of *P. latus* stages preyed (+SE) by the predatory mite *Amblyseius herbicolus*, *Neoseiulus barkeri* and *Lasioseius floridensis* on individual stage arenas. Different letters above the bars denote significant differences in the number of the individuals preyed by each predator species per broad mite stage (Wald test).

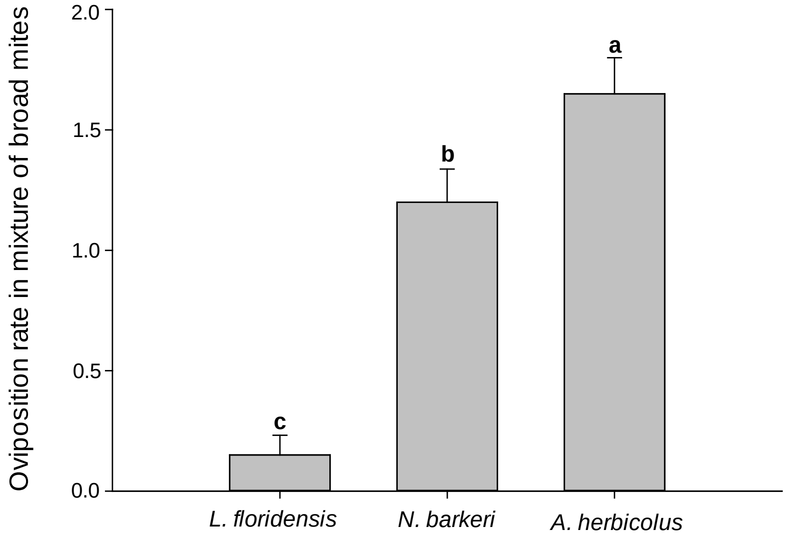

Figure 3. Oviposition rates (+SE) of *Amblyseius herbicolus*, *Neoseiulus barkeri* and *Lasioseius floridensis* feeding on mix of *P. latus* stages. Different letters above the bars denote significant differences in the number of eggs laid on each broad mite stage evaluated for each predatory mite separately (Wald test).

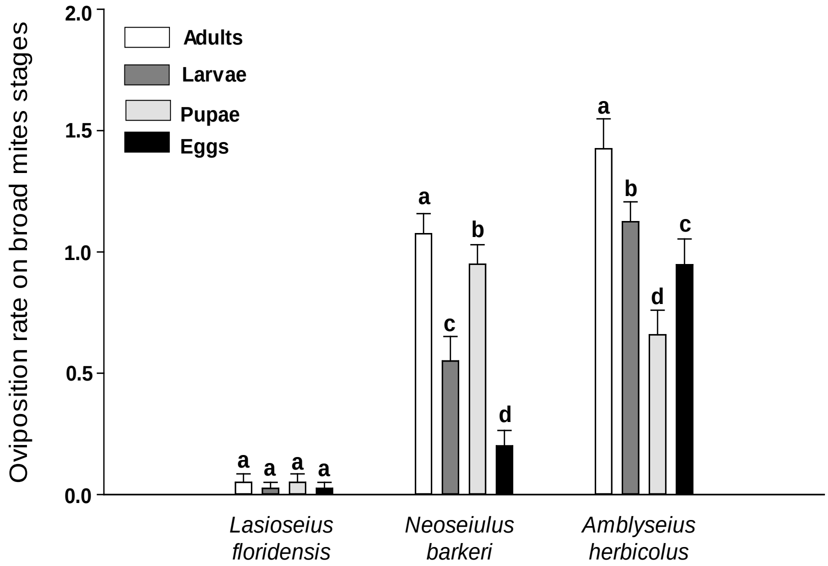

Figure 4. Oviposition rates (+SE) of *Amblyseius herbicolus*, *Neoseiulus barkeri* and *Lasioseius floridensis* when feeding on different *P. latus* stages. Different letters above the bars denote significant differences in the number of eggs laid on each *P. latus* stage evaluated for each predatory mite individually (Wald test).

CHAPTER 2: TWO SPECIES OF PREDATORY MITES ARE POTENTIAL CONTROL AGENTS OF BROAD MITES ON CHILI PEPPER PLANTS

Abstract

The broad mite *Polyphagotarsonemus latus* is considered a key pest of various crops worldwide. In Brazil, *P. latus* is the main pest of chili pepper. Two phytoseiid species have been recorded in association with this pest in Brazil, *Amblyseius herbicolus* and *Neoseiulus barkeri*. We evaluated these phytoseiid species as potential biological agents for broad mite control. Under greenhouse conditions broad mite control was evaluated on chili pepper plants infested with different predator:prey ratios. Seven days after the infestation and release of predators, chili pepper plants without predators showed severe symptoms, including foliar abscission. However, chili pepper plants with phytoseiids had low broad mite population on the different predator:prey ratios. In a second experiment, the control by these predatory mites species on broad mite populations was evaluated on chili pepper plants through time and the impact on fruit production. Chili pepper plants without predators showed higher broad mite populations with higher values on the scale notes of injury and severe symptom, including foliar abscission. The phytoseiids kept broad mite populations on low densities through time. After two months, plants with predators produced larger fruits than plants without predators. Our results show that these two phytoseiid species can control broad mites on chili peppers in different densities and over time. This is the first study that evaluated natural enemies of broad mite in greenhouses conditions. We suggest evaluating these predators under field conditions.

Key-words: *Amblyseius herbicolus*, *Neoseiulus barkeri*, *Polyphagotarsonemus latus*, *Capsicum frutensces*, biological control

1 – Introduction

The broad mite *Polyphagotarsonemus latus* (Banks 1904) (Acari: Tarsonemidae) is a widely distributed pest, occurring on more than 60 botanical families in tropical and subtropical areas (Gerson 1992). The genus *Capsicum* has low tolerance for broad mite attack (De Coss-Romero and Peña 1998) and the mite is considered a major pest of peppers in several countries such as China, the USA, New Zealand and Thailand (Riley 1992, Vichitbandha and Chandrapatya 2011, Zhang, 2008). In Brazil, broad mites are a key pest of chili pepper (*Capsicum frutensces* L.) (Venzon et al. 2006, 2011). Because of their small size (0.1 – 0.2 mm long), they go unnoticed at the beginning of the infestation; their presence only becomes evident when the plants show the first symptoms (Venzon et al. 2008). The mites mainly damage the plant apices, resulting in bronzing and curling of leaves and leaf abscission under severe attack (Gerson 1992, Weintraub et al. 2003, Venzon et al. 2011). The main control method of broad mites in Brazil is with chemical pesticides. Despite the lack of officially registered acaricides for broad mite control on chili pepper (Agrofit 2014), some Brazilian farmers apply pesticides registered for other crops in an attempt to control the pest, but control is often not successful (CMF Pinto personal communication). The misuse of this control method can lead to problems such as environmental contamination, poisoning of farmers, and residues on fruits. Although there are no records of acaricide resistance in broad mites, it is well known that other phytophagous mites, such as the tetranychids, quickly develop resistance to acaricides (Sato et al. 2005, Lin et al. 2009). Taken together, these factors have generated pressure for sustainable and environment-friendly control methods. An alternative to chemical control is the use of natural enemies to control pests (Hajek 2004, Waterfield and Zilberman 2012).

Phytoseiid mites are well known as natural enemies of pest mites (McMurtry et al. 2013), and several phytoseiid species have shown promise for control of broad mites (Fan and Petitt 1994, Peña and Osborne 1996; Weintraub et al. 2003, La et al. 2009, Rodríguez-Morell et al. 2010, van Maanen et al. 2010, Sarmento et al. 2011). In Brazil, five phytoseiids have been recorded associated with broad mites. *Amblyseius herbicolus* (Chant) was found in association with broad mites on chili pepper plants in the state of Minas Gerais (Brazil) (Matos 2006, Venzon et al. 2006), and has the ability to grow and to reproduce when fed exclusively with broad mites (Rodríguez-Cruz et al. 2013). The Brazilian race of *Neoseiulus barkeri* Hughes was recorded on a protected gerbera cultivation in association with broad mites in Mogi das Cruzes (state of Sao Paulo, Brazil) (Britto et al. 2011). This specie can it also feed and oviposited on broad mite (Rodríguez-Cruz, in prep).

Here, the potential of broad mite control by these two predators on chili pepper plants under greenhouse conditions was evaluated. Initially, broad mite control in chili pepper on different predator:prey ratios was evaluated. In a second experiment, the control by the predatory mites species through time on chili pepper plants infested with broad mites and their impact on fruits production was evaluated. Additionally, we evaluated the injury levels shown by chili pepper infested with broad mites.

2 - Materials and methods

2.1 Rearing methods

Chili pepper plants were obtained from seeds planted in a commercial substrate (Tropstrato®, HT hortaliças, Brazil) in polystyrene trays (67 x 34 x 5.5 cm) with 128 cells. Seedlings with two pairs of true leaves were transplanted into plastic pots (l L) containing a mix of soil and organic manure (3:1). Potted plants were kept inside wooden frame cages (0.70 x 0.70 x 0.70 cm) covered with a fine mesh (90 µm) in a greenhouse and were irrigated twice a week.

Polyphagotarsonemus latus was collected from infested chili pepper plants in the county of Oratórios in the experimental area of Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG) (Minas Gerais, Brazil, 20° 24' 0" S, 42° 48' 0" W). They were reared on potted chili pepper plants inside plastic pots (1L), placed inside cages as described above. When plant quality decreased due to broad mite feeding damage, new plants were introduced into the cages.

Amblyseius herbicolus was collected originally from the same area as *P. latus* and from *P. latus*-infested chili pepper plants maintained in a greenhouse in Viçosa (Minas Gerais, Brazil, 20°45´14´ S, 42°52´54´´ W). They were reared on arenas consisting of a PVC sheet (25 x 12 cm) placed on top of a foam pad (28 x 15 x 3 cm), surrounded by moist cotton wool, which served both as water source and a barrier to prevent predators from escaping. The foam was placed inside plastic trays (30 x 18 x 5 cm) filled with water. Commercial bee pollen was used as food, provided directly on the PVC sheet. A small PVC sheet (4 x 2 cm) with cotton yarns under it was provided as shelter and as oviposition site.

Neoseiulus barkeri was supplied by PROMIP® (Brazil). The predator was reared on arenas as above. A mixture of mould mite stages, *Tyrophagus putrescentiae* (Shrank) (Acari: Acaridae), was used as food. Mould mites were reared on the same arena as the predator, receiving crumbs of crackers as food source, which were supplied every 15 days. All predator arenas were kept in a climate room (25 ± 1°C, 60 ± 10% RH and 14 hours photophase).

2.2 Potential of control of *P. latus* on different predator:prey ratios in chili pepper plants

Chili pepper plants with two true leaves were transplanted into plastic pots (300 ml) containing a mixture of soil and commercial substrate (3:1). When plants had 10-12 leaves (45 days old), they were infested with either 20 or 40 adult females of *P. latus*. One hour later, two adult females of *A. herbicolus* or two recently-mated adult females of *N. barkeri* (both 1-2 days old since becoming adult) were released on the infested plant, resulting in predator:prey ratios of 2:20 and 2:40. Plants infested with 20 or 40 adult females of *P. latus* but without predators served as control. Thus, the four different predator:prey ratios were considered as treatments for each predator species.

For each predator species and treatment, six replicates were performed. Each replicate consisted of an infested plant with broad mites. Two replicate were placed inside plastic trays (50 x 35 x 15 cm) and water was added to prevent predator escape and contamination with other arthropods. Each plastic trays containing the replicates were maintained inside wooden frame cages (0.70 x 0.70 x 0.70 m) covered with fine mesh (90 µm). For each predator species, the experiments were conducted with 45 days of difference, but on summer season. Seven days after predator release, the number of adult females of *P. latus* and adults and immatures of *A. herbicolus* and *N. barkeri* were counted by detaching each leaf from the plants. The counting of individuals was done using a stereoscopic microscopic (Nikon® SMZ 645).

The number of adult female *P. latus* and predators (adults, immatures and eggs) were compared using a GLM with a Poisson or quasi-Poisson error distribution to correct for overdispersion when was necessary (Crawley 2007). Contrasts between treatments were assessed with the Wald test (Kuhn et al. 2008).

2.3 Control of *P. latus* on chili pepper plants and impact on fruit production

In the first experiment, control chili pepper showed severe symptoms including abscission foliar after seven days of infestation with broad mite. Because of this fact, a second experiment was performed on chili pepper plants with more size and age for evaluating broad mite control over time and impact on fruit production.

