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Laboratory evaluation of the effect of *Beauveria bassiana* on the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae)

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ABSTRACT

Tetranychus urticae Koch (Acari: Tetranychidae), a major pest of many agricultural crops, is mainly controlled with chemical acaricides. However, predatory mites and entomopathogens have been proposed as alternative control agents. In this study, the effect of the BotaniGard® GHA strain of *Beauveria bassiana* on the survival, longevity, fecundity, and egg hatch rate of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) were studied under laboratory conditions. When *B. bassiana* was applied directly to *P. persimilis* eggs at a concentration of 1×10^8 conidia/ml, corrected hatchability was less than 5%, and the corrected mortality of nymphs and adults was not significantly different from control 10 days after treatment. *Phytoseiulus persimilis* nymphs that hatched from treated eggs showed no significant change in their development time, adult female longevity, hatch rate, survival rates over time, or offspring sex ratio. However, significant negative effects on fecundity and life table parameters (net reproductive rate, intrinsic rate of natural increase, mean generation time, finite rate of increase, and doubling time) were found when *B. bassiana* was applied to the adult stage. Spraying *B. bassiana* at 1×10^8 conidia/ml on newly emerged adults of *P. persimilis* caused 44% reduction in the oviposition period, 26% in adult longevity, and 63% in fecundity. Due to these negative effects, *B. bassiana* should be used with careful adjustment of application timing (first spray *B. bassiana* and then release *P. persimilis*) to supplement biological mite control systems using *P. persimilis*.

1. Introduction

The spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important pest of ornamentals and vegetables, causing extensive economic losses in greenhouse and open-field crops (Grbić et al., 2011). The economic damage to crops caused by spider mites can increase rapidly due to its rapid developmental rate (Ullah et al., 2012). Chemical acaricides have been the primary strategy for controlling spider mites, but as resistance to acaricides has spread, biological control has become a more widely used alternative (Gerson and Weintraub, 2007). Predatory mites such as *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) are more selective to spider mites and safer to environment than chemical pesticides, making them more compatible with other control measures and an important element of integrated mite management programs (Skirvin et al., 2002; Shi and Feng, 2004; Alma et al., 2007; Avery et al., 2008; Vergel et al., 2011).

Phytoseiulus persimilis, a specialist predator of spider mites, has been successfully employed for regulating populations of *T. urticae* in greenhouse crops (Cote, 2002; Skirvin and Fenlon, 2001; Oliveira et al., 2007). However, different degrees of success have been obtained in different crops and conditions (Zhang and Sanderson, 1995; Kim et al., 1997; Opit et al., 2003; Naher and Haque, 2007). In addition to the use of predatory mites, growers have recently adopted the use of commercial microbial products such as entomopathogenic fungi to control populations of *T. urticae* (Alves et al., 2002; Chandler et al., 2005; Shi and Feng, 2009; Alma et al., 2007). The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin is among these (Leger et al., 1986). *Beauveria bassiana* applied against aphids, leafhoppers, and whiteflies has been shown to be effective in laboratory and greenhouse trials (Faria and Wraight, 2001; Feng et al., 2004; Hatting et al., 2004; Pu et al., 2005). Successful control of spider mites may be achieved by applying entomopathogenic fungi that are more toxic to the pest than to its natural enemies (Gonzalez et al., 2016). Because releases of *P. persimilis* may also be an effective alternative for spider mite control in field crops, identification of a selective mycopesticide that is compatible with this predator is important. The combined application of *B. bassiana* with predatory mites has been found to reduce the density of *T. urticae* by as much as 98% in greenhouse tomatoes (Chandler et al.,

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2005). However, compatibility of predatory mites and entomopathogenic fungi can be a critical issue in the field conditions (Ghazy et al., 2016). Several researchers have found *B. bassiana* having an adverse effect on the biological life history parameters of predators fed on infected prey (Agboton et al., 2013; Wu et al., 2015), and Seiedy et al. (2012) found that feeding *B. bassiana*-contaminated prey (*T. urticae*) to *P. persimilis* caused undesirable effects on the predator's life history parameters. *Beauveria bassiana* sprayed on adults of *P. persimilis* resulted in ca. 43% mortality (Duso et al., 2008; Vergel et al., 2011) and significant reduction in fecundity (Duso et al., 2008). However, interactions between arthropods and entomopathogenic fungus are complex, and the sub-lethal effects of *B. bassiana* on life history parameters of *P. persimilis* are not yet studied when sprayed on eggs and adults separately.

The aim of this study was therefore to assess the effect on *P. persimilis* life table parameters after direct application of the *B. bassiana* based commercial product BotaniGard® ES to either eggs or adult predatory mites. As a preliminary assay, we examined the hatch rate of *P. persimilis* eggs sprayed with *B. bassiana* and subsequent life history parameters of surviving mites at three different RHs. This information will help define the degree of compatibility of these two biological control agents and determine the possibility of combined applications of both agents in spider mite biological control programs.

2. Material and methods

2.1. Sources of T. urticae, P. persimilis, and entomopathogenic fungi

2.1.1. Mites

Tetranychus urticae obtained from Insect Ecology laboratory, Andong National University, Republic of Korea in 2014. Laboratory stocks of *T. urticae* were maintained on bean leaf discs (ca. 16 cm^2) of common bean, *Phaseolus vulgaris* L., placed on water-saturated polyurethane mats in plastic Petri dishes (90 mm diameter, 20 mm depth) and held at 23.0–26.9 °C, 38–61% RH, and a photoperiod of 16L: 8D h in a growth chamber (DS-11BPL, Dasol Scientific Co., Ltd, Suwon, Republic of Korea) for more than one year before the present study. Mixed stages of *T. urticae* were used to rear *P. persimilis*.

