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Two predatory mite species as potential control agents of broad mites

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Abstract The broad mite is a key pest of various crops worldwide, including chili pepper (*Capsicum frutescens*), where it is controlled with chemical pesticides. Two phytoseiid predators (*Amblyseius herbicolus* and *Neoseiulus barkeri*) and a blattisociid mite species (*Lasioseius floridensis*) occur in association with the broad mite in Brazil. Predation of broad mites and oviposition rates of *A. herbicolus* were higher than those of *N. barkeri* and lowest for *L. floridensis*. On intact plants, both phytoseiids controlled broad mite populations seven days after their release. In a separate experiment, the predators controlled broad mites on flowering plants during 15 days. After two months, plants with predators produced heavier fruits than plants without predators. Concluding, these two phytoseiid species can control broad mites on chili peppers plants at different

densities and over time, reducing production losses, and should be evaluated under field conditions.

Keywords *Amblyseius herbicolus* · *Neoseiulus barkeri* · *Polyphagotarsonemus latus* · *Capsicum frutescens* · Predation · Biological control

Introduction

The broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) occurs in tropical and subtropical areas on more than 60 botanical families (Gerson 1992). It is a key pest of several crops worldwide, including several from the genus *Capsicum* (De Coss-Romero and Peña 1998). Broad mites are a major pest of peppers in China, USA, New Zealand, Thailand and Brazil (Riley 1992; Vichitbandha and Chandrapatya 2011; Zhang 2008; Venzon et al. 2011). Because of their small size (0.1–0.2 mm long), broad mites go unnoticed at the beginning of the infestation. Their presence only becomes evident when the plants start showing damage symptoms (De Coss-Romero and Peña 1998; Peña 1990; Venzon et al. 2008, 2011). They mainly damage plant apices, resulting in bronzing and curling of leaves, followed by leaf abscission when attacks are severe (Gerson 1992; Weintraub et al. 2003; Venzon et al. 2011).

The main control method for broad mites by pepper growers worldwide is the use of chemical pesticides

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(Venzon et al. 2013; Abou-Awad et al. 2014). However, this causes environmental hazards and health risks for growers and consumers (van der Werf 1996; Travisi et al. 2006; Casida 2012). Furthermore, inefficient or illegal use of pesticides can amplify these problems. This is the case in Brazil, where several growers use unregistered pesticides for broad mite control in chili pepper (Venzon et al. 2011).

The negative effects of chemical control have generated demand for sustainable and environmental-friendly control methods such as the use of natural enemies. Several families of predatory mites are known to be natural enemies of pest mites (McMurtry et al. 2013). Many phytoseiid mite species, such as *Neoseiulus californicus* (McGregor), *N. barkeri* Hughes, *Amblyseius swirskii* Athias-Henriot, *A. herbicolus* (Chant) and *A. largoensis* (Muma), have been identified as potential biological control agents of broad mites (Fan and Pettitt 1994; Peña and Osborne 1996; Weintraub et al. 2003; La et al. 2009; Rodríguez-Cruz et al. 2013; van Maanen et al. 2010; Duarte et al. 2015). In Brazil, four phytoseiids and one species of blattisociid mite have been recorded as being associated with broad mites (Venzon et al. 2006; Sarmiento et al. 2011; Britto et al. 2012), but not all of them have been evaluated for their potential use as natural enemies for this pest.

Amblyseius herbicolus was found in association with broad mites on chili pepper plants in the state of Minas Gerais (Brazil) (Venzon et al. 2006) and can complete its life cycle feeding exclusively on broad mites (Rodríguez-Cruz et al. 2013). It is also capable of controlling broad mites in the presence of alternative food (Duarte et al. 2015). However, its predation rate when feeding on broad mites is unknown. *Neoseiulus barkeri* is a natural enemy of broad mites (Fan and Pettitt 1994; Peña and Osborne 1996) and has been found associated with broad mites in a gerbera crop (*Gerbera* sp.) (Asteraceae) in the state of São Paulo, Brazil (Britto et al. 2012). The potential of this Brazilian predatory mite for broad mite control has not been yet evaluated. The blattisociid mite species *Lasioseius floridensis* Berlese was also recorded preying on broad mites in commercial gerbera crops (Britto et al. 2012), but its potential to control broad mites remains to be tested.