Chili pepper plants with two true leaves were transplanted into plastic pots (2 L) containing a mixture of soil and commercial substrate and maintained in cages as described above, until the first flower bud appearance. These plants were irrigated twice per week and were not fertilized.

Sixty days after transplantation, chili pepper plants were infested with 20 adult female broad mites. One hour later, two adult females of *A. herbicolus* or *N. barkeri*, were released on each plant. Plants infested with broad mites but without predators served as control. Thus, three treatments were obtained: 1) Infested chili pepper plants + *A. herbicolus*; 2) Infested chili pepper plants + *N. barkeri*, and 3) Infested chili pepper plants without predators.

Ten replicates were conducted for treatment and predator species. Each replicate consisted of one chili pepper plant infested with broad mites. Two replicates were placed inside plastic trays and maintained inside cages as described on the first experiment.

Five, ten and fifteen days after predator release, the numbers of *P. latus* adult females and the specimens of predators (adult, immature and eggs) were assessed on branch with five leaves cut off nearby the place of initial infestation and counting using a stereoscopic microscopic as above. Additionally, we evaluated the symptoms of injury presented by the plants in the different treatments by visual damage scale with ratings ranging from 0 to 4 (Table 1). The evaluation of injury was current out five, ten and fifteen days after of infestation with broad mite.

Table 1: Scale notes for assessment of Injury of broad mite *P. latus* in *Capsicum frutensces* L. (adapted from Peña and Bullock 1994).

Level	**Injury**
0	No injury, smooth and well-expanded leaves with bright green color.
1	Lower brightness on leaves and leaves show slight bronzing.
2	Moderate Injury, curling, leaves showed bronzing.
3	Leves with severe curling, wilting.
4	Leaves with necrosis and leaf abscission.

Differences in numbers of broad mite in the different treatments were analysed using a Generalized Linear Mixed Effects Model (LMER) with a Poisson error distribution, with plant as random factor to correct for pseudoreplication due to repeated measures (Crawley 2007).

The notes the levels of injury were transformed into x+1 due to that in the beginning of the assessments the notes were equal to zero, especially on the treatments with predators. The notes were subjected to multivariate analysis of variance.

Two months after initial infestation with *P. latus*, the fruits were collected. In the laboratory, the fruits were counted and weighed using an electronic balance (Bioprecisa® JA3003N). Numbers of chili pepper fruits were analyzed using a GLM with a quasi-Poisson error distribution to correct for overdispersion (Crawley 2007). Contrasts between treatments were assessed with the Wald test (Kuhn et al. 2008). The weight of chili pepper fruits was subjected to ANOVA with a Tukey post-hoc test.

3 - Results

3.1 Potential of control of *P. latus* on different predator: prey ratios in chili pepper plants

The number of *P. latus* recorded on the chili pepper plants after seven days was significantly affected by the predator:prey ratio ($F_{3,16}$=124.3, p<0.001; $F_{3,18}$=15.71, *df*=3, p<0.001 for *A. herbicolus* and *N. barkeri* respectively) (Fig. 1 and Fig. 2). Despite the time difference between the experiments, no significant difference in the broad mites numbers on the predator:prey ratios with any predator ($F_{3,16}$=0.31, p=0.82).

Broad mite populations were higher on plants without predators than on plants with predators. The increase of broad mite populations on plants without predators showed that the experimental conditions were favourable for growth of this species. Broad mite populations in treatments with predators were lower than the initial populations (20 or 40 females), indicating that predators reduced the pest population (Fig. 1 and 2).

The predators increased in number during experiment period. However, there was no significant difference on the number of individuals of *A. herbicolus* between the predator: prey ratios (Dev=37.44, *df*=1, p=0.56) (Fig. 3), with presence of 4.4 (SE ±0.87) and 5.2 (±1.08) individuals for 2:20 and 2:40 predator:prey ratios, respectively. For *N. barkeri*, there was no significant difference on the number of specimens between the predator:prey ratios (Dev=16.13, *df*=1, p=0.12) (Fig. 4), with presence of 1.91 (SE ±0.28) and 2.7 (±0.48) individuals for 2:20 and 2:40 predator:prey ratios, respectively.

3.2 Control of *P. latus* on chili pepper plants in a greenhouse and impact on production

The number of broad mites differed significantly between chili plants with or without predators (X^2=692.1, *df*=2, p<0.001) and time (X^2=149.6, *df*=2, p<0.001, Fig. 5). There was no significant difference in the number of broad mites on plants with *A. herbicolus* or *N. barkeri* (X^2=0.56, *df*=1, p=0.45). The numbers of broad mite females increased over time on chili peppers plants without predators and remained low on plants with predators (Fig. 5).

Although there was an increase in the numbers of predators of the two species, there was no difference significant on the number of individuals between predator species (X^2=1.28, *df*=1, p=0.26) (Fig. 6). There was significant difference in the number of predators in the days evaluated (X^2=7.46, *df*=2, p=0.02) (Fig. 6). The average numbers of predators per plant over the three evaluations was 3.81 (±0.36 SE) for *A. herbicolus* and 2.86 (± 0.19) for *N. barkeri* (Fig. 6).

The injury caused by broad mite on physic nut plants differed significantly between plants with or without predators and with the week of evaluation, demonstrating that the broad mite attack evolves through time (Table 2) (Fig. 7).

Plants with predators produced twice as many fruits per plant than plants without them (F=0.86, *df*=2, p=0.04) (Fig. 8A). The weight of fruits of plants with predators was also higher than plants without predators (Fig. 8B, F=12.7, *df*=2, p<0.001).

Table 2: Multivariate analysis of variance of the scale notes of injury caused by *Polyphagotarsonemus latus* on chili pepper plants on the treatments evaluated. Viçosa-MG, 2012.

Source variation	Wilk`s Lambda	Num DF.	Den DF.	F	P
Treatment	0.596	2	81	27.40	<0.001
Day	0.767	2	81	12.28	< 0.001
Treatment*Day	0.649	4	81	10.92	< 0.001

4 – Discussion

The potential of *A. herbicolus* and *N. barkeri* as control agents of broad mite was evaluated in greenhouse experiments. In a first experiment, less than twelve broad mite individuals for the different predator:prey ratios for both predator species were recorded. Van Maanen et al. (2010) found low numbers of broad mites after three weeks with an initial predator:prey ratio of 1:10 and 1:20. In our experiment, broad mite populations would have decreased even more after some more time, but the control plants were already showing severe damage symptoms including severe foliar abscission, hence, the experiment was ended after seven days.

The rapid evolution of symptoms in control plants in this experiment may indicate the vulnerability of chili pepper to pest infestations in the early weeks of the crop. No studies on the effects of infestation of broad mites on the different phenological ages of the chili pepper. However, Coss-Romero and Peña (1998) recorded higher populations of broad mite in sweet pepper plants with 6 weeks of age compared with plants with more age. The age and phenological stage are similar to plants used in our experiment. Thus, the authors conclude that the genus *Capsicum* is highly susceptible to broad mite attack.

In the second greenhouse experiment, *N. barkeri* and *A. herbicolus* reduced broad mite populations on chili pepper plants when released in a predator:prey ratio of 2:20. The phytoseiid *N. barkeri* was tested for broad mite control on sweet pepper, lima and bean under greenhouse conditions on the United States (Fan and Petit 1994, Peña and Osbourne 1996). The Brazilian strain of *N. barkeri* showed a more reduction of broad mite population that American strain when similar predator:prey ratio were used. Additionally, American strain only showed a successful broad mite control on a lower predator:prey ratios than that here evaluated.

Meanwhile, this is the first study that evaluated to *A. herbicolus* as biological control agent of *P. latus* under greenhouse conditions. Our results indicate an excellent performance of this specie, confirming the outcomes obtained in laboratory tests. *A. herbicolus* showed similar or better results than other phytoseiids evaluated as natural enemies of broad mite under greenhouse conditions (Peña and Osbourne 1996, Onzo et al. 2012, van Maanen et al. 2010).

A fact recorded in this experiment was the increased of the number of individuals of the two phytoseiid species through time, even when numbers of broad mites were low. This may be caused when the predators feed on other resources, such as pollen and nectar from chili pepper flowers. It is known that *A. herbicolus* is able to feed and develop on different pollen species (Rodriguez-Cruz et al. 2013), and that *N. barkeri* is capable of ovipositing on an exclusive diet of a broad bean pollen (*Vicia faba* L.) (Nomikou et al. 2001). This is important, because predator populations may thus persist without pests and prevent infestations of the plants (Van Rijn and Sabelis 1993). Additionally, the presence of domatia in chili pepper leaves may be a factor that helps on predator establishing. Both predatory mites used as shelter and local for oviposition the domatia (Personal observation).

Chili pepper plants with presence of predators showed injury notes equal to zero or very low, without manifestation of severe symptoms from broad mite attack. High injury rating were recorded on the control chili pepper plants, including foliar abscission. Moreover, plants without predators produced fewer fruits with smaller size and weight. Stansly and Castillo (2009) recorded that sweet pepper production was significantly different in number and weight of fruits between plants with and without predator release, with production 2.3 times superior on plants with presence of predators.

Despite, the absence of standardization of size for fruit commercialization. The producers tend to reject small fruits for commercialization (CMF Pinto, personal communication). Additionally, small fruits require more effort to harvest, leading to increased costs for the producer (Embrapa 2012). Greater fruits should be contain more placenta, pericarp and seeds and generally show a higher capsaicin content (Bosland 1996, Cisneros-Pineda et al. 2007, Pandhair and Sharma 2008). This chemical compound is responsible for the chili pepper pungency, feature well appreciated in the market (CMF Pinto personal communication).

Despite better values of predation and oviposition rates showed by *A. herbicolus* on laboratory tests, the outcomes recorded on greenhouse experiments no showed differences on the effectiveness of broad mite control by the two predator species. A characteristic that can favour the performance of *N. barkeri* under greenhouse conditions is their capacity to feed on whitefly *Bemisia tabaci*

(Hemiptera: Aleyrodidae), a common pest on greenhouses (Nomikou et al. 2001). We considered that *A. herbicolus* and *N. barkeri* as potential control agents of broad mite, due to the outcomes were similar or better than that recorded by other phytoseiids evaluated on the world (Fan and Petitt 1994, Peña and Osbourne 1996, Rodríguez-Morell et al. 2010, van Maanen et al. 2010). Despite that other phytoseiids were evaluated on Brazil; this is the first study that evaluated natural enemies of broad mite in greenhouses conditions. Our outcomes indicates that *A. herbicolus* and Brazilian strain of *N. barkeri* may be alternatives to control of this pest on protected crops. We considered that field experiments should be performed to investigate the efficiency of these predatory mites for broad mite control.

References

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

Boslan PW (1996) Capsicum: Innovative Uses of an Ancient Crop. In: Janick J (Ed), Progress in New Crops. ASHP Press, Arlington, pp. 479-487.

Britto EPJ, Lindquist EE, de Gilberto GJ (2011). Redescription of *Lasioseius floridensis* Berlese, 1919 (Acari: Mesostigmata: Blattisociidae), with notes on closely related species. Zootaxa 2905: 1-15.

Cisneros-Pineda O, Torres-Tapia LW, Gutiérrez-Pacheco LC, Contreras-Martín F, González-Estrada T, Peraza-Sánchez SR (2007) Capsaicinoids quantification in chili peppers cultivated in the state of Yucatan, Mexico. Food Chemistry 104: 1755-1760.

Crawley MJ (2007). The R book. Wiley, West Sussex.

De Coss-Romero M, Peña JE (1998). Relationship of broad mite (Acari: Tarsonemidae) to host phenology and injury levels in *Capsicum annuum*. Florida Entomology 41: 515-526.

De Moraes GJ, McMurtry JA, Denmark HA, Campos CB (2004). A revised catalog of the mite family Phytoseiidae. Zootaxa 434: 1–494.

De Moraes GJ, Fletchmann CHW (2008). Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil, Holos, Ribeirão Preto, Brazil.

Empresa Brasileira de Pesquisa Agropecuaria (2014). Cultivo da Pimenta. http://www.cnph.embrapa.br/paginas/sistemas_producao/cultivo_da_pimenta/colheita_pos_colheita.htm. Accessed 2 February 2014.

Fan Y, Petit FL (1994). Biological control of broad mite, *Polyphagotarsonemus latus* (Banks), by *Neoseiulus barkeri* Hughes on pepper. Biological Control 4: 390-395.

Gerson U (1992). Biology and control of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae*).* Experimental and Applied Acarology 13: 163-178.

Hajek A (2004). Natural Enemies: An introduction to Biological Control, Cambridge University Press, Cambridge, England.

