BIOLOGICAL CONTROL

Efficiency of *Phytoseiulus longipes* Evans as a Control Agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on Screenhouse Tomatoes

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Edited by Madelaine Venzon – EPAMIG

Neotropical Entomology 39(6):991-995 (2010)

ABSTRACT - The spider mite *Tetranychus evansi* Baker & Pritchard can cause severe damage to tomato crops. The predatory mite *Phytoseiulus longipes* Evans was recently reported in association with *T. evansi* in Uruguaiana, Rio Grande do Sul State, Brazil. The objective of the present study was to evaluate the effects of *P. longipes* on the population of *T. evansi* on tomatoes under screenhouse condition. The study consisted on four experiments, in each of which 80 potted plantlets were distributed in two plots of 40 plantlets each. Two weeks later, each plantlet of both plots was infested with eight adult females of *T. evansi*; one week after, four adult females of *P. longipes* were released onto each plant of one plot. The population levels of *T. evansi* and the damage caused by these mites were significantly lower ($P \leq 0.05$; linear mixed-effect model) in the plots where *P. longipes* had been released. The results indicate the potential of this predator as a candidate for classical biological control of *T. evansi* by inoculative releases on tomato plants.

KEY WORDS: *Lycopersicon esculentum*, pest mite, predatory mite, biological control, Solanaceae

Tomato (*Lycopersicon esculentum*) is one of the most important vegetable crops around the world. It is attacked by an array of pest arthropods, including spider mite species (Acari: Tetranychidae), especially the two-spotted spider mite, *Tetranychus urticae* Koch, and the tomato red spider mite, *Tetranychus evansi* Baker & Pritchard (Jeppson *et al* 1975, Saunyama & Knapp 2003). *Tetranychus urticae* has been reported for quite a long time on tomatoes in countries of all continents. Until recently, *T.. evansi* was known from only a few countries scattered around the world, but it is now known from countries in Africa, America, Asia and Europe (Migeon & Dorkeld 2006).

Extensive damage by *T. evansi* to tomato has been reported in Africa (Saunyama & Knapp 2003), despite intensive use of acaricides (Blair 1989, Sibanda *et al* 2000). Intensive use of acaricides can lead to environmental contamination and toxic effects on animals and humans. Thus, the development of alternative methods to control *T. evansi* is desirable.

Phytoseiidae mites have received considerable attention because of their potential for biological control of phytophagous mites (Helle & Sabelis 1985, McMurtry & Croft 1997). Biological control of spider mites through the release of phytoseiids has been widely practiced, mainly in North America and Europe (Gerson *et al* 2003). Among those mites, *Phytoseiulus persimilis* Athias-Henriot is the most studied and most frequently used in protected cultivation as well as in field crops, for *T. urticae* control (Zhang & Sanderson 1995, Gerson *et al* 2003).

Several studies have been conducted to investigate the efficiency of 11 phytoseiid species as biological control agents of *T. evansi* (Moraes & McMurtry 1985, Escudero & Ferragut 2005, Rosa *et al* 2005, Vasconcelos *et al* 2008).

An extensive search for *T. evansi* in Brazil showed that it is rare. When found, population levels are usually low, what could at least partly be due to the effect of natural enemies. A promising phytoseiid predator was found in Uruguaiana, Rio Grande do Sul State, southern Brazil (Furtado *et al* 2006). Furtado *et al* (2007) and Ferrero *et al* (2007) showed that *Phytoseiulus longipes* Evans could develop and reproduce on *T. evansi* as prey, which was never observed for other phytoseiids, including an African population of *P. longipes* tested by Moraes & McMurtry (1985).

The objective of this study was to investigate the effect of *P. longipes* on the population density of *T. evansi* on tomato plants cultivated in screenhouses in Brazil.

