BIOLOGICAL CONTROL

Response of the Predatory Mite *Phytoseiulus macropilis* (Banks) to Volatiles Produced by Strawberry Plants in Response to Attack by Tetranychid Mites (Acari: Phytoseiidae: Tetranychidae)

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ABSTRACT - The attack of phytophagous mites may induce plants to produce volatiles, which in turn may attract predators. The occurrence of multiple phytophagous infestations on plants may influence predator response. In this paper, we investigated whether the attraction of the predatory mite *Phytoseiulus macropilis* (Banks) to phytophagous-infested plants would change with the simultaneous presence of two tetranichid mites *Oligonychus ilicis* (McGregor) and *Tetranychus urticae* Koch. While the former species is rarely found on strawberry plants and is only occasionally found in association with *P. macropilis*, the latter is commonly found on strawberry plants and is frequently found in association with *P. macropilis*. Y-tube olfactometer test assays demonstrated that the predator preferred plants infested with *T. urticae*, avoided plants infested with *O. ilicis*, and had no preference for plants infested with both phytophagous mite species. These results indicated that the presence of a non-prey species (*O. ilicis*) on a given plant can alter the response of the predator to one of its prey (*T. urticae*). The consequences of the predatory behavior determined in this study on the predator ability to control *T. urticae* population on strawberry plants are discussed.

KEY WORDS: Induced defense, *Fragaria*, *Tetranychus urticae*, *Oligonychus ilicis*

The attack of phytophagous mites to plants may induce the production of plant volatiles, which in turn may attract natural enemies (Dicke *et al* 1998, 2003, Arimura *et al* 2005). The role of such herbivore-induced plant volatiles (HIPV) on predator attraction has been studied under laboratory (Pallini *et al* 1997, Takabayashi *et al* 2000), greenhouse (Janssen 1999, Venzon *et al* 1999, Holtz *et al* 2003) and field conditions (De Moraes *et al* 1998, Drukker *et al* 2000, Kessler & Baldwin 2001, Heil & Kost 2006). The complexity of these studies increases with the experimental scale. Under natural conditions, for instance, the presence of more than one species of herbivore on the same plant may alter the blend of HIPV and thereby interfere with the response of natural enemies (Vos *et al* 2001, Shiojiri *et al* 2002, Moayeri *et al* 2007).

Few studies have investigated the effects of multiple herbivore infestations on natural enemy attraction. Shiojiri *et al* (2002) found that the parasitoid *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) prefers odours of cabbage plants infested with its host *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) alone, to cabbage plants infested with its host and a non-host herbivore species, *Pieris rapae* (L.) (Lepidoptera: Pieridae). In contrast, parasitism by *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) was higher on plants infested with these two herbivores than on plants infested with *P. rapae* (host) alone.

Moayeri *et al* (2007) observed a stronger attraction of the predator *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) when plants were infested by spider mites and aphids than when plants were infested by only one of these herbivores. They suggested that the attraction of this generalist predator to plants with multiple infestations would be explained by the higher profitability of a mixed diet.

Predators are expected to be attracted to more profitable plants, i.e. plants occupied by herbivores that represent food for their development and reproduction (Venzon *et al* 2002). However, it is not clear whether the presence of a non-prey would interfere in the predator attraction to plants harboring its key prey. *Phytoseiulus macropilis* (Banks) is a biological control agent of *Tetranychus urticae* Koch, a key-pest of strawberry (Oliveira *et al* 2007). The red-mite *Oligonychus ilicis* (McGregor) has not been reported as a pest of strawberry plants, although it occurs on strawberry plants produced in organic systems (Fadini *et al* 2007). The objectives of this work were to evaluate: i) the nutritional value of each phytophagous mite to *P. macropilis*, ii) the predator attraction to strawberry plants infested with each phytophagous species separately and, iii) whether the presence of both phytophagous on plants would change the predator response.

Material and Methods

Rearings. *Phytoseiulus macropilis* and *T. urticae* were colleted from strawberry plants in a commercial plantation in Barbacena, State of Minas Gerais, Brasil, in August 2003. They were reared on the underside of detached common bean leaves, *Phaseulus vulgaris*, which were put on top of a moist sponge placed inside a box (Gerbox®, 35 x 115 x 115 mm). The edges of the leaves were surrounded by moistened cotton to prevent mite from escaping. The leaves were replaced when its turgidity was severely reduced. The rearing units were kept at 25ºC under a photoperiod of L14:D10. *Oligonychus ilicis* was collected and reared on strawberry plants var. IAC Campinas in a greenhouse, where temperature ranged between 25ºC and 35ºC.