Kuhn M, Weston S, Wing J, Forester J (2008). The contrast package. http://cran. Nedmirror.nl/web/packages/contrast/vignettes. Accessed 02 March 2012.

La SY, Paik CH, Lee GH, Kim DH (2009). Biological control of *Polyphagotarsonemus latus* (Acari: Tarsonemidae) by *Amblyseius cucumeris*. Korean Journal of Entomology 48: 347-354.

Lin H, Chuan.hua X, Jin-jun W, Ming L, Wen-cai, Zhi-mo Z (2009). Resistance selection and biochemical of resistance to two acaricides in *Tetranychus cinnabarinus* (Boiduval). Pest Biochemical Physiology 93: 47-52.

Matos CHC (2006). Mecanismos de defesa constitutiva em espécies de pimenta *Capsicum* e sua importância no manejo do ácaro branco *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae). Dissertation, Federal University of Viçosa.

McMurtry J, De Moraes G, Sourassou N. F (2013). Revision of the lifestyle of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematic and Applied Acarology 18: 297-320.

Ministerio de Agricultura, Pecuaria e Abastecimento, Agrofit (2014) http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed 2 February 2014.

Nomikou M, Janssen A, Schraag, R (2001). Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. Experimental and Applied Acarology 25: 271-291.

Onzo A, Houedokoho, Hanna R (2012). Potential of the predatory, Amblyseius swirskii to supress the broad mite, *Polyphagotarsonemus latus* on the gboma eggplant, *Solanum macrocarpon*. Journal of Insect Science 12: 1-11.

Pandhair V, Sharma (2008) Accumulation of Capsaicin in Seed, Pericarp and Placenta of Capsicum annuum L Fruit. Journal of Plant Biochemistry and Biotechnology 17: 23-27.

Peña JE, Bullock RC (1994). Effects of feeding of broad mite (Acari: Tarsonemidae) on vegetative plant growth. Florida Entomologist 77(1): 180-184.

Peña JE, Osborne L (1996). Biological control of *Polyphagotarsonemus latus* (Acarina: Tarsonemidae) in greenhouses and field trials using introductions of predacious mites. Entomophaga 41: 276-285.

Pinto CMF, Salgado LT, Lima PC, Picanço M, Júnior TPJ, Moura WM, Brommonschenkel SH (1999). A cultura da pimenta (*Capsicum* sp.). EPAMIG, Belo Horizonte.

R Development Core Team (2012). R foundation for statistical computing. Vienna, Austria.

Riley D (1992). A new occurrence of Broad mite in peppers in the lower Rio Grande valley of Texas. Subtropical Plant Science 45: 46-48.

Rodríguez-Cruz F, Venzon M, Pinto CMF (2013). Perfomance of *Amblyseius herbicolus* on broad mites and on castor beans and sunnhemp pollen. Experimental and Applied Acarology 60: 497-507.

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

Rufino JLS, Penteado DCS (2006). Importancia económica, perspectivas e potencialidades do mercado para pimenta. Informe Agropecuário 27: 7-15.

Sabelis MW (1990). How to analyze prey preference when prey density varies - a new method to discriminate between effects of gut fullness and prey type composition. Oecologia 82: 279-285.

Sarmento RA, Rodrigues DM, Faraji F, Erasmo EAL, Lemos F, Teodoro AV, Kikuchi WT, Santos GR, Pallini A (2011). Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on *Jatropha curcas* plants in Brazil. Experimental and Applied Acarology 53: 203-214.

Sato ME, Da Silva MZ, Raga A, Souza-Filho MF (2005). Abamectin resistance in *Tetranychus urticae* (Acari: Tetranychidae): selection, cross-resistance and stability of resistance. Neotropical Entomology 34: 991-998.

Skirvin D, Fenlon JS (2003). The effect of temperature on the functional response of *Phytoseiulus persimilis* (Acari: Phytoseiidae). Experimental and Applied Acarology 31: 37-49.

Stansly P, Castillo J (2009). Control of broad mites, spider mites, and whiteflies using predaceous mites in open field pepper and eggplant. Processing of Florida State Horticultural Society 122: 253-257.

van Maanen R, Vila E, Sabelis M, Janssen A (2010). Biological control of broad mites (*Polyphagotarsonemus latus*) with the generalist predator *Amblyseius swirskii*. Experimental and Applied Acarology 52: 29-34.

van Rijn P, Sabelis M (1993). Does alternative food always enhance biological control? The effect of pollen on the interaction between flower thrips and its predator. IOBC/WPRS Bulletin 16: 123-125.

Venzon M, Matos CHC, Pallini A, Santos IC (2006). Pragas associadas à cultura de pimenta malagueta e estratégias de manejo. Informe Agropecuário 27: 75-86.

Venzon M, Rosado MC, Molina-Rugama AJ, Duarte V, Dias R, Pallini A (2008). Acaricidal efficacy of neem against *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). Crop Protection 27: 869–872.

Venzon M, Amaral DSSL, Perez AL, Rodríguez-Cruz FA, Togni PHB, Oliveira RFO (2011). Identificação e manejo ecológico de pragas da pimenta da cultura da pimenta. EPAMIG, Viçosa, Brasil.

Vichitbandha P, Chandrapatya A (2011). Broad mite effects on Chilli shoot damage and yields. Pakistan Journal of Zoology 43: 637-649.

Waterfield G, Zilberman D (2012). Pest management in food system: An economic perspective. Annual Review of Environmental Resources 37: 223-245.

Weintraub P, Kleitman S, Mori R, Shapira N, Palevsky E (2003). Control of the broad mite *Polyphagotarsonemus latus* (Banks) on organic greenhouse sweet peppers (*Capsicum annuum* L.) with the predatory mite, *Neoseiulus cucumeris* (Oudemans). Biological Control 27: 300-309.

Zhang L (2008). Mites of horticultural crops in the Northern Territory. Department of Regional Development, Primary Industry, Fisheries and Resources, Northern Government. 5: 1-3.

Figures

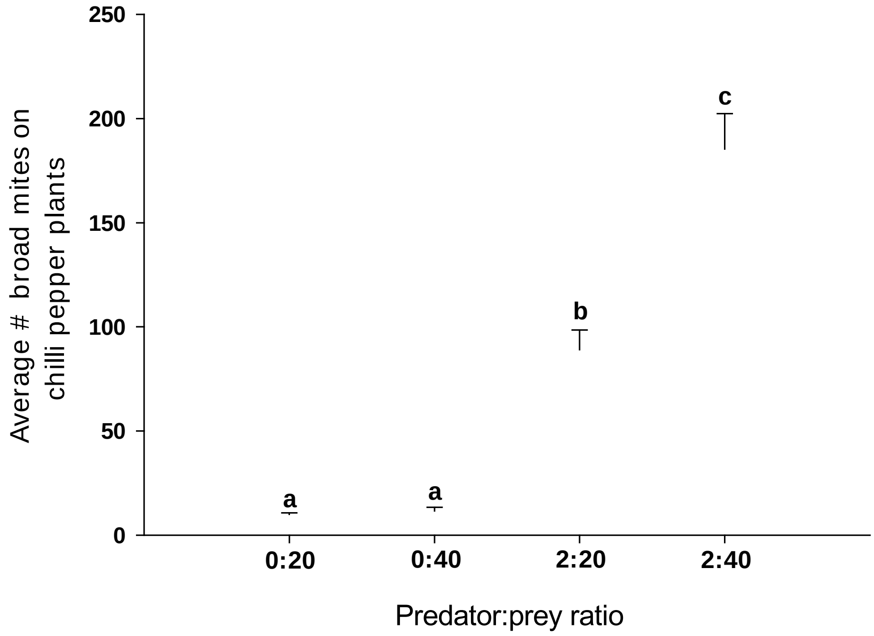

Figure 1. Average numbers (±SE) of broad mite females on chili pepper plants. Plants were either infested with 20 or 40 adult female broad mites and either 0 (0:20 and 0:40) or 2 (2:20 and 2:40) adult female *Amblyseius herbicolus* were released on the plants. Numbers were assessed seven days after initial infestation. Different letters above bars indicate significant differences (Wald test).

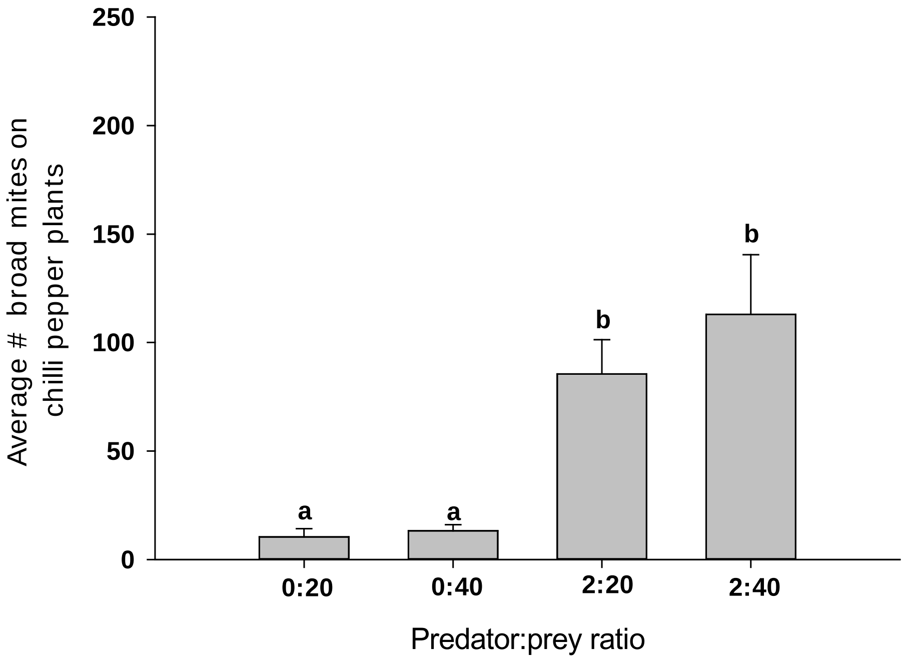

Figure 2. Average numbers (±SE) of broad mite females on chili pepper plants. Plants were either infested with 20 or 40 adult female broad mites and either 0 (0:20 and 0:40) or 2 (2:20 and 2:40) adult female *Neoseiulus barkeri* were released on the plants. Numbers were assessed seven days after initial infestation. Different letters above bars indicate significant differences (Wald test).

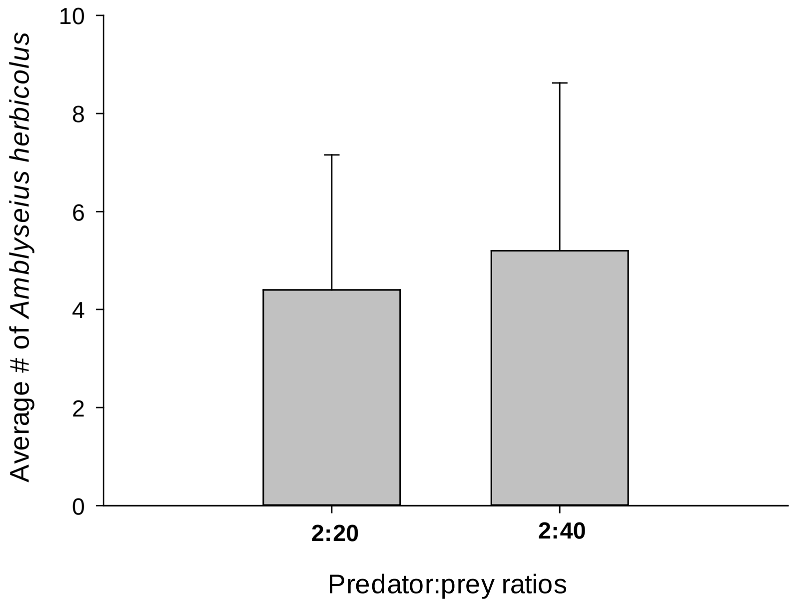

Figure 3. Average number of individuals (Adults, immatures and eggs) (±SE) of *Amblyseius herbicolus* on chili pepper plants. Initially, 2 adult females of the predator were released per plant, which contained either 20 (2:20) or 40 (2:40) adult female broad mites. Numbers were assessed seven days after initial infestation with broad mites. Viçosa (MG), Brazil, 2012.