Material and Methods

Four experiments were conducted under screenhouse conditions, three of which at Universidade Federal Rural de Pernambuco, in Recife, Pernambuco State, from September 12 to December 19, 2005, June 30 to October 6, 2006 and January 10 to April 18, 2007, respectively, and one at Escola Superior de Agricultura Luiz de Queiroz - USP, in Piracicaba, São Paulo State, from January 25 to May 3, 2007. For logistical reasons, the tomato cultivars used in the study were 'Kada Gigante' in the first two experiments and 'Yoshimatsu' in the last two experiments. These cultivars are commonly grown in the two regions where this work was done.

The specimens of *T. evansi* were taken from colonies maintained on tomato for several years at the respective universities, whereas *P. longipes* specimens were taken from colonies started with mites collected in March 2004 from tomato in Uruguaiana and maintained in the above mentioned institutions. Predators were reared in units similar to those described by McMurtry & Scriven (1965), on a mixture of all developmental stages of *T. evansi* offered on infested tomato leaves.

Temperature and relative humidity in the screenhouses were recorded daily with thermo-hygrographs. In all four experiments, the daily average temperature in the screenhouses was consistently high with total averages and extremes values of 30.5 (26.8-33.9), 30.5 (25.8-33.0), 32.0 (25.3-35.0) and 30.0°C (24.5-39.2) from the first to the fourth experiment, respectively. In the first three experiments, the average relative humidity levels and the corresponding ranges were rather similar, 66.5 (54.8-87.0), 66.9 (54.8-89.0) and 65.0% (56.0-89.0), respectively; in the fourth experiment, relative humidity was considerably lower [50.0% (31.2-87.0)].

For each experiment, three week-old plantlets were each transplanted into a 14 L polyethylene pot containing a mixture of soil and commercial compost (Plant Max Hortaliças HA®: mixture of vermiculite and shredded pine bark). Eighty plantlets were used in each experiment. The plant substrate was irrigated every other day to total holding capacity and fertilized weekly with 5 g of the formula N-P-K (19-19-19) per plant, diluted in the irrigation water. No pesticides were applied during the study. The plantlets of each experiment were maintained in a screenhouse in two separate plots of 40 plants each. In each plot, the plantlets were arranged in four rows, spaced at 0.5 m between rows and within the rows. The distance between the adjacent margins of the plots was at least 3 m, to reduce chances of mites moving from one plot to another.

Two weeks after transplanting, four adult female *T. evansi* were transferred with a brush from the stock colony onto the lower surface of each of 160 detached tomato leaflets, in the laboratory under a dissecting microscope. On the same day, the leaflets were taken to the screenhouse, fixing one of them to a leaf in the middle of the canopy and another to a leaf next to the top of each plantlet of both plots by gluing the upper surface of a laboratory infested leaflet to the upper surface of the leaflet of the plantlet with a paste made of wheat flour and water. Preliminary observations indicated that this paste would not affect the leaves, while allowing *T. evansi* to move onto the plant as the detached leaflet started to dry. At this stage, each plantlet had about eight expanded leaves.

One week after infestation with *T. evansi*, four adult female *P. longipes* were released onto each of the plantlets of one of the plots (treated plot), but not of the other (control). To facilitate this procedure, predators to be released onto a plantlet were transferred with a brush from a stock colony to a vial of 5 ml, in the laboratory under a dissecting microscope. Immediately thereafter, vials were taken to the screenhouse, and each tied to the main stem of a plantlet.

The potential of *P. longipes* as a predator of *T. evansi* was evaluated by weekly comparisons of the population density of the latter in both plots. One leaflet from the middle region of each plant of both plots was picked in each of 12 consecutive weeks, starting on the day when the predator was released, just before the release was carried out. The leaflets collected from each plot were put into 5 L paper bags, which were put into plastic bags and then into a cool box (at about 15°C) to reduce mobility of the mites during transport to the laboratory, where the numbers of all postembryonic stages of *T. evansi* and of *P. longipes* were counted and where the damage to the leaflets was estimated.

Taking into account the longitudinal structure of the data (measurements repeated over time), the numbers of *T. evansi* per leaflet in the treatment and control plots were compared using a linear mixed-effect model, i.e. the lme(nlme) function of R program, version 2.9.2 (R Development Core Team 2009). A separate analysis was carried out for each experiment. In each analysis, the number of mites per leaflet was a function of the treatment (with and without release of *P. longipes*), the period of evaluation (considered as continuous), the interaction of those factors and a random effect, attributed to the plants at each evaluation period. In the first three experiments, the assumption of homocedasticity was not met; thus, the corresponding analyses were performed taking into account the different treatment standard deviations.