As a first step in the evaluation of these three species of predatory mites as potential biocontrol agents for broad mites, we evaluated their predatory

capacity on a mix of broad mite life stages and their oviposition rate in the laboratory. Subsequently, we assessed the control of broad mites on chili pepper plants by the two most promising species, as well as their effect on fruit production in greenhouse experiments.

Materials and methods

Rearing methods

Chili pepper seeds (*Capsicum frutescens* L.) (Solana-ceae) were sown in polystyrene trays (8 × 16 cells) filled with a commercial substrate (Tropstrato[®], HT hortaliças, Brazil). Seedlings with two pairs of true leaves were transplanted to 1 l plastic pots filled with a mix of soil and organic manure (3:1). Potted plants were kept inside wooden frame cages (0.70 × 0.70 × 0.70 cm) covered with a fine mesh (90 µm) in a greenhouse and were irrigated twice a week.

Broad mites were 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 chili pepper plants placed inside cages as described above. When plant quality decreased, new plants were introduced into the cages.

Amblyseius herbicolus was collected from the same area as the broad mites and from broad mite-infested chili pepper plants maintained in a greenhouse in Viçosa (Minas Gerais, Brazil, 20°45'0"S, 42°52'0"W). They were reared on arenas made of PVC sheet (25 × 12 cm) on top of foam pads (28 × 15 × 3 cm), which were kept inside plastic trays (30 × 18 × 5 cm) filled with water. The edges of the arenas were wrapped with moist cotton wool, which served both as water source and as barrier for the predators. Commercial bee pollen (Santa Bárbara[®]) was used as food, provided directly on the PVC sheet. A small piece of PVC sheet (4 × 2 cm) was provided as shelter, under which cotton strands were placed as oviposition sites.

Neoseiulus barkeri was supplied by PROMIP (Limeira, São Paulo, Brazil) and *L. floridensis* was kindly supplied by Erika P. J. Britto and Dr. Gilberto José de Moraes (ESALQ, Piracicaba, Brazil) and were

reared on arenas as described above. Mould mites, *Tyrophagus putrescentiae* (Shrank) (Acari: Acaridae), were used as food for *N. barkeri* and *L. floridensis*. Mould mites were reared on the same arenas as the predator, receiving 11.4 g of cracker crumbs (Maizena Aymore) as food every 15 days. All predator cultures were kept in a climate room (25 ± 1 °C, $60 \pm 10\%$ RH and 14 h photophase).

Predation and oviposition rates

Predation and oviposition rates of the three predator species feeding on broad mites were measured during three days. Chili pepper leaf discs (diam. 30 mm) were placed individually in Petri dishes (diam. 45 mm) on an agar layer. The discs were placed with their abaxial side up because broad mites preferably inhabit this side. Before starting the experiment, the agar layer extending around the leaf discs was removed and water was added to the Petri dish to avoid leaf dehydration and predator escapes. Thus, the arenas were on top of an agar island surrounded by water. The arenas received 30 broad mite adults, 25 larvae and 25 pupae for *A. herbicolus* or *N. barkeri*, and 20 adults, 20 larvae and 20 pupae for *L. floridensis*. These prey densities were based on results from pilot experiments that showed that prey depletion would not occur with these densities during the experiments. Predation on the mixture of prey stages was used as a measure of predator efficiency in order to select the predators for further greenhouse experiments.

Because *A. herbicolus* reproduces thelytokously (de Moraes and Mesa 1988), only a female (1–2 days old since adult emergence) was added to each arena. For *N. barkeri* and *L. floridensis*, which reproduce sexually, copulating pairs of females and males (1–2 days old since adult emergence) were isolated from the rearing units and transferred to other rearing arenas for two days where they were allowed to mate. Subsequently, each female was transferred to a new experimental arena. Ten replicates were carried out for each predator species. Consumed prey were replaced daily. The arenas were kept at 25 ± 1 °C, $60 \pm 10\%$ RH and 14 h photophase.

The total number of adult, larval and pupal broad mites consumed (presence of remaining transparent prey cuticles) on each arena was assessed daily during

three days using a stereoscopic microscope (Nikon® SMZ 645). The predation of broad mite eggs was not quantified as pilot experiments showed that predators prefer the other prey stages.