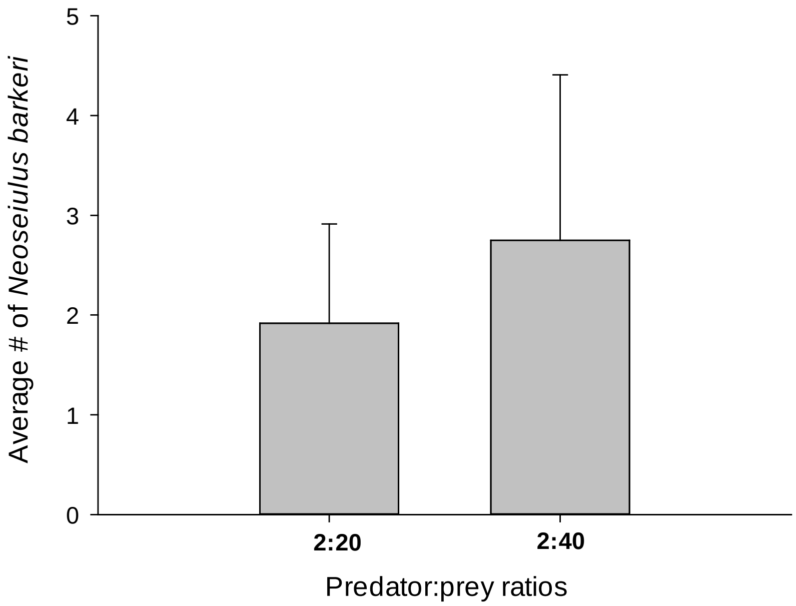

Figure 4. Average number of specimens (Adults, immatures and eggs) (±SE) of *Neoseiulus barkeri* on chili pepper plants. Initially, 2 adult females of the predator were released per plant, which contained either 20 (2:20) or 40 (2:40) adult female broad mites. Numbers were assessed seven days after initial infestation with broad mites. Viçosa (MG), Brazil, 2012.

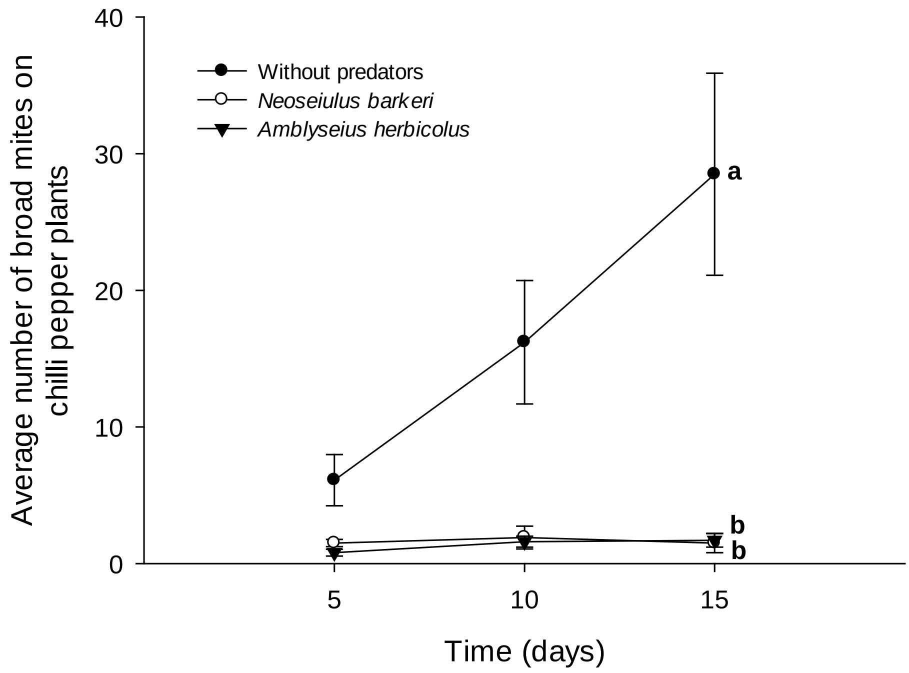

Figure 5. Number of broad mite females through time (days) (±SE) on chili pepper plants in the absence and in the presence of the phytoseiids *Amblyseius herbicolus* or *Neoseiulus barkeri*. Different letters indicate significant difference (Model simplification). Viçosa (MG), Brazil, 2012.

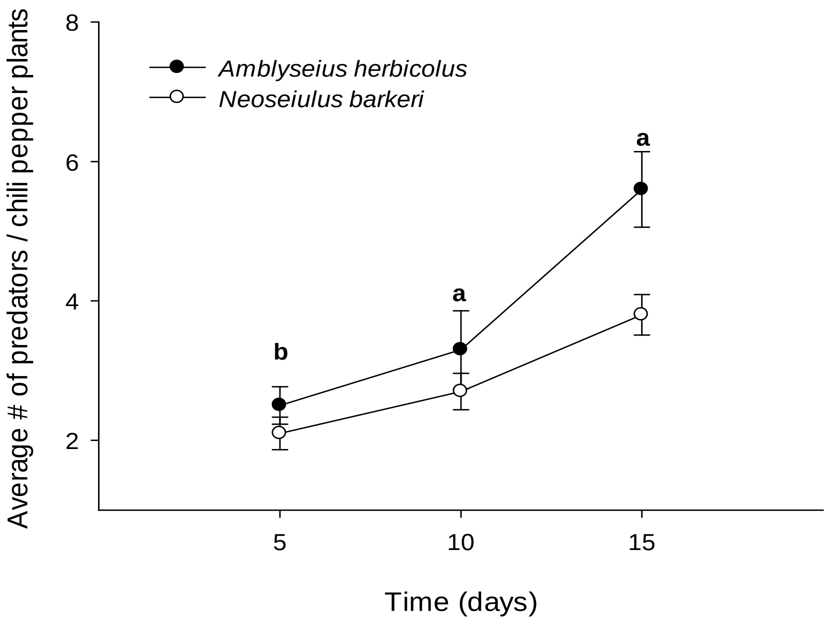

Figure 6. Number of individual predators *Amblyseius herbicolus* and *Neoseiulus barkeri* through time (days) (±SE) on chili pepper plants. Different letters indicate significant difference (Model simplification). Viçosa (MG), Brazil, 2012.

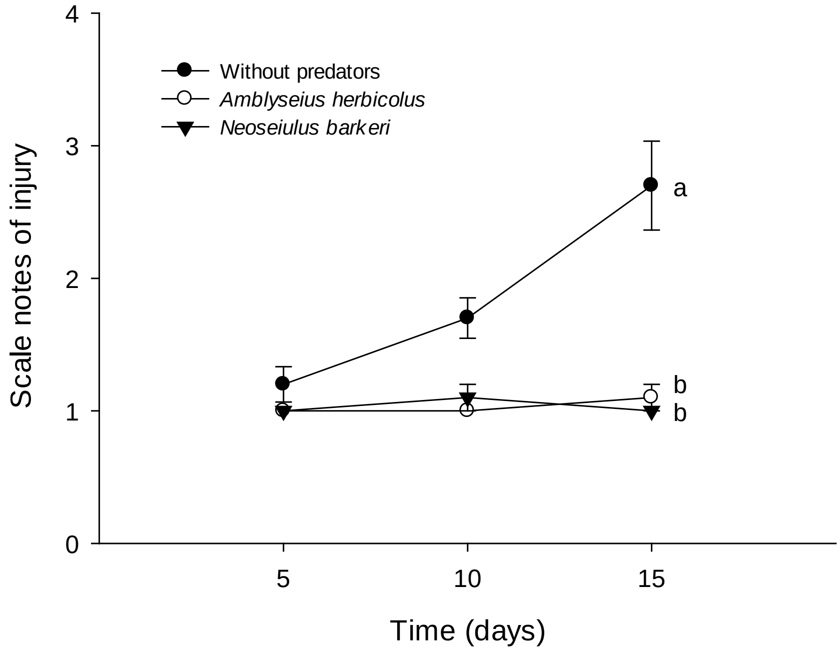

Figure 7. Variation of the scale notes of injury of broad mite *P. latus* through time (days) (±SE) on chili pepper plants. Different letters indicate significant difference (Tukey 5%). Viçosa (MG), Brazil, 2012.

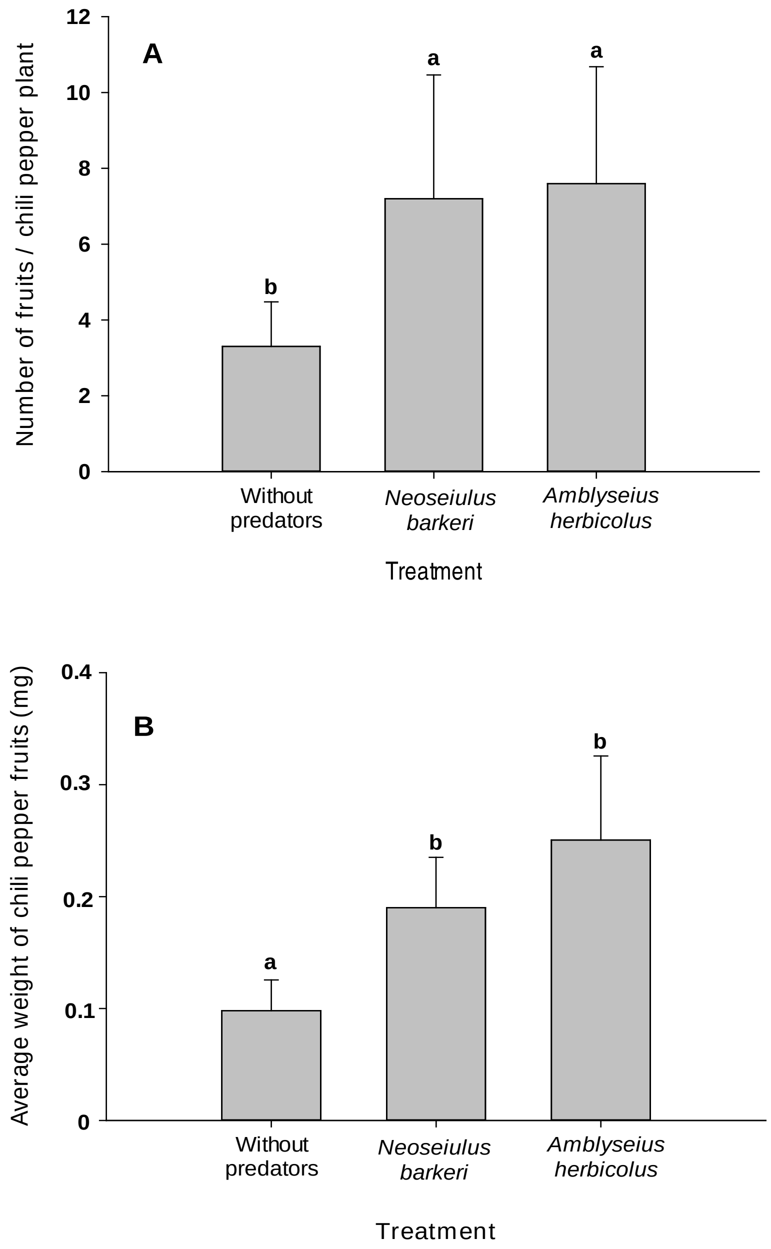

Figure 8. A) Average numbers (±SE) and (B) weight of chili peppers fruits, in the absence and in the presence of the phytoseiids *Amblyseius herbicolus* or *Neoseiulus barkeri*. Different letters above bars indicate significant difference (Wald test for number fruit and Tukey for weight of fruit). Viçosa (MG), Brazil, 2012.

CHAPTER 3: BIOLOGICAL CONTROL OF BROAD MITES ON PHYSIC NUT AND CHILI PEPPER IN THE FIELD

Abstract

Several studies have evaluated phytoseiid mites as potential biological control agents of broad mites in the laboratory and in greenhouses. However, there is little information on the use of these phytoseiid mites for broad mite control in open fields. Previously, *Amblyseius herbicolus* and a Brazilian strain of *Neoseiulus barkeri* were show to have potential for broad mite control in greenhouses in Brazil. Here, we evaluated broad mite control by these predatory mites on physic nut and on chili pepper in the field conditions. Physic nut and chili pepper plants without predators, harboured high populations of broad mite, showed more damage, including severe fall of leaves that plants with predators.

The two predators increased in numbers over time on two crops. *Amblyseius herbicolus* reared similar densities in physic nut and chilli pepper. In chili pepper, the predatory mites could have used the flower pollen as alternative food. There was no significant difference in the number and weight of chili pepper fruits between chili plants with and without predators. However, fruits from plants without predators were smaller. Despite of a single predator release, the results indicate a positive effect in chili pepper and physic nut, avoiding the presence of higher broad mite populations and the appearance of severe symptoms.

