MARCUS VINÍCIUS ALFENAS DUARTE

SUITABILITY OF ALTERNATIVE FOOD FOR *Amblyseius herbicolus* TO PROMOTE BROAD MITE CONTROL IN CHILLI PEPPER PLANTS

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

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APROVADO: 25 de Julho de 2014.

Madelaine Venzon
(Coorientadora)

Cleide Maria Ferreira Pinto

Angelo Pallini Filho
(Orientador)

OFEREÇO

À minha mãe Rita de Cássia e meu Pai Celmar

Ao meu Irmão Matheus

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BIOGRAFIA

Marcus Vinícius Alfenas Duarte, filho de Rita de Cassia Gonçalves Alfenas e Celmar Araújo Duarte, nasceu no dia 30 de Setembro de 1988, na cidade de Porto Velho, Rondônia. Aos 2 anos de idade, se mudou para Viçosa – MG, permanecendo até os 11 anos, quando se mudou novamente para West Lafayette – Indiana – EUA.

Em 2007, iniciou o curso de Agronomia, na Universidade Federal de Viçosa, graduando-se Engenheiro Agrônomo em 2012. Durante o curso de graduação, foi bolsista de iniciação cientifica no Laboratório de Acarologia sobre orientação do Prof. Angelo Pallini e no Laboratório de Entomologia da EPAMIG, sob orientação da Pesquisadora Madelaine Venzon. Trabalhou na Empresa Árvore de Natal Viçosa, coordenando a produção de mudas de espécies nativas e participando de projetos de paisagismo e reflorestamento. Foi também bolsista DTI pelo CNPq por um semestre no laboratório de Entomologia da EPAMIG.

Em Setembro de 2012 iniciou o curso de mestrado em Entomologia, na Universidade Federal de Viçosa, sob orientação do Prof. AngeloPallini e coorientação do Prof. Arne Janssen e da pesquisadora Madelaine Venzon, submetendo se à defesa de dissertação em julho de 2014.

SUMÁRIO

Resumo

DUARTE, Marcus Vinícius Alfenas, M.Sc. Universidade Federal de Viçosa, julho de 2014. **QUALIDADE DE ALIMENTOS ALTERNATIVOS PARA *Amblyseius herbicolus* PARA PROMOVER O CONTROLE BIOLÓGICO DO ÁCARO-BRANCO EM PLANTAS DE PIMENTA-MALAGUETA.** Orientador: Angelo Pallini. Coorientadores: Arnoldus Rudolf Maria Janssen e Madelaine Venzon.

Muitos artrópodes omnívoros são inimigos naturais importantes em sistemas agrícolas. Esses organismos se alimentam de plantas e presas, possuem vantagem sobre os inimigos naturais estritamente carnívoros, já que ao explorarem alimentos à base de plantas, são capazes de persistir no campo por mais tempo. Vários tipos de pólens, bem como outros herbívoros, são considerados bons alimentos para predadores. Esses alimentos alternativos podem ser utilizados para incrementar o controle biológico, uma vez que auxiliam na manutenção e crescimento das populações de inimigos naturais. O ácaro-branco *Polyphagotarsonemus latus* é considerado uma praga chave da cultura da pimenta-malagueta, uma cultura muito importante em países em desenvolvimento, como o Brasil, onde Minas Gerais é o principal produtor.. *Amblyseius herbicolus* é um predador frequentemente encontrado em plantas de pimenta-malagueta infestadas com o ácaro-branco e com o potencial para controla-lo. Foram avaliados a qualidade dos alimentos alternativos (pólen coletado por abelha, pólen de *Typha* sp., e a presa *Tetranyhcus urticae*) para *A. herbicolus,* a influencia dos alimentos alternativos no controle biológico do ácaro-branco pelo *A. herbicolus* e a atração de plantas de pimenta-malagueta com pólen de abelha e flores de pimenta-malagueta. *Amblyseius herbicolus* obteve a maior oviposição quando se alimentou dos pólens e o crescimento populacional

foi maior estando em plantas de pimenta-malagueta com pólen coletado por abelha ou em plantas da pimenta com flores, consequentemente reduzindo mais a população do ácaro-branco. Não observou atração de *A. herbicolus* por plantas de pimenta-malagueta com pólen coletado por abelhas ou plantas de pimenta-malagueta com flores e por essa razão, a liberação do *A. herbicolus* em plantas jovens com pólen coletado por abelhas parece ser uma estratégia viável para manter este predador protegendo as plantas do ácaro-branco. Pelo fato de as flores de pimenta-malagueta constituírem um alimento de qualidade equivalente ao pólen de abelha para *A. herbicolus,* a suplementação desse pólen pode ser interrompida no momento em que as plantas iniciam a produção de flores e somente as flores podem manter os predadores e reduzir a população de ácaro-branco.

Abstract

DUARTE, Marcus Vinícius Alfenas, M.Sc. Universidade Federal de Viçosa, July 2014. **SUITABILITY OF ALTERNATIVE FOOD FOR *Amblyseius herbicolus* TO PROMOTE BROAD MITE CONTROL IN CHILLI PEPPER PLANTS.** Adviser: Angelo Pallini. Co-advisers: Arnoldus Rudolf Maria Janssen and Madelaine Venzon.

Many omnivores arthropods are important natural enemies in agricultural systems. These organisms feed on both plant material and prey. Omnivores have an advantage over natural enemies that are strictly carnivorous, by exploring plant based food sources they are able to persist longer in the crop. Pollens from many plants, as well as herbivores, are considered good alternative food sources for predators. These alternative foods can be used to increase the biological control, by maintaining and incrementing the populations of natural enemies. Chilli pepper is a very important culture in developing countries, such as Brazil where the state of Minas Gerais it's main producer. The broad mite *Polyphagotarsonemus latus* is considered a serious pest of chilli pepper plants. *Amblyseius herbicolus* is a predator frequently found in chilli-pepper infested with broad mites and has already shown its potential to control broad mites. In the present work we studied the quality of different alternative foods (honeybee pollen, *Typha* sp. pollen, and the prey *Tetranychus urticae*) for *A. herbicolus*. We also evaluated how these alternative foods can influence the biological control of the broad mite by *A. herbicolus*. The attraction from *A. herbicolus* to chilli pepper plants with honeybee pollen or chilli pepper flowers was also accessed. *Amblyseius herbicolus* had the highest oviposition when it feed on pollen and the population growth was higher when it fed on honeybee pollen or pollen from

chilli pepper plants and consequently causing a higher reduction of broad population. Neither honeybee pollen nor chilli pepper were attractive to *A. herbicolus.* Since no attraction to honeybee or chilli pepper flower, the release of the *A. herbicolus* in younger plants along with honeybee pollen seems like a viable strategy to maintain this predator protecting the plant from broad mites. Since flowers were an equally good source as honeybee pollen for *A. herbicolus* the supplementation of this pollen can be ceased once the plants start to produce flowers and the flowers alone can maintain predators and reduce broad mite population.