Assessment of prey quality to the predatory mite. The intrinsic rate of population growth (r_m) was measured when *P. macropilis* was fed a diet of either *T. urticae* or *O. ilicis* at controlled conditions (25 ± 1ºC, 60 ± 5% RH and 13:11 L:D). Sixty strawberry leaf discs (13 mm Ø, var. IAC Campinas) were placed individually into a disposable petri dish (35 mm Ø; 15 mm high), over a 5 mm thick 2% carrageenan solution. Thirty leaf discs were placed upside down and other 30 discs, upside up. The first group of discs was used for *T. urticae* and the second, for *O. ilicis*. This was done due to their preferences to colonize the under and the upper sides of strawberry leaves, respectively (Fadini *et al* 2007). Five adult females of either one of the two phytophagous mites were randomly taken from their stock colonies and transferred to each of the leaf discs. After three days, two one-day-old eggs of *P. macropilis* were placed onto each leaf disc. After eclosion of the first larva, the remaining egg was removed. At this time, the average number of phytophagous mite eggs was about 200 per disc. This number is high enough to ensure sufficient food for the predator (our personal observation). Phytophagous mite density was kept about constant during the experiments by adding new adult females of *T. urticae* and *O. ilicis* whenever necessary.

Twice a day (9 a.m. and 3 p.m.), the duration and survival of the predator immature phases were registered. Just after the final molting of each predator female, one predator male was introduced to the corresponding disc. Female oviposition and longevity were evaluated daily. The data scored were used to estimate the intrinsic rate of population growth (number of females per female per day) using the Lotka´s equation (Carey 1993).

Olfactometer experiments. A Y-tube olfactometer was used to study the response of predatory mites to plants infested with the phytophagous mites (Sabelis & van de Baan 1983, Pallini *et al* 1997). The base of the olfactometer was connected to an air pump which generated airflow from the arms of the tube to its base. During the experiment, the wind speed was kept at 0.40 m/s in each arm of the tube.

Adult females of *P. macropilis* were randomly collected from the stock colony and isolated in petri dishes where they remained without food for 1h, before starting the test. Each odour source consisted of a set of two potted strawberry plants, both of which were either uninfested, infested with *T. urticae*, infested with *O. ilicis* or infested with both species. Pots of each set were placed in a tray (46 x 30 x 8 cm) that was placed inside a second tray (60 x 40 x 4.5 cm) partially filled with water. A plexiglass container (50 x 36 x 43 cm) was used to cover the plants of each set, resting in the water-containing outer tray; water prevented phytophagous mites from escaping, serving also as an air tight seal for the container. Each container had one air inlet and one air outlet (diameter 4 cm) in opposing walls. The inlet and the outlet were covered with mite-proof gauze. Each infested plant had about one thousand immatures and adults of either *T. urticae*, *O. ilicis*, or a mixture of about 500 immatures and adults of each species. Four experiments were carried out using different sets of plants: *i*) uninfested plants *versus* clean air; *ii*) uninfested plants versus *T. urticae*-infested plants; *iii*) uninfested plants versus *O. ilicis*-infested plants; and *iv*) uninfested plants versus *T. urticae* and *O. ilicis*-infested plants. The first experiment was used as a control. Each experiment was replicated three times, using different sets of plants and mites. For each comparison, 20 female predators were used per replicate. After the evaluation of five females, the Y-tube was cleaned with 70% ethanol and dried for 5 min at room temperature before further use. Differences in the proportions of *P. macropilis* females choosing between the odour sources were tested using the Replicated Goodness-of-Fit Test at a 5% critical level (Sokal & Rohlf 1995).

Results

Prey quality. The predator *P. macropilis* was not able to complete its life cycle when fed only eggs and adults of *O. ilicis*. Larvae survived for 3.0 ± 0.1 (mean ± standard deviation) days, but then died. However, when fed eggs and adults of *T. urticae*, the predator completed its life cycle. On the latter prey, the duration of the larval, protonymphal and deutonymphal stages of the predator were 1.4 ± 0.4, 2.5 ± 0.2 and 0.6 ± 0.1 days, respectively. The longevity of the adult females was 12.0 ± 1.0 days, the pre-oviposition period was 5.3 ± 0.4 days and the total number of eggs per female was 11.1 ± 0.1. The corresponding intrinsic rate of population growth (r_m) was 0.295 day^{-1}.