Key-words: *Amblyseius herbicolus*, *Neoseiulus barkeri*, *Polyphagotarsonemus latus*, *Capsicum frutensces*, *Jatropha curcas*, Biological control

1 – Introduction

The broad mite is a small mite (0.1-0.3 mm in length) with a worldwide distribution, capable of attacking more than 60 botanical families (Gerson and Weintraub 2012, De Moraes and Flechtmann 2008). Among these are two important crops, in which the broad mite is considered a key pest in Brazil, physic nut (*Jatropha curcas* L.) and chili peppers (*Capsicum frutescens* L.) (Venzon et al. 2006, 2013, Lopes 2009, Sarmento et al. 2011, Evaristo et al. 2013).

The physic nut is a large shrub of Mexican origin, distributed worldwide in tropical and subtropical zones (King et al. 2009, Pandey et al. 2012). Due to its tolerance to degraded soils and soils with low fertility, drought and its high yield of oil in their seeds, it is considered as a promising species for biodiesel industry (King et al. 2009, Pandey et al. 2012, Parawira 2010). In Brazil, smallholders have begun to plant it as monoculture (Sarmento et al. 2011), and a result, pest attack has been increased. On the physic nut, broad mites are found on the apical region (Lopes 2009) causing the loss of natural sheen, curling and deformation of leaves (Fig. 1B). Additionally, the broad mite attack can cause emission disorder of leaves and premature fall of leaves in severe infestations (Fig. 1C and 1D).

Chili pepper (*Capsicum frutescens* L.) is an important crop in the state of Minas Gerais State (Brazil), with a strong social component, because its production uses mainly family labour (Pinto et al. 1999, Venzon et al. 2006). Due to small size of broad mite, farmers usual only detect it when the first symptoms appear in the crop (Venzon et al. 2013). The symptoms manifested by attacked plants include curling and bronzing of leaves, especially of the apices; in severe attacks of broad mite high defoliation is recorded (Venzon et al. 2013).

The main method for combating broad mite in the world and Brazil is chemical control (Peña 1988, Gerson 1992, De Moraes and Flechtmann 2008). However, for physic nut and chili peppers, no acaricides are officially registered for broad mite control in Brazil (Agrofit 2014). Nevertheless, Brazilian farmers apply pesticides registered for other crops in an attempt to control the pest, especially in chili pepper, but control is often not successful (CMF Pinto personal communication). Experimentally, abamectin shows good control of broad mites in physic nut, but due to a lack of registration, its use is not recommended in commercial plantations (Albuquerque 2008). The misuse of chemicals can lead to problems such as environmental contamination, poisoning of farmers, and residues on fruits in the case of chili pepper (Peña 1988, Pinto et al. 2012).

Biological control of pest mites is considered as an excellent alternative for chemical control. Predatory mites of the family Phytoseiidae are key natural enemies of pest mites (McMurtry et al. 2013). Five phytoseiids have been registered in association with broad mites in Brazil. *Amblyseius herbicolus* (Chant) was found in chili pepper plants infested with the pest in the state of Minas Gerais (Matos 2006). This species grows and can reproduce exclusively on broad mites (Rodríguez-Cruz et al. 2013). A second species, a Brazilian strain of *Neoseiulus barkeri* Hughes, was collected on infested gerbera in greenhouses in the state of São Paulo (Britto et al. 2011). *Iphiseiodes zuluagai* Denmark & Muma and *Euseius concordis* Chant are the most common natural enemies associated with broad mites in physic nut in the state of Tocantins, Brazil (Sarmento et al. 2011). Additionally, *Typhlodromus transvaalensis* Nesbit, was recorded on chili pepper infested on Minas Gerais (Rodríguez-Cruz, unpublished data). Pest control provided by natural enemies is considered as an environmental service (Myers 1996). This feature may be of great importance for smallholders, due to lack of resources for pest control (Sarmento et al. 2011) and it is especially relevant in crops with poor or no phytosanitary support, as is the case of physic nut and chili pepper.

Two studies have evaluated the potential of these four species as biological control agents of broad mite in Brazil (Sarmento et al. 2011, Rodríguez-Cruz et al. in prep). However, these studies were carried out on laboratories and greenhouses. Additionally, these studies generally are carried out with release of only predator species. However, generally there are several of predatory mites inhabiting the same plant. Lopes (2009) recorded six different predatory mites species associated on physic nut when study the bioecology of broad mite on this crop. Despite this fact, there is no literature on the interactions that can occur between different species of predatory mites evaluated as biological control agents of broad mite.

Here, we evaluated in the field broad mite control by the predatory mites *A. herbicolus* and *N. barkeri* when released individually and in combination on physic nut and released individually on chili pepper plants. Additionally, we evaluated the symptoms of injury presented by the plants in the different treatments by visual damage scale as described on the chapter two.

2 – Material and methods

2.1 Physic nut and chili pepper seedlings

Physic nut seedlings were obtained by sowing the seeds directly in plastic pots (2L) containing a mix of soil and organic manure (3:1). Forty-five days after germination, seedlings received chemical fertilization (Biofert®, Brazil). Monthly fertilization were done with the same product. Potted plants were kept in a greenhouse until time for transplantation. Seedlings were irrigated once a week, due their drought tolerance.

Chili pepper seedlings with four true leaves were obtained from a local provider (Semearte, Minas Gerais, Brazil) and kept in a greenhouse until time for transplantation in field. Chili pepper seedlings were irrigated daily.

2.2 Mite rearing

The broad mites and predator species were obtained from populations maintained in the laboratory of entomology of the Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG) (Viçosa, Brazil, 20° 24' 0" S, 42° 48' 0" W), as described in the previous chapter.

2.3 Physic nut field experiment

The physic nut experiment was carried out in the experimental area of EPAMIG in Oratórios (state of Minas Gerais, Brazil, 20° 24' 0" S, 42° 48' 0" W). Seedlings were transplanted to an area of 30 x 28 m, with two meters of space between plants and between rows, with a total of 182 physic nut plants. Each hollow for physic nut seedling, received two litters of organic manure a week before transplantation, which was set on August 16, 2013. Chemical fertilization was applied 40 and 80 days after transplantation, 70 grams per plant (Heringer ® 20-05-20, NPK), to promote leaf emission taking into account that the species is deciduous. Plants were irrigated once a week.

The broad mite infestation was done artificially. Infestation was done by attaching a piece of infested chili pepper leaf (about 3 cm^2 and average of 15 broad mite females) on the upper third part of the plant. Attachment was done with adhesive tape, ensuring that the abaxial sides remain facing each other, because broad mites preferably inhabit this side of the leaf.

Physic nut plants were infested 90 days after transplantation. A week after this activity, a sheet next to the side of the point of infestation was cut, placed in a paper bag and stored in a polystyrene box for transport to the laboratory and subsequent evaluation of the success of the infestation under a microscope stereoscopic (Nikon® modelo SMZ 645). The infestation was considered successful when we recorded the presence of different broad mite stages on the leaves evaluated.

For predators releasing, pipette tips (2 ml) were adapted as container. The narrower end was cut off and sealed with silicone. The wider end was sealed with PVC film after storage of the predators. To allow the predators breathing, the PVC film received several piercing. Predators were kept starving for about 12 hours before the release.

Pipettes containing the predators were fastened with the aid of a cotton thread (approximately 20 cm of long) placed through the tip and the silicone on the same leaf on the which the infestation with broad mite was realized (Fig. 2). The PVC film was completely removed while the silicone was partially removed to allow dispersion of the predators.

The release of predatory mites occurred one week after infestation confirmation, as described above, resulting in four treatments: (1) *A. herbicolus* (10 females); (2) *N. barkeri* (10 females); (3) *A. herbicolus* + *N. barkeri* (five females for each predator species); (4) no predatory mites. The predator:prey ratio was approximately of 1:4.5. A randomized complete block design with four replicates per treatment was used. Each replicate included four physic nut plants (two plants x two rows). Each replicate was isolated from the others replicates by one plant physic nut and one row of physic nut. The outermost plants were not considered to avoid edge effects.

2.4 Chili pepper field experiment

The chili pepper experiment was done in Duas Barras, district of Viçosa (state of Minas Gerais, Brazil, 20° 24' 0" S, 42° 48' 0" W). Seedlings were transplanted to an area of 20 x 20 m, with 0.80 meter of space between plants and between rows of plants, with total of 625 chili pepper plants. During the experimental period, cultural treatments recommended for the species as fertilization, irrigation and weed control were carried out according to technical recommendations (EPAMIG, Informe Agropecuário 2006). Transplantation occurred in early October.

Chili pepper plants were infested 70 days after transplantation as described for physic nuts. A week after of infestation with broad mites, a branch (with four leaves) next to the infestation place was cut off, stored inside of a paper bag and transported to the laboratory to evaluate the infestation success under a stereomicroscope as described for physic nut. The infestation was considered successful when we recorded the presence of different broad mite stages on the leaves evaluated.

The release of predatory mites was conducted a week after infestation confirmation, resulting in three treatments: (1) *A. herbicolus* (5 females); (2) *N. barkeri* (5 females); (3) no predatory mites. The predator:prey ratio was approximately 1:2.3. In this experiment, a severe reduction in the number of chili pepper plants available was recorded. This reduction was caused by lower development of the plants, which did not reach a height of ten centimeters, being discarded for the use. Thus, the treatment with combined release of predators no was realized.

A randomized complete block design with four replicates per treatment was used. Each replicate included nine chili pepper plants (three plants x three rows). Each replicate was isolated from the other through two lines of plants and two rows. The outermost plants (two plants x two rows) were not considered for sampling to avoid the edge effect.

2.5 Mite sampling

Seven days after predator release, the mite sampling was initiated for both crops. For physic nut, three leaves from each plant of the replicate were collected near to the infestation point. The leaves were put in a paper bag, stored inside a polystyrene box and transported to the laboratory for evaluation. For chili pepper, a branch with five leaves from each plant of the replicate was cut near the infestation point. These branches were put in Petri dishes, stored in a paper bag and transported to the laboratory for evaluation.

Sampling was continued for six and eight weeks after the predators releasing, for chili pepper and physic nut, respectively. The number of broad mite females as well as motiles and eggs of predators were counted under a stereomicroscope (Nikon® modelo SMZ 645). Similarly to second experiment on greenhouse, the injury caused by broad mite was recorded according to the visual damage presented on the chapter two. The evaluation was carried out every week, coinciding with the counting of mite populations on the two crops.

Additionally to mite sampling, we collected the mature chili pepper fruits from each plants of the replicate on three treatments evaluated. The fruits were stored in paper bag and transported to laboratory for counting and weighting using an electronic balance (Bioprecisa® JA3003N). The fruits collect was done during three weeks

2.6 Statistical analysis

Data on the average number of broad mite females per 12 leaves for each replicate of the physic nut experiment and nine branches for each replicate of the chili pepper experiment were analyzed using mixed-effects models (lmer of the library lme4 of R, R Development Core Team 2012) with plant as random factor to correct for repeated measures (Crawley 2007). The contrasts between treatments were assesed through model simplification.

The notes the levels of injury were transformed into x+1 due to that in the beginning of the assessments the notes were equal to zero, especially on the treatments with predators. The notes were subjected to multivariate analysis of variance.

Numbers of chili pepper fruits from each replicate of three treatments evaluated were analyzed using generalized linear models (GLM) with a quasi-Poisson error distribution to correct for overdispersion (Crawley 2007). The weight of chili pepper fruits was subjected to ANOVA. The analyses were performed using the statistical software R 2.15 (R Development Core Team 2012).

3 – Results

On physic nut plants, broad mite populations increased gradually during four weeks and them declined until the last week of evaluation, especially on plants with *A. herbicolus* or *N. barkeri* released independently (Fig. 3). The number of broad mites differed significantly among physic nut plants with released of predatory mites, each species independent or in combination, and plants without predators (X^2=3309.9, *df*=3, p<0.001) (Fig. 3). The broad mite populations in physic nut plants without predatory mites were significantly higher than on plants with predatory mites (Fig. 3, contrasts through model simplification: X^2=95.06, *df*=1, p<0.001; X^2=38.91, *df*=1, p<0.001; X^2=18.29, *df*=1, p<0.001, for *A. herbicolus*, *N. barkeri* and *A. herbicolus* + *N. barkeri*, respectively). The differences in broad mite populations among physic nut plants with predatory mites were significant with all values of p<0.01 (Fig.3).

