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Article

Feeding preference of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) towards untreated and *Beauveria bassiana*-treated *Tetranychus urticae* (Acari: Tetranychidae) on cucumber leaves

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Abstract

Determination of the host preference of predatory mites when receiving signals related to either untreated and *Beauveria bassiana*-treated *Tetranychus urticae* (Acari: Tetranychidae) is important in predicting the predatory mite's reaction when encountering pathogens due to their coincident presence in greenhouses and fields. Here, we have studied the preference of female predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) when choosing between untreated and *B. bassiana*-treated *T. urticae* (72 hours after treatment of spider mite by fungus) on leaves. This investigation showed a highly significant preference of *P. persimilis* towards untreated *T. urticae* (P< 0.0001). We concluded that the predatory mite is capable to recognize fungus-treated prey and prefers to prey on the uninfected ones. This suggests that the predatory mite and the fungus may be compatible agents of biological control.

Key words: Biological control, prey, host preference, predatory mite.

Introduction

Spider mites are major agricultural pests, which often cause severe damage to a variety of crops (Gotoh *et al*. 2004). Since resistance to acaricides in *T. urticae* spreads rapidly, biological control tactics are crucial to manage spider mite populations (Gerson & Weintraub 2007).

The predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) has been used in integrated pest management programs for *T. urticae* Koch (Acari: Tetranychidae) suppression (Cote 2001; Skirvin & Fenlon 2001). *Phytoseiulus persimilis* is a predatory mite that in absence of vision relies on the detection of herbivore induced plant odors to locate its prey, the two-spotted spider mite, *T. urticae* (Sabelis & van der Weel 1993). Entomopathogenic fungi can also be promising alternatives to harmful synthetic pesticides in agricultural, horticultural, and forest systems (Inglis *et al*. 2001). *Beauveria bassiana* (Balsamo) Vuillemin is one of the major fungal entomopathogens (Rashki *et al*. 2009) and products based on *B. bassiana* have been reported as being capable of controlling many arthropod pests, including *T. urticae* (Wright & Kennedy 1996).

There is increasing evidence that arthropods foraging both for food and oviposition sites assess their environment by chemical cues related to risks of predation, either to themselves or their offspring (Offenberg *et al.* 2004). Detectable cues to assess microhabitat quality are therefore a selective advantage for food and ovipositing female.

In the present study, host preference of *P. persimilis* was investigated when offered *T. urticae* infected adults by *Beauveria bassiana* vs. untreated *T. urticae* on the leaf. The aim was to understand if the predatory mite was able to detect the presence of an entomopathogenic fungus and avoid feeding on fungus infected prey.

Materials & Methods

Plants

Cucumber plants (*Cucumeris sativus* L. variety PS) were reared in plastic pots (13 cm diameter, 10 cm height) in a climate-controlled greenhouse room (25±1°C, 60–70٪ RH, 16:8 h (L: D).

Rearing of herbivorous & predatory mites

The two spotted spider mite, *T. urticae* was collected from cucumber plants in a greenhouse in Karaj, Iran and reared on cucumber plants (*Cucumeris sativus* L. var. PS), for one year in the laboratory at 25±1°C, 60-70٪ RH and a photoperiod of 16:8 h (L: D). To obtain adult *T. urticae* of uniform age, 25 adult females were taken from the mite culture and put on leaf discs placed on wet cotton wool in Petri dishes (9 cm diameter) and allowed to lay eggs for 18 h. After which the females were removed and the eggs remained till adult.

The predatory mite colony, *P. persimilis* was obtained from Koppert Biological Systems Inc., The Netherlands. The colony was reared according to Seiedy *et al*. (2012a).

Preparation of conidia

Beauveria bassiana isolate *DEBI008* which observed to cause non significant mortality on *P. persimilis* (Unpublished data) in comparison with two other isolates (*EUT105, EUT116*) (percentage of mortality among each of these three isolates was recorded as 39.37, 33.75 and 16.87 for *EUT105, EUT116* and *DEBI008*, respectively) was used for this experiment (with discriminatory single concentration (1×10^6 conidia/ml) that produced LC_{50} for *T. urticae*). This isolate was obtained from a *Chorthippus brunneus* Thunberg (Orthoptera: Acrididae), that had been stored in the "Pathogen Bank" at the Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran. It was cultured on Sabouraud dextrose agar plus 1٪ yeast extract (SDAY) at 25 ±1°C, 60−70% RH and in darkness for 10–14 days to obtain conidia. Spore concentration was provided according to Seiedy *et al*. (2013).