Olfactometer experiments. *Phytoseiulus macropilis* preferred clean air when the alternative odour source corresponded to uninfested plants (Gp = 5.48, d.f. = 1, p = 0.019; G_h = 3.23, d.f. = 2, p = 0.199; G_t = 8.71, d.f. = 3, p = 0.033) (Fig 1a); it preferred odours from plants infested with *T. urticae* over those from uninfested plants (Gp = 13.59, d.f. = 1, p < 0.001; G_h = 1.07, d.f. = 2, p = 0.586; G_t =

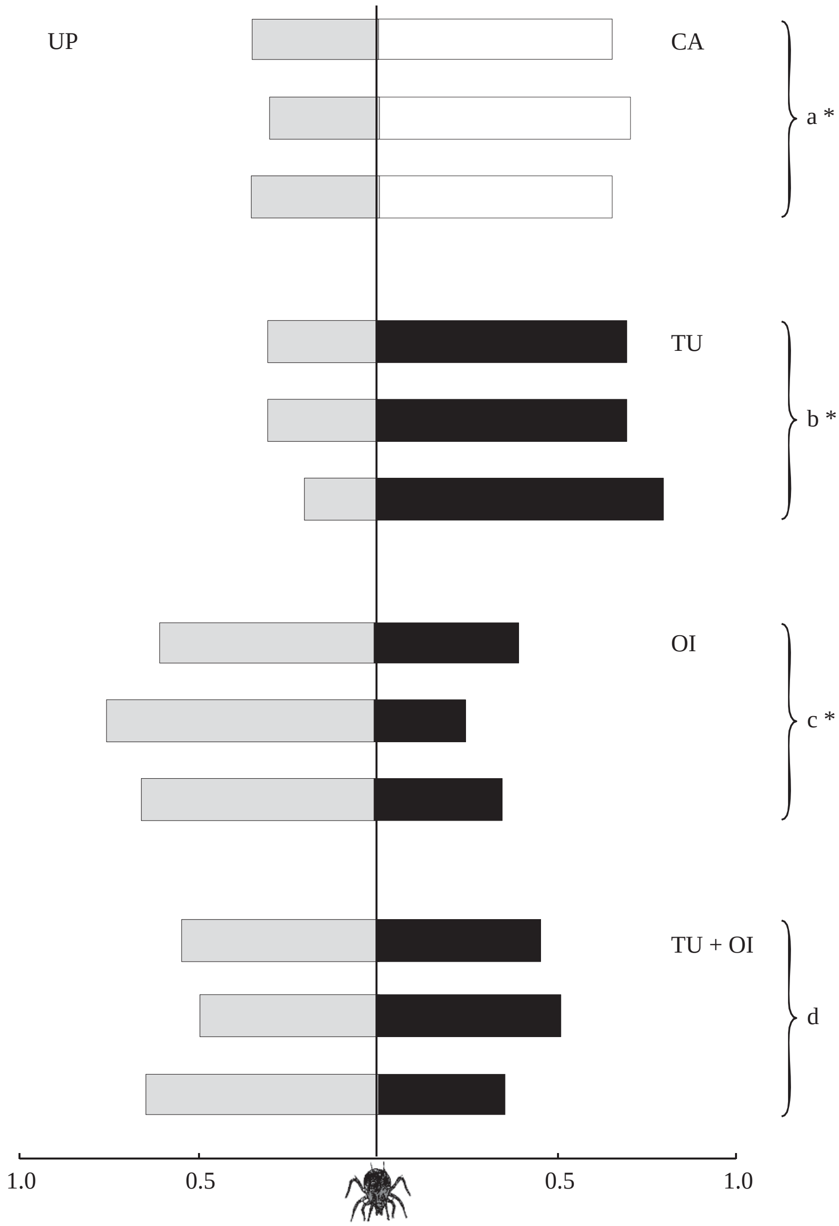

Fig 1 Proportion of *Phytoseiulus macropilis* females in Y-tube olfactometer. a) uninfested strawberry plant (UP) versus clean air (CA); b) uninfested strawberry plants (UP) versus strawberry plants infested with *Tetranychus urticae* (TU); c) uninfested strawberry plants (UP) versus strawberry plants infested with *Oligonychus ilicis* (OI); and d) to uninfested strawberry plant (UP) versus strawberry plants infested with *T. urticae* and *O. ilicis*. Each bar represents a single replicate experiment involving n = 20 mites (Gp; *P < 0.05; see text for explanation).