Predator populations increased through the time on physic nut plants. The number of individuals differed significantly among plants with and without predators (X^2=101.48, *df*=3, p<0.001) (Fig. 4). *Amblyseius herbicolus* showed more number of individuals that *N. barkeri* (X^2=13.75, *df*=1, p<0.001), combined release (X^2=33.94, *df*=1, p<0.001) or plants without predators (X^2=95.06, *df*=1, p<0.001) (Fig. 4). The number of predators on physic nut plants with *N. barkeri*, *A. herbicolus* + *N. barkeri* and plants without them were significant with all values of p<0.01 (Fig.4).

The injury caused by broad mite on physic nut plants differed significantly between plants with or without predators and with the week of evaluation, demonstrating that the broad mite attack evolves through time (Table 1) (Fig. 5).

On chili pepper plants, broad mite populations increased gradually during four weeks and them declined until the last week of evaluation, especially on plants with *A. herbicolus* (Fig. 6). The number of broad mites differed significantly among chili pepper plants with and without predators (X^2=134.21, *df*=2, p<0.001) (Fig. 6).

Table 1: Multivariate analysis of variance of the scale notes of injury caused by *Polyphagotarsonemus latus* on physic nut plants on the treatments evaluated, Oratórios (MG), Brazil, 2013.

Source variation	Wilk`s Lambda	Num DF.	Den DF.	F	P
Treatment	0.22	3	108	127.59	<0.001
Week	0.38	8	81	21.78	< 0.001
Treatment*Week	0.58	24	81	3.30	< 0.001

The densities of broad mites in chili pepper plants without predators were significantly higher than on plants with predators (Fig. 5, contrasts through model simplification: X^2=119.44, *df*=1, p<0.001; X^2=67.79, *df*=1, p<0.001, for *A. herbicolus* and *N. barkeri*, respectively). The densities of broad mites in chili peppers plants differed significantly among *A. herbicolus* and with *N. barkeri* (X^2=13.45, *df*=1, p<0.001). (Fig. 6).

Predators increased through time in chili pepper plants. The number of individuals differed significantly among plants with and without predators (X^2=26.85, *df*=2, p<0.001) (Fig. 7). *Amblyseius herbicolus* showed the higher number of individuals through time than *N. barkeri* (X^2=5.26, *df*=1, p=0.002) or *Typhlodromus transvaalensis* (X^2=26.07, *df*=1, p<0.001). The number individuals of *N. barkeri* and *T. transvaalensis* differed significantly (X^2=3.94, *df*=1, p=0.047) (Fig. 7).

Similarly to it recorded on physic nut, the damage caused by the pest on chili pepper plants differed significantly between plants with and without predators and with the week of evaluation, indicating that injury evolves through time (Table 2) (Fig. 8).

All chili pepper plants produced fruits. The number of fruits did not differ significantly on plants with and without predators ($F_{2, 21}$=0.67, *df*=2, p=0.52) (Fig. 9A). The fruit weight was not different between plants with presence of predators and plants without them ($F_{2, 21}$=0.65, p=0.53) (Fig. 9B).

The presence of individuals of *T. transvaalensis* Nesbit (Acari: Phytoseiidae) were recorded both in physic nut (1.12±0.51) and chili pepper (1.3±0.42), on plants without release of predators. (Fig. 4 and 7).

Table 2: Multivariate analysis of variance of the scale notes of injury caused by *Polyphagotarsonemus latus* on chili pepper plants on the treatments evaluated, Viçosa (MG), Brazil, 2013.

Source variation	Wilk`s Lambda	Num DF.	Den DF.	F	P
Treatment	0.60	2	168	56.00	<0.001
Week	0.47	6	168	31.60	< 0.001
Treatment*Week	0.68	12	168	6.51	< 0.001

4 – Discussion

An increase of broad mite populations was recorded on physic nut plants over time, indicating that conditions were appropriate for pest development. The broad mite populations increased until the fourth week of evaluation. After that, a reduction of broad mite populations in all plants of physic nut was observed (Fig. 3). In plants without predators, this reduction may be related to severity of the damage caused by the broad mites that resulting in severe defoliation according the scale notes of injury (Fig. 5). Thus, the pest lost a source of resources. In physic nut plants with predators this reduction may be related to the increased number of predators due to increased use of food resource (Fig. 4). However, the growth pattern of broad mite, a rapid exploitation of the resource in the first week followed by a fall as result of overexploitation of the resource, may be an intrinsic characteristic of the species due to their short life cycle and strong attack to the host

(Lopes 2009, Gerson and Weintraub 2012). Tetranychids, other important phytophagous mites, showed the same pattern of growth (Krips et al. 1998). Broad mites on eggplants and sweet pepper plants showed a similar growth pattern in absence of natural enemies on field conditions (Stansley and Castillo 2009, Vichitbandha and Chandrapatya 2011).

The independent release of the predatory mites *A. herbicolus* or *N. barkeri*, had a positive effect on physic nut plants. These plants had lower pest populations during the evaluation period. The injury notes were low, although some plants showed leaves with shine loss and with some degree of curling, but these symptoms were not severe and foliar abscission was not recorded. The combined release of phytoseiids showed a lower efficient reducing broad mite populations compared to the independent release of each species. There are no previous studies on combined released of predators for broad mite control. Here, our outcome may be related with the foraging behaviour of the predators. *Amblyseius herbicolus* is more active in prey search than *N. barkeri* and consume indiscriminately broad mite as immatures and eggs of the *N. barkeri* (personal observations). *Amblyseius herbicolus* is catalogued as generalist predator, able of using a wide range of foods (McMurtry et al. 2013). However, *N. barkeri* is able to consume the immatures of other species of phytoseiids (Schausberger and Croft 2000). Thus, this behaviour can affect negatively the control of the pest. However, it is not clear the mechanism by which this result was recorded.

In physic nut, the phytoseiids remained and increased in number over time, especially when release was independent. The highest number of *A. herbicolus* was recorded in the third and fourth weeks of evaluations. The growth of this species probably indicates the use of feed resource (broad mite) in abundance. *Amblyseius herbicolus* has the ability to complete its life cycle in exclusive diet of broad mite (Rodríguez-Cruz et al. 2013). Additionally, in laboratory experiments, *A. herbicolus* consumed a greater number of broad mite individuals in comparison with *N. barkeri*, resulting in a higher oviposition rate of the former species (Rodríguez-Cruz et al. in prep).

In chili pepper, broad mite populations were recorded on all plants over time, indicating that conditions were appropriate for development of the pest. Broad mite populations in chili pepper showed a similar growth pattern to recorded in physic nut (Fig. 6). The scale notes of injury showed lower values that recorded on physic nut, resulting on a slight defoliation on control plants. Therefore, reduction of broad mite population in these plants may be related to the effects of rainfaill. During the experimental period were recorded the highest rates of rain for the region (INMET 2014). Although there no literature showing the effect of rainfall on broad mites, rainfall has a negative effect on populations of other phytophagous mites on coffee and physic nut (Pedro Neto et al. 2010, Cruz et al. 2013).

The predators remained and increased in number of individuals on the chili pepper plants. *Amblyseius herbicolus* showed a higher number compared to *N. barkeri*. Additionally to broad mite consumption, chili pepper plants provide alternative resources such as nectar and pollen. Although both predators evaluated are able to use pollen from different plant species (Nomikou et al. 2001, Rodriguez-Cruz et al. 2013), there is indirect evidence that *A. herbicolus* is more it effective using chili pepper pollen than *N. barkeri*. In a greenhouse experiment, *A. herbicolus* populations increased in number in chili pepper plants with low broad mite populations, but with flowers present (Rodriguez-Cruz et al. in prep).

There was no difference in the number and weight of chili pepper fruits between chili pepper plants with or without predators. Although chili pepper plants showed the typical symptoms of broad mite attack, the defoliation was not severe. Thus, these plants could recover from the pest attack and compensate the damage caused by broad mite due to its modular growth. Additionally, some individuals of the phytoseiid *T. transvaalensis* were recorded on these plants. This species was recorded in association with broad mites in *Ageratum conyzoides*, a non-crop plant usually found on areas of chili pepper crop (Rodríguez-Cruz, unpublished data). This specie can feed on broad mite (Cañarte, unpublished data). Thus, this species can was able to exercise some control over the broad mite populations, despite the low number of registered individuals. We only recorded individuals of *T. transvaalensis* on chili pepper control plants. However, it is possible that this species occurred in the other treatments, but in a smaller number due to predation of *A. herbicolus* or N. *barkeri*.

Despite a single release of the predators, their presence resulted positive for physic nut and chili pepper plants, avoiding the development of high broad mite populations and the appearance of severe symptoms. There is no information about the effect of multiple releases of predatory mite for the broad mite control on the plants evaluated. However, multiple releases of *A. cucumeris* and *A. swirskii* resulted in a successful broad mite control on sweet pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) in open-field when compared to control with predator or chemical control (Stansly and Castillo 2009). Thus, multiple release of predators may result in low broad mite populations and better benefits for physic nut and chili pepper.

For the both crops, *N. barkeri* showed a lower performance that *A. herbicolus.* This fact may be related to a better adaptation to the regional environment. *Amblyseius herbicolus* was recorded in association with broad mite in the region of these studies. Although is it not a native species, may be better adapted to environmental characteristics that Brazilian strain of *N. barkeri*. Some phytoseiids has been a shown lower efficiency between the sampling site and the site of release. Australian strain of *A. herbicolus* is capable of controlling phytophagous mites in orange (New South Wales, Australia), but is much less effective and not set on orange orchards in Israel (Argov et al. 2002).

Both phytoseiid species evaluated showed their potential as biological control agents for broad mite. Thus, *A. herbicolus* and N. *barkeri* can be of great importance for pest control and may be used in integrated management strategies of crops with poor or no phytosanitary support such as physic nut and chili pepper or in organic crops. Producers can benefit by reducing the application of pesticides, which represent about 10% of production costs (Vilela et al. 2008). Furthermore, chili peppers produced without pesticides can result in more profit for farmers, especially in certain markets such as organic products. Finally, the potential as biological control agents of these predatory mites can be exploited in other crops susceptible to broad mite attack in open field or on greenhouse conditions.

References

Albuquerque FA (2008). Metodologia para detecção da presenca do ácaro branco em pinhão manso. EMBRAPA, Centro Nacional de Pesquisa de Algodão, Documento 195.

Britto EPJ, Lindquist EE, de Gilberto GJ (2011). Redescription of *Lasioseius floridensis* Berlese, 1919 (Acari: Mesostigmata: Blattisociidae), with notes on closely related species. Zootaxa 2905: 1-15.

Crawley MJ (2007). The R book. Wiley, West Sussex.

Evaristo AB, Venzon M, Matos FS, Freitas RG, Kuki NK, Dias LAS (2013). Susceptibility and physiological responses of *Jatropha curcas* accessions to broad mite infestation. Experimental and Applied Acarology 60, 485-496.

Gerson U (1992). Biology and control of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae*).* Experimental and Applied Acarology 13: 163-178.

Gerson U, Weintraub PG (2012). Mites (Acari) as factor in greenhouse management. Annual Review of Entomology 57: 229-247.

Informe Agropecuário (2006). Cultivo da Pimenta. Empresa de Pesquisa Agropecuária de Minas Gerais 235.

King AJ, He W, Cuevas JA, Freudenberger M, Ramiaramanama D, Graham IA (2009). Potential of *Jatropha curcas* as a source of renewable oil and animal feed. Journal of Experimental Botany 60: 2897-2905.

Krips OE, Witul A, Willems PEL, Dicke M (1998). Intrinsic rate of population increase of the spider mites Tetranychidae urticae on the ornamental crop gerbera: intraspecific variation in host plant and herbivore. Entomologia Experimentalis et Applicata 89: 159-168.

Lopes EN (2009). Bioecologia de *Polyphagotarsonemus latus* em acessos de pinhão manso (*Jatropha curcas*). Dissertation, Federal University of Viçosa.

Matos CHC (2006). Mecanismos de defesa constitutiva em espécies de pimenta *Capsicum* e sua importância no manejo do ácaro branco *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae). Dissertation, Federal University of Viçosa.

Ministerio de Agricultura, Pecuaria e Abastecimento, Agrofit (2014) http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed 02 February 2014.

McMurtry J, De Moraes G, Sourassou N. F (2013). Revision of the lifestyle of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematic and Applied Acarology 18(4): 297-320.

Moraes GJ, Fletchmann CHW (2008). Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil, Holos, Ribeirão Preto, Brazil.

Myers N (1996). Enviromental Services of Biodiversity. Proceeding of the Natural Academy of Sciences 93: 2764-2769.