Preference experiments on cucumber leaf discs

Experimental arenas were constructed by placing a cucumber leaf disc, 60 mm in diameter excised from cucumber plants on top of a layer of cotton in a 9 cm Petri dish filled with sterile distilled water. A layer of cotton was put around the leaf in order to prevent predatory mite from escaping. Ten newly emerged adult with uniform age were placed on a side of freshly cucumber leaf discs and 10 newly emerged adult with interval 72-h post-inoculation (conidial suspension of *B. bassiana* DEBI008 was prepared at 1×10^6 conidia/ml as LC50 for the adult of *T. urticae*) (Seiedy *et al*. 2010) were placed on the other half of the leaf. One milliliter of this concentration was applied against the adults of *T. urticae* in Petri dish using a potter spray tower (Burkard, Uxberge, UK) with a nozzle size of 0.25 mm diameter and 0.7 kg/cm^2 pressure). The infected adult of *T. urticae* were sluggish, darker than untreated individuals. A single *P. persimilis* female (starved for 24 h) was placed in the center of the main vein. Adult females 2 days after moulting were used for this experiment. Eggs of the prey were removed from the arenas every hour for the duration of the experiment. The number of consumed untreated and *B. bassiana*-treated *T. urticae* adults in each Petri dish was assessed after 24 h. Thirty replicates were conducted for this experiment. The experiments were conducted at 25 ±1°C, 60–70% RH and a photoperiod of 16:8 h (L: D).

Statistical analysis

In order to determine whether the predator preferred *T. urticae* infected adults by *B. bassiana* DEBI008 with interval; 72 hours after infection vs. untreated *T. urticae* offered on the cucumber leaf, Manly's preference index (*β*) (Manly 1974) was calculated from the number of prey consumed:

$$\beta = \frac{Log\overline{P}i}{\sum_{j=1}^{m} Log\,\overline{P}j}$$

Where *β*= Manly's *β* for prey kinds (untreated *T. urticae* & *T. urticae* infected adults by *B. bassiana*) i; P_i= proportion of prey *I* remaining at the end of the experiment relative to the origin input (*i*= 1, 2, 3, 4,…, *m*); P_j= proportion of all prey kinds together remaining at the end of the experiment relative to the original input (*j*= 1, 2, 3, 4,…, m); and *m*= number of prey kinds, Manly's β can vary between zero and unity. In a two prey (untreated *T. urticae* and infected prey by *B. bassiana* (*m*= 2), the value 0.5 represents no preference, value larger than 0.5 indicate a preference for prey *A* and a smaller value indicate a preference for prey *B*. This method takes into account the prey density depletion by predation during experiments (Sherratt & Harvey 1993).

Results

When adults of both untreated prey and infected prey by *B. bassiana* were offered simultaneously, *P. persimilis* consumed more untreated *T. urticae* (5.26 ± 0.17 (mean ± SE)) than *T. urticae* infected adults by *Beauveria bassiana* DEBI008 that had been infected for 72 hours (0.93 ± 0.19). The Manly's *β* preference index (*β*) for untreated *T. urticae* and for *T. urticae* infected adults by *B. bassiana* DEBI008 that had been infected for 72 hours were 0.95 ± 0.01 and 0.045 ± 0.01, respectively. These results showed that *P. persimilis* prefers untreated *T. urticae* rather than *T. urticae* infected adults by *B. bassiana* DEBI008 with interval 72 hours after infection (Fig. 1).

Discussion

Our data analysis showed that when untreated and *B. bassiana*-treated *T. urticae* were offered simultaneously, *P. persimilis* consumed more untreated *T. urticae* than *T. urticae* infected adults by *B. bassiana* DEBI008 with interval 72 hours; because fungal control agents have the potential to negatively affect natural enemies through their effects on the quality of prey (Seiedy *et al*. 2012a). In this experiment, the quality of the prey seems to have been significantly affected through infection by the fungus and consequently it affects feeding preference of *P. persimilis*.

We speculated this predator can avoid feeding on *T. urticae* adults infected with *B. bassiana*. Earlier studies showed that behavioral factors such as feeding, preference and activity of predators were altered by the presence of fungus in the prey or its habitat. Indeed a significant decrease in feeding of predator *Anthocoris nemorum* (Linnaeus, 1761) on aphid treated with *B.*

bassiana was observed (Meyling & Pell 2006); the predator *Hippodamia convergens* Guerin-Meneville did not feed on aphids treated with *Paecilomyces fumosoroseus* (Wize) (Pell & Vandenberg 2002); *Orius albidipennis* (Reuter) reduced its feeding time and predation rate on *Thrips tabaci* (Lindeman) larvae treated with *Metarhizium anisopliae* (Metchnikoff) (Pourian *et al.* 2011), *P. persimilis* showed increased handling time of on *B. bassiana*-treated adults of *T. urticae* if infected for 48 and 72 h (Seiedy *et al.* 2012a); and finally, whitefly nymphs treated with *Isaria fumosorosea* Wize were not accepted by the predator *Dicyphus hesperus* Knight (Alma *et al.* 2010). Avoidance of feeding of the predatory mite on *T. urticae* infected adults by *B. bassiana* is evident on the leaf and also Seiedy *et al*. (2013) showed that *P. persimilis* was attracted towards control plants if tested against plants with spider mites infected with *B. bassiana* for 72 h. This indicated that the predator was able to detect the presence of *B. bassiana* in the 72 h treatments. Therefore, live *T. urticae* infected with the fungus for 72 h caused avoidance behavior of the predator not only in the olfactometer but also on the cucumber leaf too.