The number of predator eggs laid was also accessed daily during three days. Predation and oviposition data from the first day were not included in the analysis because of effects of previous diet (Sabelis 1990). The data of the other two days were summed, and differences in predation rates among predators were analysed using generalized linear models (GLM) with a Poisson error distribution (log link function) (Crawley 2007). Because oviposition rates in phytoseiids are strongly correlated to population growth rates (Janssen and Sabelis 1992), we also compared these rates among predator species. They were summed over the last two days of the experiment and analysed as above. Contrasts among predators were determined with general linear hypothesis testing (function `glht` of the package `lsmeans` in R, Lenth 2016). All analyses were performed using the statistical software R 2.15 (R Development Core Team 2012).

Potential to control broad mites

Chili pepper plants with 10–12 leaves (45 days old) were infested with either 20 or 40 adult female broad mites. One hour later, one group of plants from each of the two prey densities received two recently mated adult females of *N. barkeri* (1–2 days old since emergence), another group received two *A. herbicolus* females (1–2 days old since emergence), and a third control group did not receive any predators. Each treatment was replicated on five plants, with the exception of the controls for the treatments with *N. barkeri*, which had six plants. All plants were maintained inside separate cages in a greenhouse as described above (26 ± 2 °C and $70\% \pm 10\%$ RH). Seven days after predator release, adult female broad mites and motile predator stages were counted by detaching each leaf from the plants and examining it under a stereoscopic microscope. Numbers of broad mites were analysed with a GLM with a Poisson error distribution corrected for overdispersion (log link function). Numbers of predators were analysed similarly, but without correction for overdispersion. Contrasts were assessed as above.

Control of broad mites and fruit production in a greenhouse

A second experiment was performed to evaluate the effect of predators on fruit production. Chili pepper seedlings with two true leaves were transplanted to 2 l pots filled with a mixture of soil and commercial substrate, and maintained individually in cages as described above, until the first flower bud appeared. The plants were irrigated twice per week and were not fertilized.

Sixty days after transplantation, when plants started flowering, they were infested with 20 adult female

broad mites. One hour later, two adult females of *A. herbicolus* or two recently mated adult females of *N. barkeri* were released on two groups of the infested plants. Plants infested with broad mites but without predators served as control. Each treatment was replicated on ten plants, which were placed inside plastic trays (50 × 35 × 15 cm). Water was added to each tray to prevent predators from escaping. The plastic trays were kept inside cages as described above.

Five, ten and fifteen days after predator release, the number of adult female broad mites and motile stages of predators were assessed on a detached branch with five leaves, close to the place of initial infestation, using a stereoscopic microscope, as described above. The numbers broad mite females were log ($x + 1$)-transformed and tested with a linear mixed effects model (LME) with plant identity as random factor to correct for repeated measures. Contrasts were assessed as above. Two months after the initial infestation with *P. latus*, chili pepper fruits were collected, counted and weighed using an electronic balance (Bioprecisa JA3003 N). Numbers of fruits per plant were log ($x + 1$)-transformed and compared among treatments using GLM with Gaussian error distribution (identity link). The average weights of fruits per plant were log ($x + 0.1$)-transformed and compared among treatments using a GLM with a Gaussian error distribution (identity link). Contrasts were assessed as above.

Fig. 1 **a** Average numbers (+SE) of broad mites consumed per two days by the predatory mites *A. herbicolus*, *N. barkeri* and *L. floridensis* on a diet of mixed life stages of broad mites. **b** Average oviposition rates (+SE) (eggs per female per 48 h) of *A. herbicolus*, *N. barkeri* and *L. floridensis* fed on mixed life stages of broad mites. Different letters above the bars denote significant differences among treatments (contrasts after GLM, $p < 0.05$)

Results

Predation and oviposition

All predator species fed on a mixture of broad mite stages. The number of preyed broad mites differed significantly among predator species (GLM, $\chi^2 = 290.9$, d.f. = 2, $p < 0.001$). The predation rate was higher for the two phytoseiid species than for *L. floridensis*, and *A. herbicolus* had a higher predation rate than *N. barkeri* (Fig. 1a).

Oviposition also differed significantly among predator species (GLM, $\chi^2 = 15.6$, d.f. = 2, $p < 0.001$). *Amblyseius herbicolus* had a higher oviposition rate than *L. floridensis*, the other oviposition rates did not differ significantly (Fig. 1b).