Pandey VC, Singh K, Singh JS, Kumar A, Singh B, Singh R (2012). *Jatropha curcas*: A potential plant for sustainable environmental development. Renewable and Sustainable Energy Reviews 16: 2870-2883.

Parawira W (2010). Biodiesel production from *Jatropha curcas*: A review. Scientific Research and Essays 5: 1796-1808.

Peña JE (1988). Chemical control of broad mite (Acarina: Tarsonemidae) in limes (*Citrus latifolia*). Processing of Florida State of Horticultural Society 101: 247-249.

Peña JE, Osborne L (1996). Biological control of *Polyphagotarsonemus latus* (Acarina: Tarsonemidae) in greenhouses and field trials using introductions of predacious mites. Entomophaga 41: 276-285.

Pinto CMF, Salgado LT, Lima PC, Picanço M, Júnior TPJ, Moura WM, Brommonschenkel SH (1999). A cultura da pimenta (*Capsicum* sp.). EPAMIG, Belo Horizonte.

Rodríguez-Cruz F, Venzon M, Pinto CMF (2013). Perfomance of *Amblyseius herbicolus* on broad mites and on castor beans and sunnhemp pollen. Experimental and applied acarology 60: 497-507.

Sarmento RA, Rodriguez DM, Faraji F, Erasmo FAL, Lemos F, Teodoro AV, Kikuchi WT, dos Santos GR, Pallini A (2011). Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on *Jatropha curcas* plants in Brazil. Experimental and applied acarology 53: 203-214.

Stansly P, Castillo J (2009). Control of broad mites, spider mites, and whiteflies using predaceous mites in open field pepper and eggplant. Processing of Florida State Horticultural Society 122: 253-257.

Venzon M, Matos CHC, Pallini A, Santos IC (2006). Pragas associadas à cultura de pimenta malagueta e estratégias de manejo. Informe Agropecuário 27: 75-86.

Venzon M, Oliveira RM, Perez AL, Rodríguez-Cruz, FA, Filho, SM (2013). Lime sulfur toxicity to broad mite, to its host plants and to natural enemies. Pest Management Science 69: 738-743.

Vichitbandha P, Chandrapatya A (2011). Broad mite effects on Chilli shoot damage and yields. Pakistan Journal of Zoology 43: 637-649.

Vilela NJ, Ribeiro CSC, Madail JCM (2008). Eficiência técnico-econômico de quatro sistemas de produção de pimentas *Capsicum*. Embrapa, Comunicado Técnico 56.

Figures

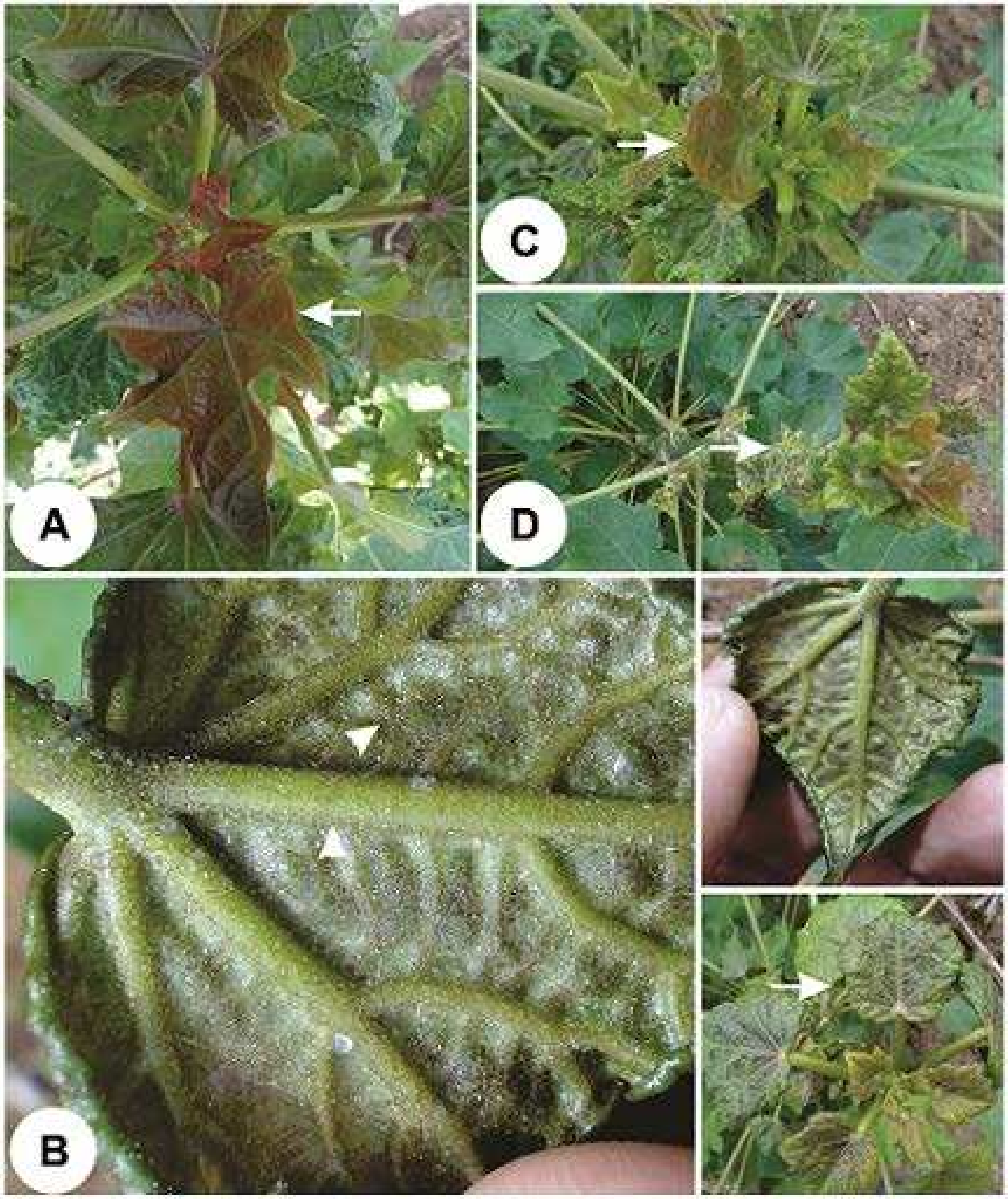

Figure 1. Physic nut plants presenting different attack levels for broad mites. A) Without attack, see natural shine; B) Attacked leaf, presenting deformation by action of broad mite attack (arrow); C) Plant attacked presenting disordered emission of leaves (arrow); D) Aspect of a physic nut plant, after the premature fall of leaves provoked by a severe infestation of broad mites (arrow). Oratórios (MG), Brazil, 2013.

Figure 2. Pipette tip adapted as container for predator releasing on physic nut and chili pepper plants. The narrower end was cut and sealed with silicone. The larger end was sealed with PVC film.

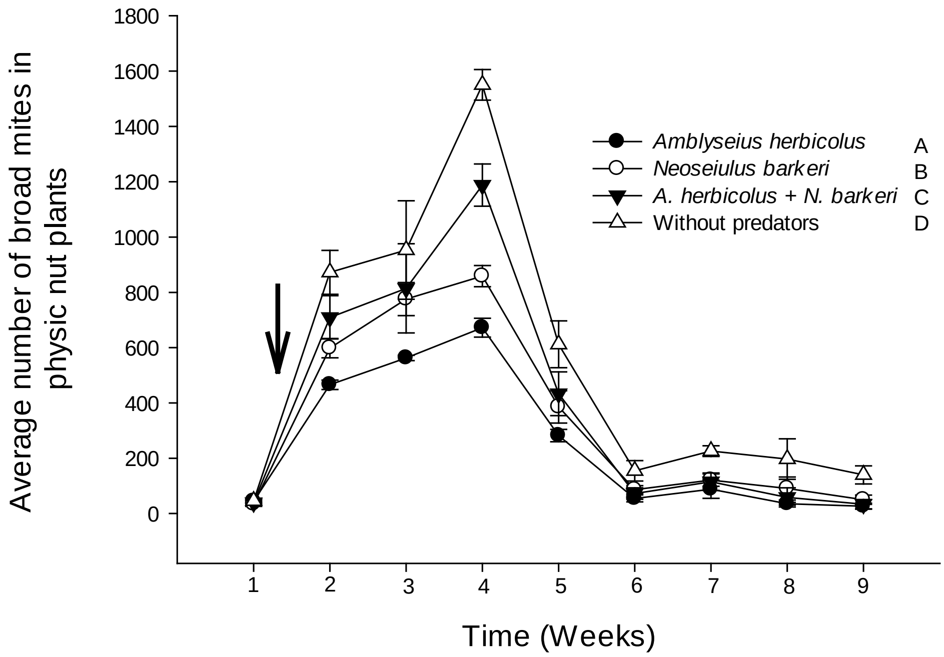

Figure 3. Number of broad mite females through time (weeks) (±SE) on physic nut plants under field conditions. 1) *Amblyseius herbicolus*; 2) *Neoseiulus barkeri*; 3) *Amblyseius herbicolus* + *Neoseiulus barkeri* and 4) Without predators. Different letters beside the treatment name indicate significant difference (Model simplification). The arrow indicates the predators release. Oratórios (MG), Brazil, 2013.

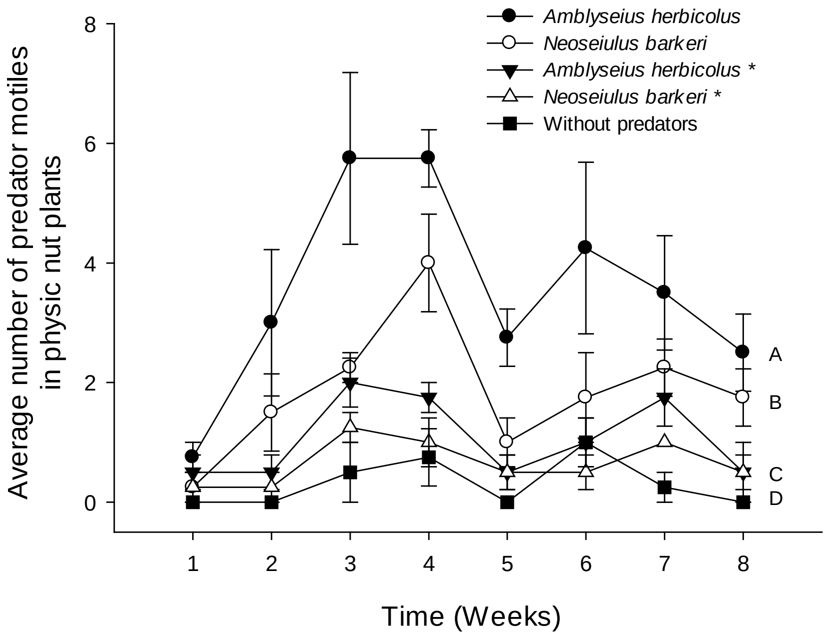

Figure 4. Number of predators through time (weeks) (±SE) on physic nut plants under field conditions. 1) *Amblyseius herbicolus*; 2) *Neoseiulus barkeri*; 3) *Amblyseius herbicolus**+ *Neoseiulus barkeri** and 4) Without predators (individuals of *Typhlodromus transvaalensis* recorded). Different letters indicate significant difference (Model simplification). Oratórios (MG), Brazil, 2013.

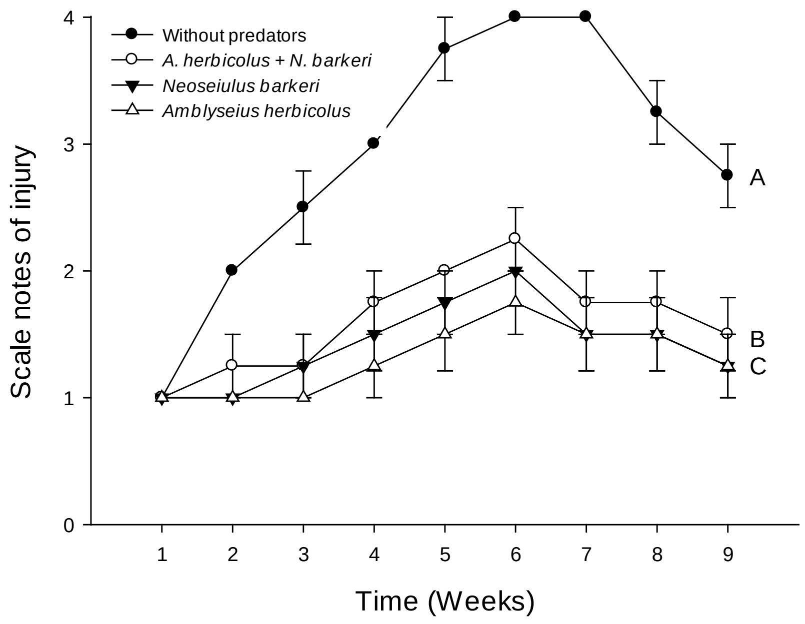

Figure 5. Variation of the scale notes of injury of broad mite *P. latus* through time (days) (±SE) on physic nut plants. Different letters indicate significant difference (Tukey 5%). Oratórios (MG), Brazil, 2013.