Introduction

Omnivorous arthropods are organisms that feed on both plant material and prey (Coll & Guershon, 2002). Many important natural enemies in biological control are omnivores, such as predatory bugs (Bonte and De Clercq 2011; Pumariño et al. 2012; Wong and Frank 2013), ladybugs (Burgio et al. 2006; Amaral et al. 2013), lacewings (Venzon et al. 2006; Nunes Morgado et al. 2014) and predatory mites (van Rijn and Tanigoshi 1999; Goleva and Zebitz 2013). When feeding on food sources from plants, omnivorous natural enemies persist longer in the crop compared to enemies that are strictly carnivorous, which could be advantageous for biological control (Symondson et al. 2002).

Pollen of many plant species is a good food source for predators and it is used for mass rearing of natural enemies on biological control programs (van Rijn and Tanigoshi 1999; Nomikou et al. 2003; Gnanvossou et al. 2005). Pollen has also been used to maintain and increase predator populations in crops with the intention of improving biological control by these predators (van Rijn et al. 2002; Duso et al. 2004; González-Fernández et al. 2009). Plants can also offer other food for natural enemies such as extrafloral nectar, which can also enhance biological control by natural enemies (Wäckers et al. 2005; Rezende et al. 2014). The feeding of predators on plant materials results in an increase of predator densities through the availability of extra resources for the predators. These increased densities subsequently cause a reduction of the pest populations. The effects of the addition of extra food for predators is reminiscent of a predator-mediated interaction between two prey species, called apparent competition. Hence, it seems as if the alternative food and the herbivore population are “competing”, but the interaction is mediated by a shared predator, and not by a shared food source (Holt 1977; van Veen et al. 2006; Messelink et al. 2008; Nomikou et al. 2010).

Besides pollen, other herbivores can also serve as alternative prey/food for predators (Langer and Hance 2004; Messelink et al. 2008; Park et al. 2011; Goleva and Zebitz 2013), resulting in control of several pests with one species of predator (Messelink et al. 2010). Alternative prey can promote persistence of natural enemies in agro-ecosystems, thus improving biological control by these natural enemies (Pickett and Bugg 1998; Altieri 1999). Besides offering a higher quantity of food, a mixed diet of two prey species can also provide food of higher nutritional value for the predator than single-prey diets, which can also lead to better biological control (Messelink et al. 2008).

The release of non-pest herbivore mite as an alternative food has already shown its potential as strategy to increase biocontrol (Karban et al. 1994). *Tetranychus urticae* is a polyphagous herbivore mite that feeds on many plant species (Jeppson et al. 1975; Moraes and Flectchmann 2008; Migeon and Dorkeld 2014). Although this two-spotted spider mite has been reported to attack chilli pepper plants (*Capsicum frutescens*) (Venzon et al. 2011), we never observed significant spider-mite damage on chilli pepper plants while doing field experiments at the Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG). Herbivores that do not cause significant damage and serve as food for predators could offer opportunities to maintain natural enemies on these plants. Hence, the artificial infestation of pepper plants with this alternative prey could have a beneficial effect for the biocontrol of other pests on these plants by promoting persistence of populations of natural enemies (apparent competition, that is known to be competition between two preys mediated by a common natural enemy).

Besides having positive effects on biological control, the presence of alternative food can also result in negative effects. By promoting satiation of the natural enemies, the presence of alternative food can reduce the efficiency of biological control in the short term (so-called apparent mutualism, mutualism between two preys mediated by a common predator).However, in the long term, this often results in positive effects on biological control through apparent competition (Van Maanen et al. 2012; Bompard et al. 2013). Plants with a great variety of food sources for predators would be ideal to study interactions in food web such as apparent competition.

Chilli pepper (*Capsicum frutescens*) is a very important crop, especially in developing countries, such as Brazil, where the main production takes place in the state of Minas Gerais (Pinto 2006). It is mainly cultivated by small farmers due to its high demand in terms of labour (Pinto 2006). Chilli pepper is attacked by many herbivores such as aphids, whiteflies, mites and fruit borers (Venzon et al. 2011). Among this great variety of herbivores, the broad mite *Polyphagotarsonemus latus* deserves special attention due to its serious economic damage to chilli pepper plants (Venzon et al. 2011; Venzon et al. 2013).

The broad mite is a polyphagous arthropod that feeds on more than 60 families of plants (Gerson 1992). They are very small (0.10 to 0.30 mm), which makes them hard to detect. They are usually noticed only when the plant shows damage symptoms, which usually occurs when the damage threshold has already been reached (Moraes and Flectchmann 2008; Venzon et al. 2013). Plants from the genus *Capsicum* are highly susceptible to damage from these mites; an infestation with a small number of individuals (10 individuals per plant) is sufficient for causing significant damage and reducing the yield from these crops (Coss-Romero and Peña 1998, Rodriguez-

Cruz, 2014). Moreover, there are no pesticides registered in Brazil to control this herbivore on chilli pepper plants (Agrofit, 2014). Hence, alternative methods such as biological control should be used to control pests.

Besides being attacked by many herbivores, chilli pepper plants have characteristics that facilitate the persistence of populations of predators in a crop, even when pest densities are low. For example, they possess domatia, which are specific structures that give shelter to predators (O'Dowd and Willson 1991). These structures are involved in reduction of intraguild predation (predation that occurs between two member of the same guild, predator that feeds on another predator for instance (Polis et al. 1989)), cannibalism (Schausberger 2003) and offer protection from environmental condition (Faraji et al. 2002; Ferreira et al. 2008; Rowles and O'Dowd 2009; Ferreira et al. 2011). Pepper flowers produce pollen and nectar that can serve as food sources for predatory mites (Phytoseiid) (Ramakers 1990; Avery et al. 2014) and predators that reproduced in these pepper plants are capable of moving to adjacent culture and controlling pests, serving as banker plants (Frank 2010; Xiao et al. 2012).

Phytoseiid predatory mites are important natural enemies in many agricultural systems (Moraes and Flectchmann 2008). Phytoseiids have a great variety of feeding habits, from species that feed exclusively on a specific family of herbivores (such as *Tetranychidae*) to species that feed preferently on pollen. Many phytoseiids are omnivores and are capable of feeding on great variety of prey and plant material (McMurtry and Croft 1997; McMurtry et al. 2013).