Fig. 2 Average numbers (+SE) of broad mite females per chili pepper plant. Plants were either infested with 20 or 40 adult female broad mites and either 0 (0:20 and 0:40) or 2 (1:10 and 1:20) adult females of **a** *A. herbicolus* or **b** *N. barkeri*. Numbers of *Polyphagotarsonemus latus* females were assessed seven days after initial infestation. Different letters above bars indicate significant differences among treatments (contrasts after GLM, $p < 0.05$). **c** Average numbers (+SE) of motile stages of *A. herbicolus* (white bars) and *N. barkeri* (gray bars) with predator:prey ratio 2:20 and 2:40. There was no significant difference in predator densities among predator:prey ratios for *A. herbicolus* and *N. barkeri*

Potential to control broad mites

The number of broad mites on the chili pepper plants after seven days was significantly affected by the initial predator:prey ratio, with higher broad mite densities on plants without predators than on plants with predators (Fig. 2a, b: GLM, $F_{3,16} = 124.3$, $p < 0.001$; $F_{3,18} = 15.7$, $p < 0.001$, for *A. herbicolus* and *N. barkeri*, respectively). Broad mite densities on plants with predators at the end of the experiment were lower than the initial densities, indicating that predators reduced the densities (Fig. 2). There was no significant difference in broad mite densities between plants with *N. barkeri* and plants with *A. herbicolus* (ratio 2:20: GLM, $F_{1,8} = 0.339$, $p = 0.576$; ratio 2:40: $F_{1,8} = 3.28$, $p = 0.108$). The densities of *A. herbicolus* and *N. barkeri* increased during the experimental period (Fig. 2c), and there was no significant effect of the initial predator:prey ratio on their densities (Fig. 2c: GLM, $\chi^2 = 0.448$, d.f. = 1, $p = 0.50$ and $\chi^2 = 1.80$, d.f. = 1, $p = 0.18$, respectively).

Control of broad mites and fruit production in a greenhouse

The interaction between treatment and time had a significant effect on the number of adult female broad mites (LME, $\chi^2 = 39.8$, d.f. = 2, $p < 0.001$). Broad mite numbers increased over time on chili pepper plants without predators and remained low on plants with predators (Fig. 3a). Densities of *N. barkeri* increased significantly over time (Fig. 3b, $\chi^2 = 15.0$, d.f. = 1, $p < 0.001$), but those of *A. herbicolus* not ($\chi^2 = 1.25$, d.f. = 1, $p = 0.263$). Although plants with predators produced twice as many fruits as plants without predators, this effect was not significant (GLM, $F_{2,27} = 1.77$, $p = 0.19$) (Fig. 4a). The presence of predators had a significant effect on the individual weight of the fruits (GLM, $F_{2,27} = 4.04$, $p < 0.029$): fruits produced by plants with *A. herbicolus* were heavier than those of plants without predators (Fig. 4b).

Discussion

We evaluated the predation and oviposition rates of three predatory mite species that were previously

Fig. 3 **a** Average numbers ($\pm$ SE) of broad mite females per chili pepper plant through time (days) in the absence or presence of *A. herbicolus* or *N. barkeri*. Different letters indicate significant differences among treatments (contrasts after LME, $p < 0.05$). **b** Average numbers ($\pm$ SE) of *A. herbicolus* and *N. barkeri* females per chili pepper plant through time (days)

recorded in association with broad mites in Brazil (Venzon et al. 2006; Sarmiento et al. 2011; Britto et al. 2012). The predators were able to feed and reproduce on a diet of mixed life stages of broad mites. Whereas *L. floridensis* showed very low predation and oviposition rates, the phytoseiids *A. herbicolus* and *N. barkeri* had higher rates. Janssen and Sabelis (1992) reported a strong correlation between oviposition rate and intrinsic growth rate of phytoseiids when preying on spider mites. If this can be extrapolated to other prey types, the oviposition rates found here will give a reasonable idea about the predator population growth rates and their ability to control broad mites. Indeed, the two phytoseiids tested here reduced the densities of broad mites when compared to control plants and increased fruit numbers and weight. However, it is

Fig. 4 **a** Average numbers of fruits per chili pepper plant ($\pm$ SE) and **b** average weight of individual chili pepper fruits per plant in the absence and presence of the phytoseiids *A. herbicolus* or *N. barkeri*. Different letters above bars indicate significant differences among treatments (contrasts after GLM, $p < 0.05$)

important to note that the predator:prey ratios used here were relatively high. Nonetheless, this demonstrates that broad mites can be controlled using predatory mites. An alternative to releasing high numbers of predators would be to supply the predators with alternative food, such as pollen (van Rijn et al. 2002; Rodríguez-Cruz et al. 2013; Duarte et al. 2015; Janssen and Sabelis 2015).