In the third and fourth experiments, the damage caused by *T. evansi* to the same sampled leaflets was also estimated, using the following scale: 1) leaflets without damage or with initial signs of damage; 2) leaflets with discreet lesions; 3) leaflets with lesions in the initial stage of coalescence and large numbers of different developmental stages of *T. evansi*; 4) leaflets with coalescent lesions forming whitish, shrunk areas covered with dense webbing and with high densities of *T. evansi*; 5) leaflets with large whitish areas, strongly shrunk and partly dry, covered with dense webbing and on which *T. evansi* density was declining.

Because damage grades could not be considered as a continuous variable, the damages caused by *T. evansi* in both treatments were compared with a generalized linear mixed-effect model, with a logit link using glmmPQL(MASS) function of R program (version 2.9.2). Given the time necessary for the predator to reduce the pest population and, as a consequence, for damage to be reduced, the analysis of damage was performed taking into account only the evaluations performed after the first six weeks of the release of the predator. A separate analysis was conducted for each experiment.

Results

In all experiments, population levels of *T. evansi* from both plots increased slowly up to the week 3 after infestation (Fig 1). In the plots where the predator was not released, *T. evansi* reached the highest population levels towards the end of the observation period.

In the first experiment, the population of *T. evansi* increased more quickly between the weeks 3 and 9, especially after the week 7. The population level decreased between the weeks 9 and 12. In the subsequent experiments, the population of *T. evansi* rose gradually, reaching the maximum densities in the week 11.

In all experiments, the patterns of population growth of *T. evansi* were similar in the plots where *P. longipes* had been released. The population levels of *P. longipes* increased only marginally until the period between the weeks 3 and 5 after it was released, reaching relatively high densities towards the end of experiments one to four.

In all four experiments, the number of *T. evansi* was significantly lower on plants onto which *P. longipes* had been released (Table 1). The fixed effects of the mixed model [intercept, treatment, date and interaction (treatment x date)] were significant in all experiments.

The mean damage caused by *T. evansi* to tomato leaflets tended to increase up to the week 8 or 9 in the control plots, leveling off afterwards (Fig 2). Similarly to what was observed in relation to the density of *T. evansi*, in both experiments (3 and 4), lower damage levels were observed on plants onto which *P. longipes* had been released. Intercept, treatment and date effects were significant in both experiments ($P \leq 0.001$; d.f.: 399, 78 and 399 respectively). However, the interaction treatment x date was not significant ($P \geq 0.124$ and 0.140 in