Several predatory mites, such as *Neoseiulus cucumeris, Amblyseius swirskii*, *Iphiseiodes zulugai, Euseius concordis* and *Amblyseius herbicolus* have shown potential to control broad mite populations (Weintraub et al. 2003; van Maanen et al. 2010; Sarmento et al. 2011; Rodríguez-Cruz 2014). *Amblyseius herbicolus* is frequently found in association with chilli pepper plants infested with broad mites in Minas Gerais (Matos 2006, Rodriguez-Cruz 2014). It is capable of reproducing and completing its development when feeding either on pollen or on broad mites (Rodríguez-Cruz et al. 2013), and of suppressing broad mite populations under greenhouse and field conditions (Rodríguez-Cruz 2014). Being a local predatory mite with the ability to control broad mites, this predator is ideally suited to investigate how alternative foods will influence the settlement of these natural enemies and the biocontrol done by them.

We conducted a series of experiments to determine the ability of *A. herbicolus* to reproduce on several types of alternative food that were used in the rearing (Method developed by Rodriguez-Cruz at EPAMIG) of this predatory mite such as a) honeybee and *Typha* sp. pollen, b) an non-pest herbivore for chilli pepper *T. urticae* and c) chilli pepper pollen a food sources that this predatory mite will encounter in chilli pepper plants in the field. The population growth of these predatory mites on these alternative foods on entire plants was also evaluated. We also tested how the best alternative food sources affected the biological control of the broad mite by the predatory mite *A. herbicolus.* It would be interesting if the alternative foods that have the greatest potential to maintain and increase populations of *A. herbicolus* could also attract them in the field, thus averting the necessity of releasing predatory mites along with the alternative food. Therefore, experiments were done to evaluate the attractiveness of the food sources that showed the highest potential for application in biological control.

Materials and Methods

Rearing methods

Plants

Chilli pepper seeds were sown in polystyrene trays (8 x 16 cells) filled with a commercial substrate (Bioplant®, a mixture of vermiculite and organic fertilizer) in a greenhouse. Plants of 21 days old were transplanted to 2L pots filled with the same substrate. Plants were watered daily and a commercial fertilizer (Biofert®, 6-4-4 and micronutrients) was applied every fifteen days according to the seller's instructions.

Pollen

Honeybee (*Apis melifera*) pollen was bought from a local store (Vivenda Naturalis) in a dehydrated form. It consisted of a mixture of pollen of various plant species, collected by honeybees (*Apis mellifera*). Pollen from a local *Typha sp.* was collected from plants found in the rural area of Viçosa (Minas Gerais, Brazil, 20° 45' 0" S, 42° 52' 0" W). Chilli pepper pollen was collected from recently opened flowers from plants that grew in a greenhouse that belong to the Department of Entomology from the Federal University of Viçosa. All pollen was dried in a drying oven at 60^{o} C for 48 hours.

Herbivores

Polyphagotarsonemus latus were obtained from naturally infested chilli pepper plants greenhouses that belong to the Department of Entomology from the Federal University of Viçosa in 2012 and rearing kept in the Laboratory of Entomology at EPAMIG. They were reared on chilli pepper plants placed inside cages (100 x 70 x 70 cm) covered with fine mesh (90µm) inside a greenhouse. New plants where placed in the cages every week, and plants with reduced quality due to high mite infestation where removed.

Tetranychus urticae was collected from naturally infested bean plants at the campus of the Federal University of Viçosa in 2002. They were reared at the Laboratory of Acarology on *Canavalia ensiformis L.* leaves placed on top of foam pads (4 cm height), covered with cotton wool, which was submersed in a tray (29 x 15 x 4 cm) filled with water. These arenas were kept in climate-controlled chambers (25 ± 1°C, 70±10% humidity, 12:12 h photophase). Leaves were replaced each 4 days; the old leaves were cut and placed on top of the new leaves.

Predators

A rearing of *A. herbicolus* was started with mites provided by Rodríguez-Cruz (EPAMIG). These mites were reared in the same way that was done during these experiments. They were collected from chilli pepper plants in Viçosa and Oratórios (Minas Gerais, Brazil, 20° 45' 0" S, 42° 52' 0" W and 20° 24' 0" S, 42° 48' 0" W respectably). The rearing was kept on arenas made of PVC sheets (15x10 cm) on top of foam pads (4 cm height), which were kept in plastic trays (29 x 14x 4 cm) filled with water. The edges of the arenas were wrapped with wet Kleenex® paper tissue, which was suspended into the water and thus served as both barrier and water source. A small tent-shaped piece of PVC sheet was placed on the arena to serve as shelter, and cotton treads were put under it as oviposition substrate. *Typha sp.* pollen and honeybee pollen were offered as food. These arenas where kept in climate-controlled chambers (25 ± 1°C, 70±10% humidity, 12:12 h photophase).

Experiments

Oviposition with different food sources

Females of *A. herbicolus*, 10-day-old since egg (3 to 4 days-old since adult), were used to test the quality of the different diets. These females were placed on 3 cm chilli pepper leaf discs that were placed, abaxial side up, in a 5 cm Petri dishes on top of cotton wool in water. Predatory mites tend to avoid direct light by hiding on specific parts of plants (Onzo et al. 2010). Preliminary experiments showed that, when placed on the leaf discs, the small tent-shaped pieces of PVC effectively prevented the mites from escaping; hence, they were used in these experiments as well. The arenas were kept in climate chambers (25 ± 1^{o}C, 70±10% humidity, 12:12 h photophase). The mites were offered different types of food: chilli pepper pollen, honeybee pollen, *Typha sp.* pollen and a mixture of different stages of *T. urticae.* This mixture was obtained by placing twenty adult females on the discs, allowing them to oviposit for 2 days, and subsequently adding fifteen larvae and deutonymphs. This number of prey was found to be sufficient food in previous experiments. Predator's oviposition was recorded for 3 days; oviposition of the first day was not included in the analysis because it will have been affected by the diet of previous days (Sabelis 1990). The eggs were counted while removing them from the arenas. Since these oviposition data did not fit a parametric distribution, a non-parametric Kruskal Wallis test was used to assess the effect of diet on oviposition, and a Wilcoxon rank sum test with Holm correction was used to compare the different food sources.