14.29, d.f. = 3, p = 0.002) (Fig 1b), but avoided odours from plants infested with *O. ilicis* (Gp = 6.79, d.f. = 1, p = 0.009; G_h = 1.07, d.f. = 1, p = 0.586; G_t = 7,86, d.f. = 3, p = 0.044) (Fig 1c). When plants were simultaneously infested with *T. urticae* and *O. ilicis*, the predator showed no preference for these plants over clean plants (Gp = 1.07, d.f. = 1, p = 0.301; G_h = 0.96, d.f. = 2, p = 0.619; G_t = 2.03, d.f. = 3, p = 0.567) (Fig 1d). Results from Replicated Goodness-of-Fit Tests showed that for all experiments the p-values associated to Gh values were not significant, indicating that there was not heterogeneity between replicates.

Discussion

The results indicated that *O. ilicis* is a non-suitable prey for *P. macropilis*, as the predator did not complete its life cycle when fed on this prey. *Tetranychus urticae*, however, proved to be a suitable prey for *P. macropilis*, as the predator had a positive intrinsic rate of population growth when feeding on it. Silva *et al* (2005) also found that *T. urticae* was suitable for development and reproduction of *P. macropilis*. However, they found different values for the biological parameters evaluated, which could be explained by the different geographical origin of these predator populations and/ or by the methods used.

The *P. macropilis* preference for clean air in opposition to uninfested plants is not an unusual response by phytoseiid predators (Shimoda *et al* 2005), but the reason for this type of response is still unknown. Probably, the cues emitted by non-attacked plants signal to the predator the absence of a prey and lead it to search for a prey elsewhere. However, the predator was repelled by strawberry plants infested with *O. ilicis* when tested against clean plants. *Oligonychus ilicis* is found on strawberry plants in greenhouses (Fadini *et al* 2007), but it is not a suitable prey for *P. macropilis* as shown here. Furthermore, the presence of *O. ilicis* together with *T. urticae* apparently impairs the attraction of the predator to the latter (a favorable prey), as indicated by the absence of preference for plants attacked simultaneously by both phytophagous species.

Rasmann & Turlings (2007) also studied a system in which the simultaneous occurrence of two phytophagous pests changed the attraction of a natural enemy to its host. They found that the parasitic wasp *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) was strongly attracted to maize plants infested with the leaf feeder *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). However, the parasitoid attraction was significantly reduced when *S. littoralis* and the root feeders *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) were attacking the same plants. According to these authors, the emission of the main leaf attractant was reduced under double infestation. Likewise, this could have happened in our system; it seems possible that *T. urticae* reduces its feeding on plants occupied by *O. ilicis* because of a reduction in the emission of volatiles by the plant that attract *P. macropilis*. Alternatively, *O. ilicis* could release volatiles that repel *P. macropilis*.

In another study, Boer *et al* (2008) showed that when *T. urticae* and *Spodoptera exigua* (Hübner) were simultaneously infesting lima bean and cucumber plants, the attraction of the predatory mite *Phytoseiulus persimilis* Athias-Henriot to these plants was increased, in comparison with its attraction to plants infested only with *T. urticae*. They reported that multiple-species infestation resulted in the release of a larger number of compounds by lima bean plants as compared to single infestation. In contrast, multiple infestations led to the suppression of emission of two volatiles of cucumber plants in comparison to plants with single infestation. This suppression however did not decrease, but actually increased the attraction of the predator, indicating that plant species is an important determinant in which herbivore-induced defense pathways are triggered upon multispecies herbivory.

Multiple phytophagous mite infestation may negatively influence biological control programs of *T. urticae* using *P. macropilis* due to the reduced attraction of the latter to such infested plants, when other phytophagous species are unsuitable preys. However, it has been shown that in systems where the established food web is less complex, as in protected environments, the associative learning could be improved by producing or selecting predators that have experience with the blend of volatiles of the target phytophagous (Drukker *et al* 2000, De Boer & Dicke 2006). Under field conditions, multiple infestations by more than two species are very common, very often involving different taxonomic classes of organisms. Our data suggest that different performances of *P. macropilis* and perhaps even of other natural enemies are to be expected under the diversity of associations under such conditions.

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