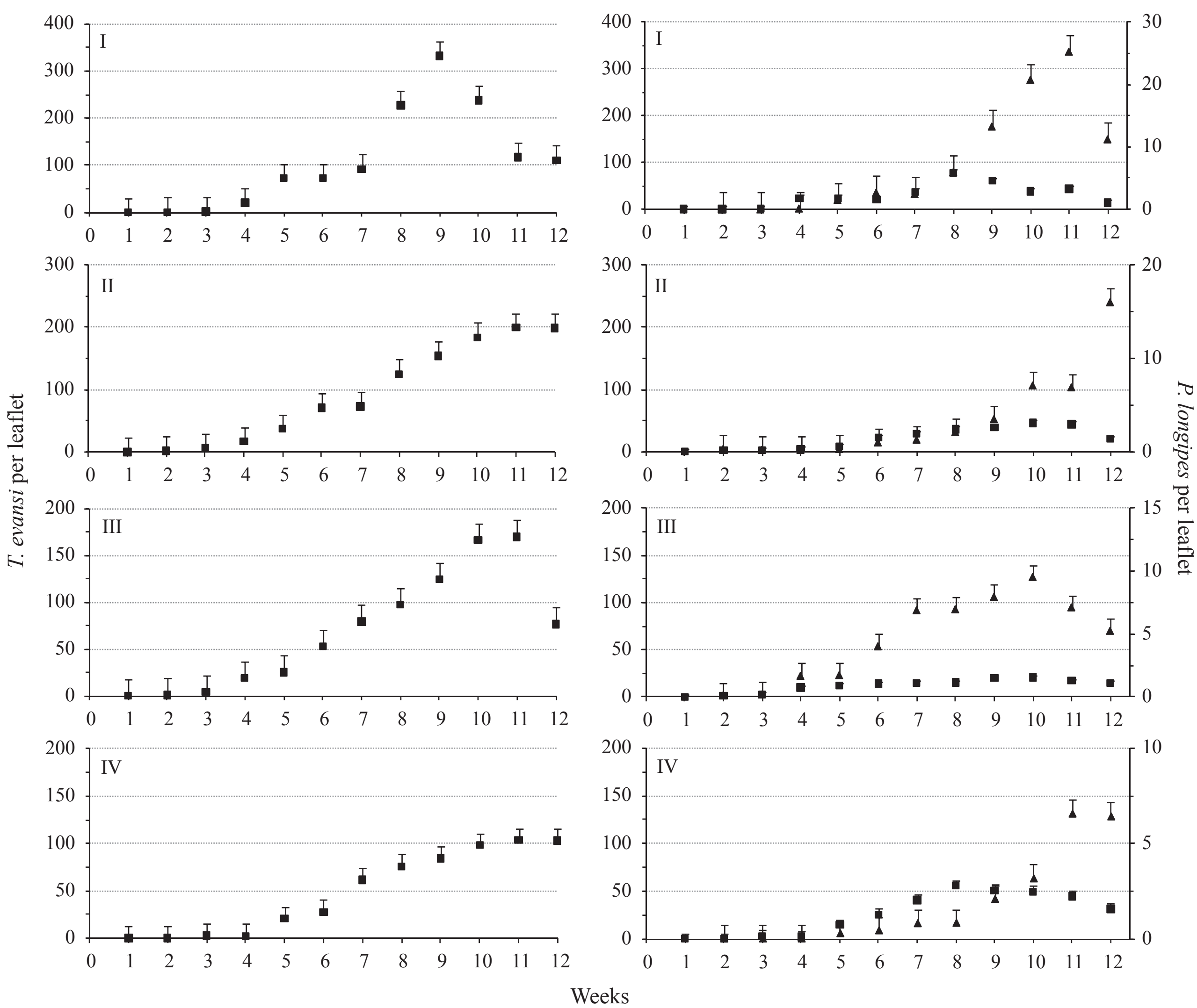

Fig 1 Average numbers of *Tetranychus evansi* (■) [plots without *Phytoseiulus longipes* release (left) and with *P. longipes* release (right)] and *P. longipes* per tomato leaflet (▲), at each sampling date in each experiment (number of experiment indicated in the upper left corner of each graph; vertical lines indicate standard error).

Table 1 Levels of significance (P-values) of the different factors of the statistical analyses of the effect of *Phytoseiulus longipes* on the density of *Tetranychus evansi* on tomato leaflets (linear mixed-effect model; see Material and Methods for details of each experiment).

Experiment	Intercept (878)[1]	Date (878)	Treatment (78)	Date * treatment (878)
1	≤ 0.021	≤ 0.000	≤ 0.047	≤ 0.000
2	≤ 0.000	≤ 0.000	≤ 0.000	≤ 0.000
3	≤ 0.000	≤ 0.000	≤ 0.000	≤ 0.000
4	≤ 0.000	≤ 0.000	≤ 0.000	≤ 0.000

[1]Degrees of freedom

the third and fourth experiments, respectively; d.f.: 399 for both experiments).

Discussion

The rapid population growth of *T. evansi* in the control plots was expected, given the high biotic potential of the pest (Moraes & McMurtry 1987, Bonato 1999) and the fact that prevailing conditions in protected cultivation often favor the development of spider mites (Sabelis 1985, Bonato 1999).