Although other *Lasioseius* species have been reported as efficient predators of tarsonemid mites (Zhang and Lin 1991; Zhang and Fan 2010; de Moraes et al. 2015), we found no evidence that *L. floridensis* is effective against *P. latus*. Britto et al. (2012) reported that this species can survive and reproduce on a diet of broad mites but with a low intrinsic growth rate. One possible explanation for its bad performance is that

broad mites are no part of its natural diet because the genus *Lasioseius* is usually found in environments with higher humidity, such as in the soil, and not on aboveground plant parts, where broad mites reside.

Both phytoseiids were able to significantly reduce broad mite densities on chili pepper plants. In the experiment where the potential to control broad mites was assessed, the numbers of broad mites were very low seven days after the initial infestation (Fig. 2). Similarly, van Maanen et al. (2010) found low numbers of broad mites (fewer than four) per sweet pepper plant three weeks after releasing *A. swirskii* at an initial predator:prey ratio of 1:20 than on plants without predators. In our study, broad mite densities might possibly have decreased even more given more time, but the control plants showed severe damage symptoms after seven days, including leaf loss, and the experiment was therefore ended. Therefore, another greenhouse experiment was performed with older and larger plants, which resisted damage by broad mites for a longer period. In this experiment, both *N. barkeri* and *A. herbicolus* also significantly reduced broad mite densities during the first 15 days, and prevented severe symptoms associated with broad mite attacks (A. Rodríguez-Cruz, pers. obs.).

Plants without predators produced smaller-sized fruits than plants with predators. Small fruits are of lower commercial value and the market tends to reject them. Additionally, small fruits require more effort to harvest, leading to increased costs for the grower. Heavier fruits contain more seeds and have a higher content of capsaicin, the chemical compound responsible for the burning sensation caused by chili pepper and the principal reason for consumers to purchase chili peppers (Pinto et al. 2012). Thus, the predatory mites have the potential to add value to the production chain of chili pepper without the use of acaricides.

The densities of the two predators increased over time, even when numbers of broad mites were low. The predators probably fed on other resources such as pollen and nectar from chili pepper flowers. *A. herbicolus* can reproduce when feeding on pollen from castor bean (*Ricinus communis* L., Euphorbiaceae) or chili pepper (*C. frutescens*) and its populations increase on chili pepper plants with pollen (Rodríguez-Cruz et al. 2013; Duarte et al. 2015). It is unknown whether *N. barkeri* is capable of reproducing on a diet of chili pepper pollen, but it can do so on other pollen species (van Rijn and van Houten 1991;

Nomikou et al. 2001). These life history traits are important because they indicate that the two species could be used in inundative or conservation biological control programs by releasing these predators together with pollen or on flowering plants (van Rijn et al. 2002; Nomikou et al. 2002; Liu et al. 2006; Messelink et al. 2008; Adar et al. 2014; Duarte et al. 2015; Delisle et al. 2015; Kumar et al. 2015; Leman and Messelink 2015; see Janssen and Sabelis 2015 for a short review). Perhaps the predators could also be used combined with banker plants (Kumar et al. 2014).

In summary, our results demonstrate the potential of two phytoseiids for the biological control of broad mites. Both *N. barkeri* and *A. herbicolus* were able to control this pest under greenhouse conditions. Both species can also feed on the eggs of whiteflies (Nomikou et al. 2001; Cavalcante et al. 2015), which are the vector of broad mites. This can further contribute to the control of broad mites (Natarajan 1988; Flechtmann et al. 1990; Palevsky et al. 2001; van Maanen et al. 2010). Hence, we propose that additional experiments should be performed to investigate the efficiency of these predatory mites in the field under crop conditions.

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- Madelaine Venzon** does research on agroecological strategies for pest control focused in biological control.