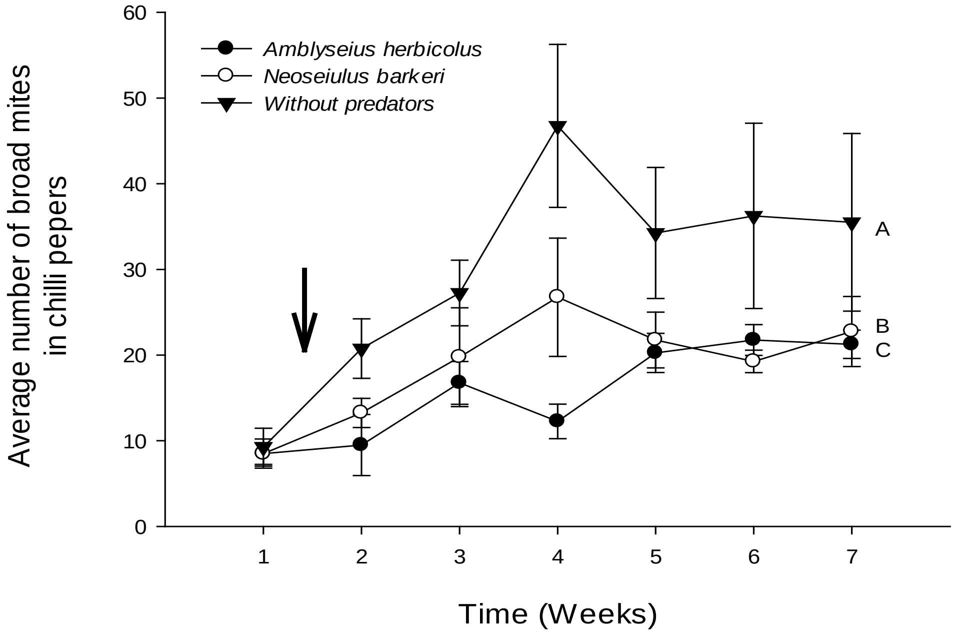

Figure 6. Number of broad mite females through time (weeks) (±SE) on chili pepper plants under field conditions. 1) *Amblyseius herbicolus*; 2) *Neoseiulus barkeri* and 3) Without predators. Different letters indicate significant difference (Model simplification). The arrow indicates the time of release predators. Viçosa (MG), Brazil, 2013.

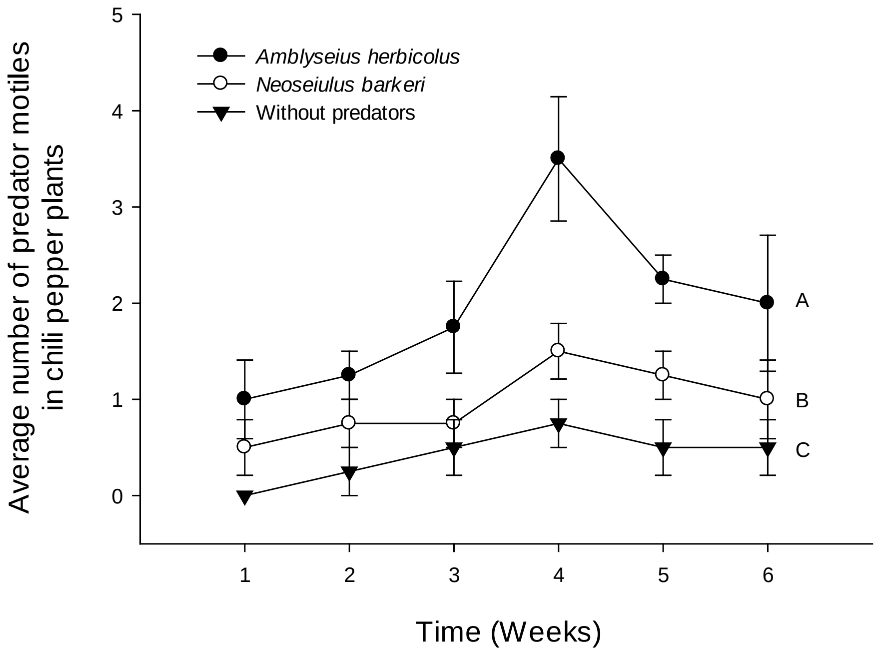

Figure 7. Number of predators through time (weeks) (±SE) on chili pepper plants under field conditions. 1) *Amblyseius herbicolus*; 2) *Neoseiulus barkeri* and 3) Without predators (individuals of *Typhlodromus transvaalensis* recorded). Different letters indicate significant difference (Model simplification). Viçosa (MG), Brazil, 2013.

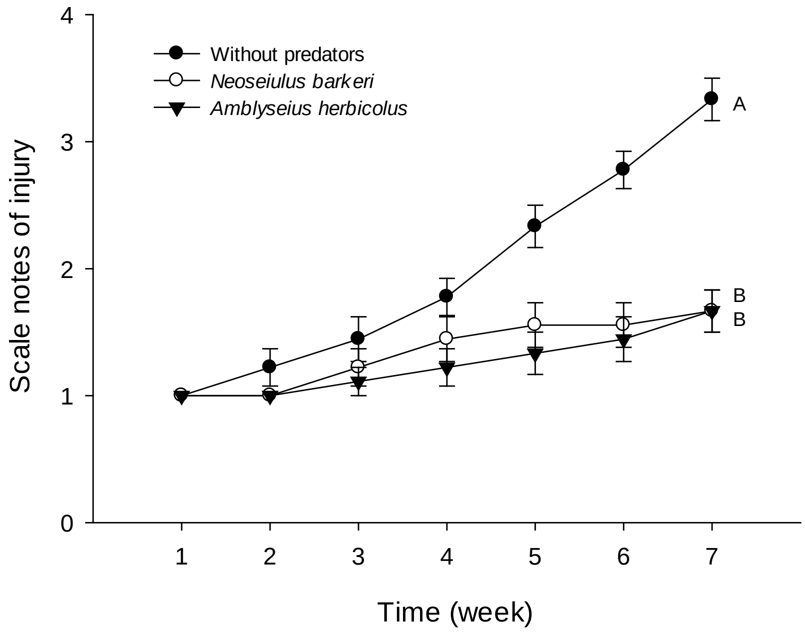

Figure 8. Variation of the scale notes of injury of broad mite *P. latus* through time (weeks) (±SE) on chili pepper plants. Different letters indicate significant difference (Tukey 5%). Viçosa (MG), Brazil, 2013.

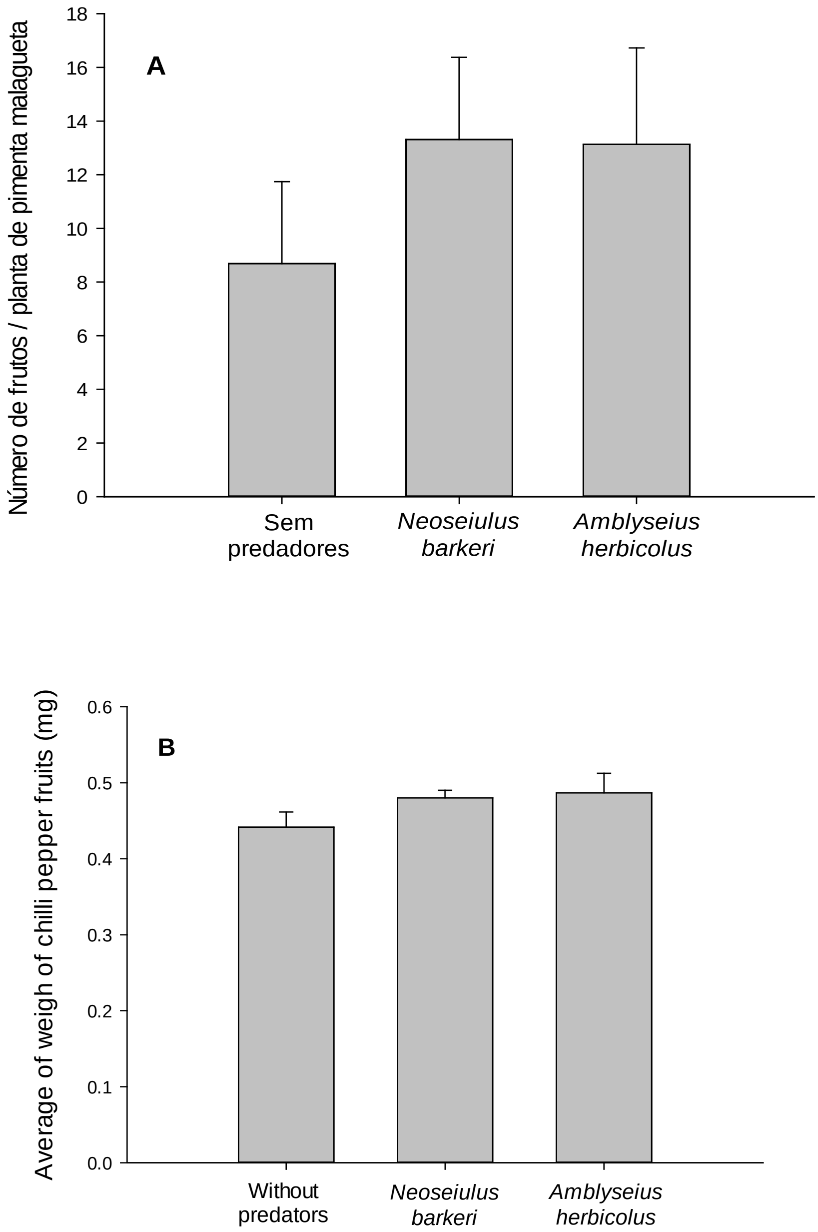

Figure 9. A) Average numbers (±SE) and (B) weight of chili peppers fruits in the absence and in the presence of the phytoseiids *Amblyseius herbicolus* or *Neoseiulus barkeri*. No significant difference was presented. Viçosa (MG), Brazil, 2013.

GENERAL CONCLUSIONS

- The phytoseiids *Amblyseius herbicolus* and *Neoseiulus barkeri* are able to feed on the different broad mite stages and oviposited as result of the consumption of broad mite.

- The consumption of broad mites recorded for *Lasioseius floridensis* was very low as well as their oviposition;

- In laboratory tests, the species *Amblyseius herbicolus* showed higher consumption of broad mites and a higher oviposition that *Neoseiulus barkeri*;

- The phytoseiids *Amblyseius herbicolus* and *Neoseiulus barkeri* are able to control broad mite populations in different predator:prey ratios on chili pepper plants under greenhouse conditions;

- The presence of *Amblyseius herbicolus* and *Neoseiulus barkeri* benefit the chili pepper plants, increasing the production of fruits;

- Chili pepper plants without presence of predators showed severe symptoms of broad mite attack, including leaf abscission. Resulting on low production of fruits, which showed low size and weight;

- First time on Brazil potential control agents of broad mite are evaluated under greenhouse conditions;

- Under field conditions, *Amblyseius herbicolus* and *Neoseiulus barkeri* are able to reducing the broad mite control on physic nut and chili pepper plants;

- The presence of *Amblyseius herbicolus* and *Neoseiulus barkeri* benefit the physic nut and chili pepper plants, avoiding the development of severe symptoms of broad mite attack;

- When *Amblyseius herbicolus* and *Neoseiulus barkeri* were released in combination, a lower reduction of broad mite was recorded that when the predators were released independently;

- First time in Brazil was evaluated the effect of the combined release of natural enemies of broad mite;

- The phytoseiids *Amblyseius herbicolus* and *Neoseiulus barkeri* are able to reducing and controlling broad mite populations under different conditions;

- The phytoseiids *Amblyseius herbicolus* and *Neoseiulus barkeri* can be considered as good biological control agents of broad mite;

- The control exercised by *Amblyseius herbicolus* and *Neoseiulus barkeri* can be used in other crops suspctibles to broad mite attack both in greenhouse as on open-field.