Predator population dynamics on chilli pepper plants with different food sources

To evaluate the quality of this food sources in plants the population growth of *A. herbicolus* with different food sources was tested on chilli pepper plants (60 days old) kept in a climate-controlled room (26 ± 2^{o} C and 70±10 % RH). Each treatment had a total of six plants were each one of the plants were considered a replicate. One group of plants (each group were made of six plants) had flowers, which supply pollen as food. Flowers were removed from the other plants, of which one group was supplied with honeybee pollen, another group contained a mixture of *T. urticae* stages, and the last group contained no food (control). The mixture of *T. urticae* was obtained by placing 200 *T. urticae* females on the plants one week prior to the experiment, thus all stages were present during the experiment. The plants were placed in water-filled trays to avoid escape of mites and migration to others plants. Five *A. herbicolus* females (10-days-old since egg) were placed on each plant. On the second, third, forth, sixth and eight day, the number of motile predators was assessed on the intact plants using a hand-held 10x magnifying glass. The numbers of predators per plant were compared among treatments with a linear mixed-effects model with a Poisson error distribution. On the tenth day, the plants were taken to the laboratory, the leaves were removed one by one and the numbers of motile predatory mites present was assessed using a stereoscopic microscope (Zeiss® Stemi 2000-c). The numbers of predators found during this destructive sampling were analysed using a non-parametric Kruskal Wallis ANOVA followed by a Wilcoxon rank sum test with Holm correction to compare the different treatments.

Population dynamics of *P. latus* and *A. herbicolus* in the presence of alternative food

This experiment was also done in a climate-controlled room (26 ± 2^{o} C and 70±10 % RH). Chilli pepper plants (90 days old) had the different treatments which were: a) chilli pepper plants without flowers or pollen (control), b) chilli pepper plants without flowers but with honeybee pollen (easiest pollen to acquired) and chilli pepper plants with flower but without honeybee pollen. The pollen (0,15g) was placed in a single place in the third completely expended leaf. Each treatment consisted had a total of eight plants, where each plant each one of the plants were a replicate. Each replicate received five 10-day-old *A. herbicolus* females. Ten days later, plants were infested with twenty adult *P. latus* females. Seven and fourteen days after this infestation, three leaves were removed from the top third and from the middle third of the plant, and the number of eggs and motile mites were counted using a stereoscopic microscope as described above. The numbers of mites were compared among treatments with a linear mixed-effect model with a Poisson error distribution, and with plant identity as a random factor to correct for repeated measures. Non-significant interactions and factors were removed from the model, and contrasts among treatments were assessed by stepwise model simplification through aggregation of non-significant factor levels (Crawley 2013).

Olfactometer bioassay

The attractiveness of chilli pepper plants (60 days old), with the different treatments: a) without flowers but with honeybee pollen (as described for the previous experiment) or b) with flowers but without honeybee pollen were compared with the control made of plants without flowers and without honeybee pollen was tested in a Y tube olfactometer (Sabelis and Baan 1983; Janssen 1999). This olfactometer consists of a Y-shaped glass tube (27 cm in length x 3.5 cm in diameter) with a Y-shaped metal wire inside it, running parallel to the tube walls. The two arms of the olfactometer were connected to glass boxes (50 x 36 x 43 cm) with an air inlet and outlet, in which the different odour sources were kept (Clean x Flower; Clean x Pollen). The base of the olfactometer was connected to a pump that pumped air out of the Y tube, thus forming a flow from the containers through the olfactometer. The air flow in each arm was kept at 0.5 m/s, hence, 1.0 m/s in the base. The wind speed was measured with an anemometer and controlled with valves placed at the exit of each glass box. The test subjects for this experiment were *A. herbicolus* females, 10-day-old since egg, starved for 24 hours before the experiments on arenas similar to those used for rearing, but without food. After disconnecting the base of the Y tube from the pump, a female was released on the metal wire at the base of the Y tube, where after the pump was reconnected. Subsequently, the female could walk upwind to the junction, where she had to choose for one of the arms. The female was removed from the olfactometer after having reached ¾ of the length of an arm and her choice was recorded. Females that did not reach this point before 5 minutes were also removed from the olfactometer and they were scored as not having made a choice. Four replicates were done for each combination of odour sources, and twenty predatory mites were tested per replicate. The data of olfactometer bioassay were analysed using a log-linear model for contingency tables with Generalized Linear Models (GLM) using a Poisson error distribution (Crawley 2013).

All statistical analyses were done using the computer software R version 3.1.0 (R Project for Statistical Computing, http://www.r-project.org).

Results

Oviposition with different food sources

The different diets significantly affected the oviposition of *A. herbicolus* (Chi^2= 36.9; d.f.= 3; p< 0.001). Treatments with pollen (honeybee, chilli pepper and *Typha sp.*) did not differ significantly from each other (p< 0.45), whereas *A. herbicolus* feeding on *T. urticae* had a lower oviposition rate (p< 0.001)(Figure 1).

Predator population dynamics on chilli pepper plants with different food sources

The number of motile predators on plants with different treatments differed significantly (Figure 2; Chi^2= 287.2, d.f.= 6, p< 0.001). Time was not significant (Chi^2= 3.2, d.f= 1, p= 0.7), however, the interaction between time and treatment was significant (Chi^2= 95.25, d.f= 3, p< 0.001). The number of motile predators on plants without food (Control) was lower than in the other treatments (Chi^2= 151.4, df= 2, p< 0.001; X^2= 202.5, df= 2, p< 0,001; X^2= 1.,36, df= 2, p< 0.01, for the treatments with flowers, honeybee pollen and *T. urticae* respectively). Plants infested with *T. urticae* had fewer motile predators than the treatment with flowers and honeybee pollen, whereas the latter two treatments did not differ significantly (Figure 2).

The destructive sampling also showed that the number of motile predators differed depending on the treatment (Figure 3: Chi^2= 20.91, d.f.= 3, p< 0.001). The number of motile predators was highest in the treatment with honeybee pollen (p< 0.05). There was a larger number of mites in the treatment with flowers than on plants infested with *T. urticae* and on plants without food; the treatment with *T. urticae* had a higher number of motile predators than the control (Figure 3).

Population dynamics of *P. latus* and *A. herbicolus* in the presence of alternative food

Time did not have an effect (Chi^2= 0.07; d.f.= 1; p= 0.79) on the total number of *P. latus* allowing to combine data from day 7 and day 14.The total number of *P. latus* differed significantly among treatments (Figure 4; Chi^2= 215.7; d.f.= 2; p< 0.001). The number of *P. latus* was higher in the control treatment than in the other treatments. No significant difference was found in the number of *P. latus* on plants with flowers or with honeybee pollen (Chi^2= 1.53; d.f.= 1; p= 0.22, Figure 4).