Within limits, the development of spider mites is generally favored by low humidity and high temperature, as summarized by Sabelis (1985). It seems that in all four experiments conducted the prevailing temperatures were adequate for the development of *T. evansi* (Moraes & McMurtry 1987, Bonato 1999). The increase of *P. longipes* population in the course of each experiment was also expected, as this predator has a high potential for population growth when feeding on *T. evansi* (Furtado *et al* 2007). In addition, the high temperatures also contributed to the development of the population of the predator population (Ferrero *et al* 2007).

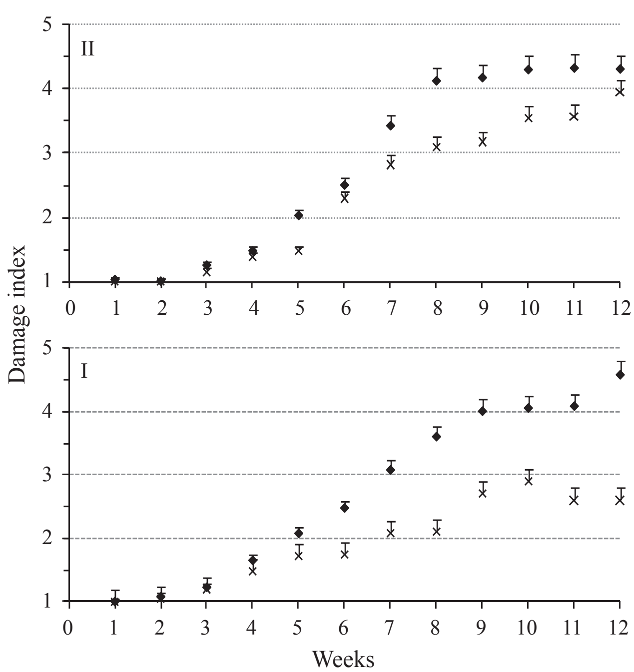

Fig 2 Mean damage levels by *Tetranychus evansi* to tomato leaflets [plots without *Phytoseiulus longipes* release (♦) and with *P. longipes* release (X)] at each sampling date of third (above) and fourth (below) experiments (damage scale given in Material and Methods). Vertical lines indicate standard error.

Detrimental effects of low relative humidity have been reported for *P. persimilis* (Williams *et al* 2004), a mite species taxonomically close to *P. longipes*. Despite the unavailability of information in relation to the effect of humidity on *P. longipes*, the results of the present study suggest that it may withstand relatively low levels of relative humidity. The performance of *P. longipes* as control agent of *T. evansi* might be better than determined in the present study at higher relative humidity levels.

All experiments indicated the potential of *P. longipes* to reduce the density of *T. evansi* and its damage to tomato plants. Thus, the results further support the conclusion that this predator has good potential as candidate for use in classical biological control projects against this pest.

The set up of the experiments did not allow a comparison of the effects of the predator between varieties. However, on both varieties ('Kada Gigante' and 'Yoshimatsu') the effect of the predator was significantly positive.

The number of predators released in the present study is compatible to the lowest numbers suggested by Hussey & Scopes (1985) for the release of *P. persimilis* to control *T. urticae* on tomatoes in greenhouses, as well as to what has been recommended commercially today for that purpose. The experiments conducted indicated the potential of the predator to reduce the population of *T. evansi*. However, higher predator-prey ratios at the time of predator release seem necessary for the effective control of the pest, given that even in the plots where the latter had been released plants were damaged.

Therefore, the practical use of *P. longipes* in inoculative release programs for the control of *T. evansi* would still require complementary studies to determine the most appropriate number of predators to be released, taking into account the population level of the pest, the stage of development of the plant and other environmental factors. Additionally, given the positive results obtained by Furtado *et al* (2007) under laboratory conditions, the results of the present study indicate that it would be also worthwhile to investigate the potential of *P. longipes* as a control agent of *T. urticae* on tomato plants grown in protected environments.

Acknowledgments

To the Brazilian Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq as well as to the German Federal Ministry for Economic Cooperation and Development (BMZ) for their financial support.

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Received 22/VII/09. Accepted 23/III/09.