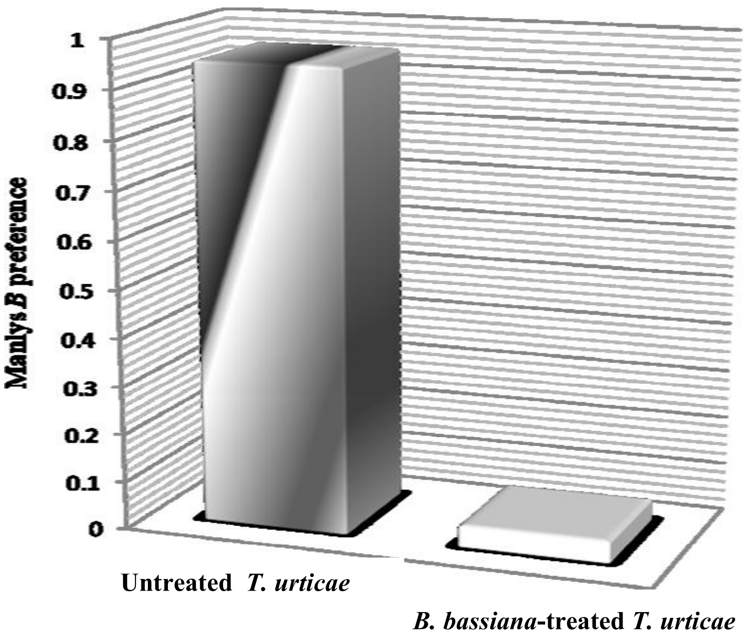

Figure 1. Manly's preference index of *Phytoseiulus persimilis* when feeding on untreated and *Beauveria bassiana*-treated *Tetranychus urticae* infected for 72 h treatments in assay with two choices.

An insect or mite may gain selective advantage if it is able to detect entomopathogenic fungi from a distance and respond via behavioral avoidance or through post-contact responses such as grooming (Chouvenc *et al.* 2008; Seiedy *et al.* 2012b). Baverstock *et al*. (2005) have shown that entomopathogenic fungi produce a range of volatile chemicals, so it is possible to assume that the fungus produces one or some compounds that the predatory mite can detect and that trigger the avoidance behavior. This response can be interpreted as the capability of the predatory mite to recognize dangerous conditions based on odour, and to adjust its foraging behavior accordingly (Faraji *et al*. 2001).

Our research indicates that fungus-treated hosts can affect the behavior of *P. persimilis*. Sun *et al*. (2008) found that organic mulches supplemented with *M. anisopliae* significantly repelled foraging of *C. formosanus* and reduced mulch consumption up to 71%. Ormond (2007) found that the seven-spot laybird *Coccinella septempunctata* (L.) detected and avoided *B. bassiana* on leaves and in soil.

Avoidance of the fungus by the predator would reduce the impact of intra-guild interactions and allow coexistence under laboratory conditions. These data may advance our understanding of tritrophic interactions by revealing an understudied association between a predator (*P. persimilis*) and an entomopathogenic fungus (*B. bassiana*).

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ترجیح غذایی کنه شکارگر ***Phytoseiulus persimilis*** **Athias-Henriot** **(Acari: Phytoseiidae)** به سمت کنه تارتن دو لکه‌ای ***Tetranychus urticae*** **(Acari: Tetranychidae)** سالم و تیمار شده با قارچ بیمارگر ***Beauveria bassiana*** روی برگ‌های خیار

مرجان سیدی

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تعیین ترجیح میزبانی کنه شکارگر *Phytoseiulus persimilis* Athias- Henriot (Acari: Phytoseiidae) پس از دریافت سیگنالهای حاصل از کنه تارتن دو لکه‌ای *Tetranychus urticae* (Acari: Tetranychidae) سالم و نیز کنه تارتن تیمار شده با قارچ *Beauveria bassiana* در تعیین واکنش کنه شکارگر در مواجهه با عوامل بیمارگر در زمان کاربرد هم‌زمان‌شان در گلخانه و مزرعه مهم است. در این بررسی، ترجیح کنه ماده شکارگر را زمانی که انتخابی بین کنه تارتن سالم و کنه تارتن تیمار شده با قارچ روی برگ‌ها (۷۲ ساعت پس از تیمار شدن کنه تارتن با قارچ) داشت، مطالعه شد. این پژوهش ترجیح زیاد معنی‌دار کنه شکارگر در تغذیه از کنه‌های تارتن دو لکه‌ای سالم ($P < ۰/۰۰۰۱$) را نشان داد. مشخص شد که کنه شکارگر قادر به تشخیص شکار تیمار شده با قارچ است و ترجیح می‌دهد روی شکارهای غیر آلوده تغذیه کند. این نتیجه‌گیری، سازگاری زیاد کنه شکارگر با قارچ به عنوان عامل مفیدی در کنترل بیولوژیک را نشان می‌دهد.

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