Time did not have and effect on the density of *A. herbicolus* (Chi^2= 0.01; d.f.= 1; p= 1) allowing to combine data from day 7 and day 14 Densities of *A. herbicolus* also differed significantly among treatments (Figure 5: Chi^2= 94.7; d.f.= 2; p< 0.001). The number of predators was lower in the control than in the other treatments. The densities of *A. herbicolus* did not differ between the treatments with flowers and honeybee pollen (Figure 5; Chi^2= 0,31; df= 1; p= 0.58).

Olfactometer bioassay

When offered a choice between odours of plants with out honeybee pollen or flowers and plants with bee pollen, *A. herbicolus* females did not show a preference for either of the odours (GLM: Dev= 4.0 x 10^{-15}, df= 1, p= 1.0) (Figure 6). There was also no preference for odours of plants with flowers when offered together with odours of plants without flowers or pollen(GLM: Dev= 0.05, df= 1, p= 0.82)(Figure 7).

Discussion and Conclusions

As shown previously, pollen is an excellent food source for predatory mites (van Rijn and Tanigoshi 1999; Nomikou et al. 2003; Gnanvossou et al. 2005; Rodríguez-Cruz et al. 2013). When these food sources were offered on entire chilli pepper plants, the results were consistent with those found in the oviposition experiment, where honeybee pollen and chilli pepper pollen were superior food sources compared to *T. urticae*. It is known that *A. herbicolus* is not able to develop to adulthood when fed only with *T. urticae*, possibly because it cannot cope with the web produced by *T. urticae* (Oliveira et al. 2009). In our experiment, a population of *A. herbicolus* persisted on chilli pepper plants with a population of *T. urticae*, but the numbers did not increase as much as on the plants with honeybee pollen or with flowers. This may be due to the fact that chilli pepper plants are not a good host for *T. urticae*, not allowing their population to grow much, consequently producing less web, allowing *T. urticae* to serve as food for *A. herbicolus*. A non-pest herbivore that is not capable of reaching high populations and causing significant damage could bring the benefit of alternative food without the cost the herbivory mite imposes (Karban et al. 1994), but chilli peppers seem to be such a bad host for *T. urticae* that the spider mites cannot persist and may have to be released weekly, similar to the application of pollen. Therefore the application as *T. urticae* as an alternative food for *A. herbicolus* would be less interesting, due to the necessity of rearing this herbivore, while honeybee pollen can be store bought.

Amblyseius herbicolus is already known for its potential to control broad mites on chilli peppers in greenhouses and in the field (Rodríguez-Cruz 2014). In a field experiment with chilli peppers, the release of *A. herbicolus* resulted in a reduction of the number of broad mites, yet when the release of the predator after the prey is presenta relatively high number of broad mites (over twenty broad mite females in a 5 leaf sample) were found during some weeks (Rodríguez-Cruz 2014). This level of infestation will probably result in significant reduction of chilli pepper yield (Coss-Romero and Peña 1998). These high pest numbers might have been caused by a late release of predators; by this time a number of *P. latus* might have already infested the plants. The fact that broad mites are very small, hard to be detected and cause high amounts of damage to chilli pepper plants, the detection of the broad mite can happen when it is already to late. By placing honeybee pollen (until the plants start to produce flowers) to maintain *A. herbicolus* could be a way to reduce the damage done by the broad mite to chilli pepper plants.

Previous work has demonstrated how pollen can be used to improve biocontrol (van Rijn et al. 2002; Duso et al. 2004; González-Fernández et al. 2009) and that the application of pollen can result in persistence of a population of predatory mites on plants without pests (Ramakers 1990; Avery et al. 2014). Here we demonstrate that this increment in the number of predatory mites in the absence of the prey can be a strategy to "shield" the plant from infestation of pest, such as the broad mite, similar to what was found by Ramakers (1990) for thrips.

No attraction was found from *A. herbicolus* to chilli pepper plants with flowers or honeybee pollen. Rodríguez-Cruz (2012) found that *A. herbicolus* were attracted by volatiles from chilli pepper plants infested with *P. latus*. Predatory mites can have a genetic innate response to a specific component of a volatile blend directing them to food sources (Sznajder et al. 2011). The mixture of pollen present in honeybee pollen or chilli pepper flowers are food sources on which *A. herbicolus* probably feeds in nature and may have been selected to be attracted to, therefore an innate response from volatiles from these food sources could have been found (Same as Sznajder et al. 2011 found for plant volatile produced by damaging herbivores), however no response was found.

Because *A. herbicolus* were not attracted to the volatiles from honeybee pollen, an early release of this predatory mite in young plants with honeybee pollen and the weekly addition of honeybee pollen could be enough to maintain the predatory mites on chilli pepper plants. Our results also show that chilli pepper flowers are just as good as food resource as honeybee pollen, demonstrating that the addition of pollen can be ceased when chilli pepper plants start to flower. Chilli pepper flowers are produced facing the leaves under the flowers (Figure 8) as if they were meant to make the pollen rain down on the leaves. Being that pollen is a good food source for predatory mites (van Rijn and Tanigoshi 1999; Nomikou et al. 2003; Gnanvossou et al. 2005), the production of excess pollen could be a characteristic selected to protect plants from herbivory by providing pollen as alternative food for predator such Phytoseiid mites.

Large-scale experiments in the greenhouse or in the field are needed to verify if the addition of food and predatory mites results in better control of broad mite populations. There is the risk involved in supplying plants with pollen that other organisms may profit from this pollen, which is not necessarily to the benefit of the plant. These competitors for pollen can potentially feed on the predatory mite of interest (intraguild predation) or monopolize the resource making it more difficult for *A. herbicolus* to benefit from it and consequently protect the plant (van Rijn et al. 2002). If the phenomena shown here also hold at larger spatial scales and within the context of a community of herbivores, predators and omnivores, the release of predatory mites along with honeybee pollen prior to the infestation, followed by the weekly addition of this pollen until the plants start to produce flowers is a promising strategy for pest management.

Figures

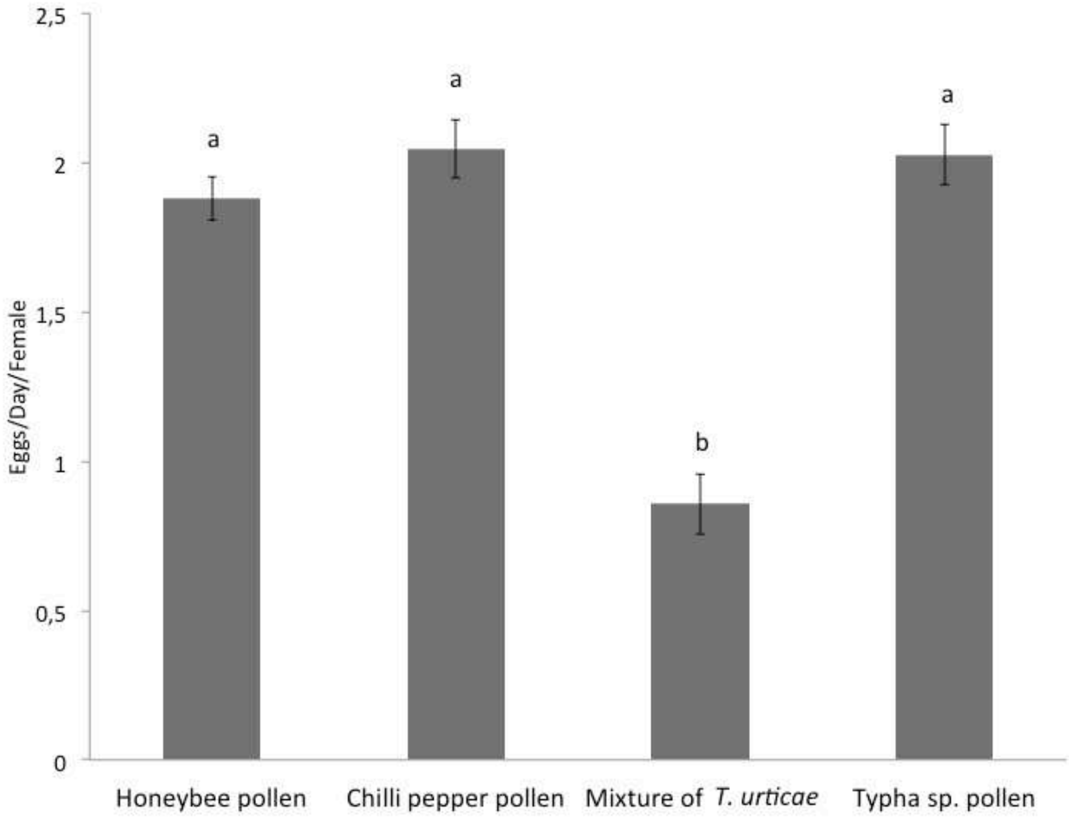

Figure 1: Average oviposition (Day 2 and 3) by female *Amblyseius herbicolus* (±SEM) when fed on different diets. Bars with different letters indicate significant differences among diets (Wilcoxon rank sum test with Holm correction; $p < 0.05$).

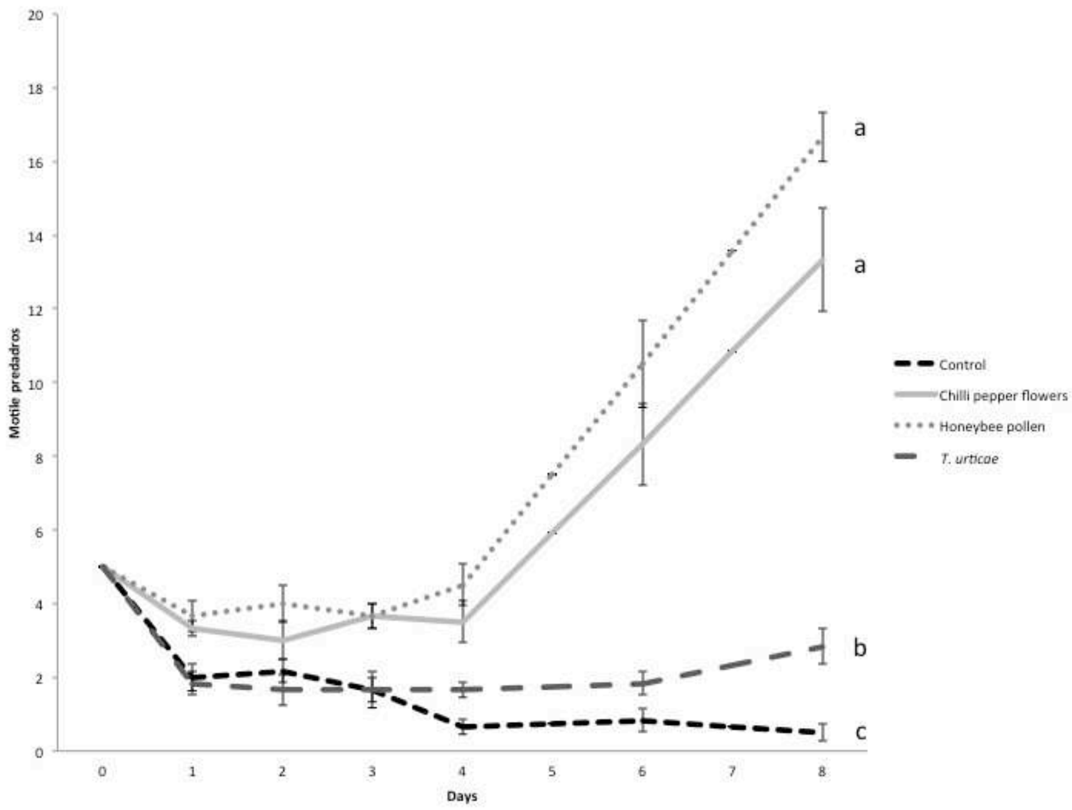

Figure 2: Average number of motile *A. herbicolus* (±SEM) on chilli pepper plants without flowers or food (Control), on plants with flowers supplying pollen, plants supplied with bee-collected pollen, and plants with a population of *T. urticae*. Lines with different letters differ significantly (linear mixed-effect model with a Poisson error distribution; $p < 0.05$).

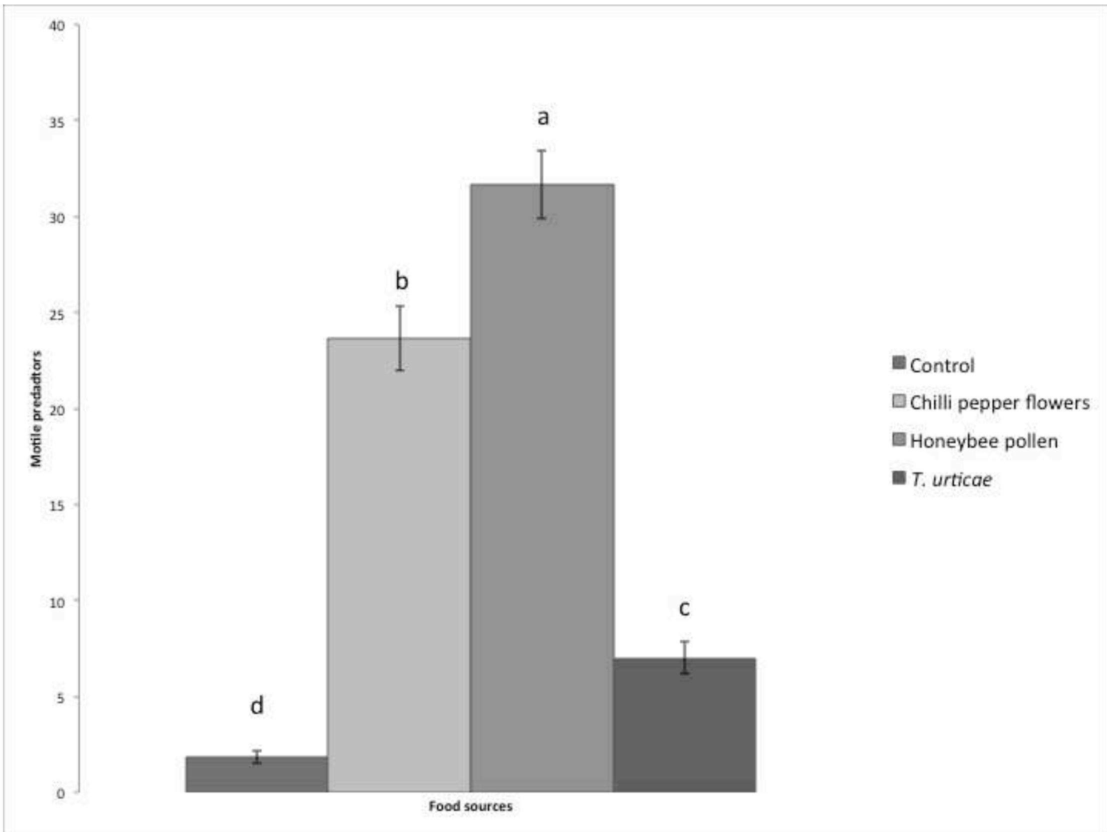

Figure 3: Average number of motile *A. herbicolus* (±SEM) on chilli pepper plants with different food sources at the end of the experiment (destructive sampling). Bars with different letters indicate a significant difference among the treatments (Wilcoxon rank sum test with Holm correction; $p < 0.05$).

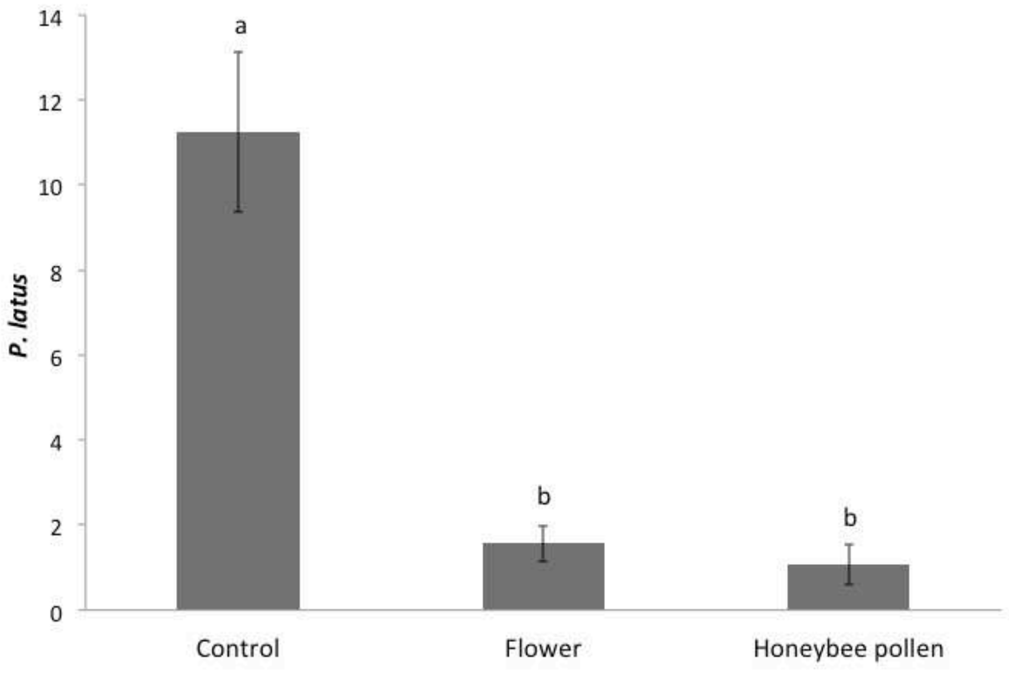

Figure 4: Average number of *P. latus* (adults, larvae, pupae and eggs) (±SEM) on plants with the different food sources. Bars with different letters indicate a significant difference between the treatments (linear mixed-effect model with a Poisson error distribution; $p < 0.001$).

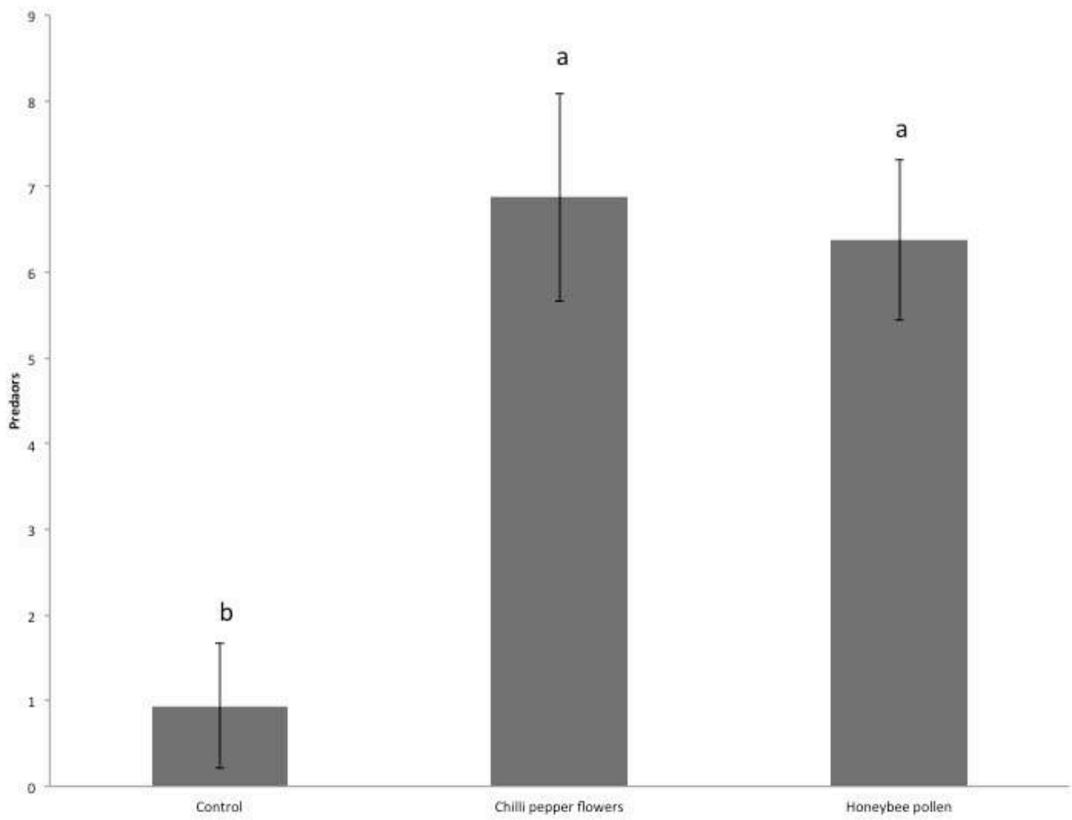

Figure 5: Average number of motile predators and eggs (±SEM) sampled on plants without food (control), with flowers providing pollen and on plants supplied with honeybee-collected pollen. Bars with different letters indicate a significant difference among the treatments (linear mixed-effect model with a Poisson error distribution; $p < 0.001$).

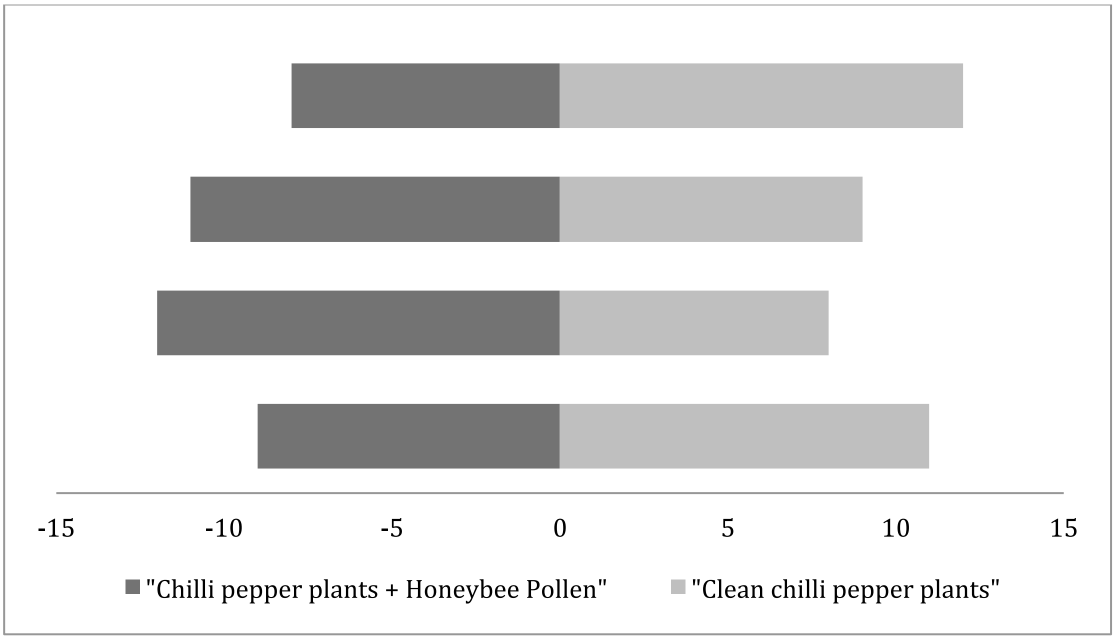

Figure 6: Choice of female *Amblyseius herbicolus* when offered odours from chilli pepper plants with honeybee pollen vs. clean chilli pepper plants. Each bar represents the result of one replicate in which 20 mites were tested (GLM: Dev= 4.0×10^{-15}, df= 1, p= 1.0, ns).

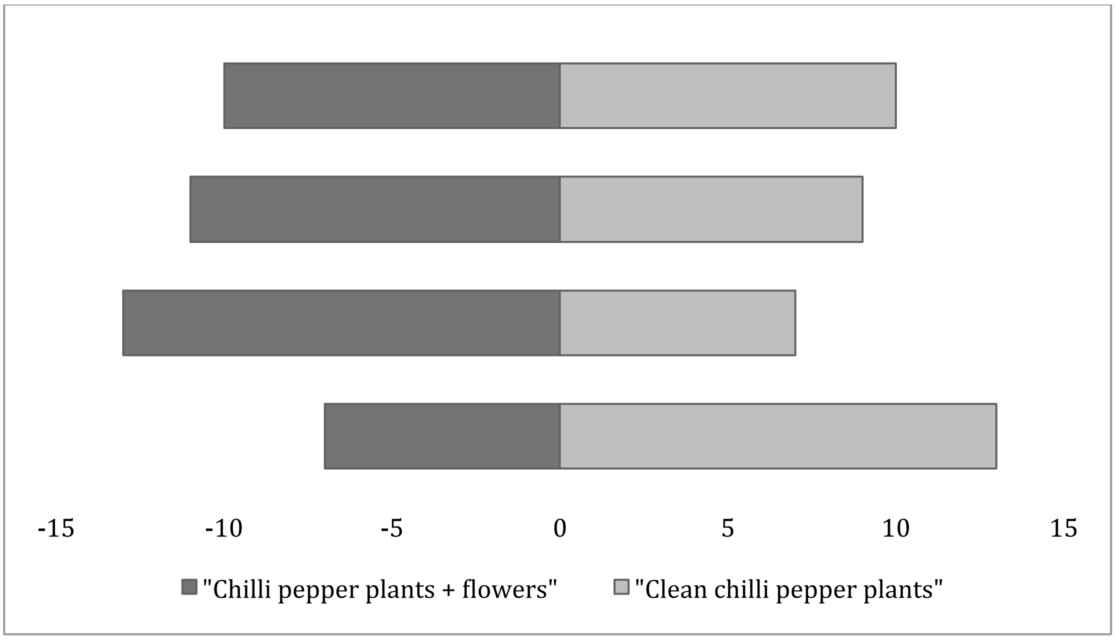

Figure 7: Choice of female *Amblyseius herbicolus* when offered odours from chilli pepper plants with flowers vs. clean chilli pepper plants. Each bar represents the result of one replicate in which 20 mites were tested (GLM: Dev= 0.05, df= 1, p= 0.82, ns).

Figure 8: Chilli pepper flower facing down to rain down the pollen over the plants.

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