2.1.2. Phytoseiulus persimilis

Phytoseiulus persimilis was collected from the Dongbu Farm Ceres Company in 2014. Laboratory stocks of *P. persimilis* were maintained using all stages of *T. urticae* (green form) reared on bean leaf discs (ca. 16 cm^2) placed on water-saturated polyurethane mats in plastic Petri dishes (90 mm diameter, 20 mm depth) for more than one year in a rearing chamber (23.0–26.9 °C, 38–61% RH, and a 16L: 8D h photoperiod). The leaf discs were renewed as necessary.

2.1.3. Fungal pathogen and preparation of conidial suspension

The entomopathogenic fungus tested was obtained as the commercial product BotaniGard® ES (*Beauveria bassiana*, GHA strain, Arysta LifeScience, Tokyo, Japan). As a viability test, subcultures were grown on Sabouraud Dextrose Agar (SDA) in Petri dishes and maintained in the dark at 25 ± 1 °C for 10–14 days. Conidia were harvested from surface cultures by scraping and were then suspended in 10 ml of sterile distilled water containing 0.05% Triton X-100 using universal bottles containing glass beads. Conidial suspensions were vortexed for 5 min, and spore concentrations were determined using a haemocytometer (Neubauer-improved haemocytometer, Lauda-Königshofen, Germany). Conidial viability was determined before the bioassay by spread-plating 0.1 ml of conidial suspension titrated to 1×10^4 conidia ml^{-1} on SDA plates (Seiedy et al., 2012). Plates were incubated at 25 ± 1 °C, and the percentage germination was determined after 24 h from 100-spore counts by placing a sterile microscope coverslip on each plate and counting germinants under a microscope (100× magnification, Nikon, Eclipse E200, Japan). Each plate was replicated four times. Quality control tests of *B. bassiana* showed 90% conidial viability. Conidial suspension was prepared at a concentration of 1×10^8 conidia ml^{-1} diluted with distilled water just before assay.

2.2. Effect of B. bassiana on P. persimilis survival

2.2.1. Eggs

Beauveria bassiana conidia were assayed using a direct spray method to evaluate their ovicidal effect on *P. persimilis* eggs. Four 5-day-old adult female *P. persimilis* were taken from the stock predator culture and transferred on a kidney bean leaf (4×4 cm^2 leaf disc) infested with mixed stages of *T. urticae*. Those adult predators were allowed to lay eggs for 24 h and then removed, and the eggs laid were collected and used for bioassay. There were four discs (2×2 cm^2 leaf disc), each with 15 predator eggs from a cohort of the population was kept for ovicidal effect test. Subsequently, 1×10^8 conidia ml^{-1} suspension of *B. bassiana* was sprayed onto the leaf disc (2×2 cm^2 leaf disc) with a hand sprayer (4 ml in 2×2 cm^2 leaf disc). In order to standardize the use of the sprayer to deliver a consistent dose, five consecutive sprays were performed on Petri dishes (50 mm diameter, 15 mm depth), each in triplicate, and the deposits were quantified (5.0 ± 0.02 ml). No significant differences were obtained (data not shown), and so hand application was used in this manner in the main experiment. For a control, pure distilled water was sprayed on eggs of *P. persimilis* present on leaf discs. After air drying, the sprayed spider mite eggs were held at one of three RHs (55, 75, and 95%), as this variable often affects spore germination and hence infection rates. The three RHs were achieved by dissolving $Mg(NO_3)_2{\cdot}6H_2O$, NaCl, and K_2SO_4 in distilled water in desiccators (140 mm diameter, Scienceware®, Wayne, New Jersey, USA) to prepare 55, 75, and 95% RH, respectively (Rockland, 1960). Temperature and RH were measured using a data logger (U10-001; Onset Computer Corporation, Cape Cod, MA). All treated and control Petri dishes were held at 24.7 ± 0.05 °C in an incubator, and the number of eggs hatched was recorded daily until there was no change for three consecutive days. Later, the eggs were individually examined under an inverted microscope (40× magnification, Leica microsystems, GmbH Wetzlar, Germany) for verification of fungal infection. Finally, all unhatched eggs were transferred to moist chambers for three days to detect any fungal outgrowth, as evidence of egg mortality due to fungal infection.

2.2.2. Protonymphs and newly eclosed adults

Protonymphs and newly eclosed adults of *P. persimilis* collected from the predator colony were sprayed with a 1×10^8 conidia ml^{-1} suspension of *B. bassiana* strain GHA onto the leaf disc with a hand sprayer (4 ml in 2×2 cm^2 leaf disc), and their survival was assessed. In separate trials, for each stage, mites were exposed to the fungal pathogen through direct spray and then transferred to plastic Petri dishes (50 mm diameter, 15 mm depth) lined with freshly excised bean leaf discs and held at 24.7 ± 0.05 °C, a photoperiod of 16L: 8D h, and one of three RHs. Mixed stages of *T. urticae* were provided on leaf discs as food. After air drying, the leaf discs with treated protonymphs or adults of *P. persimilis* were held under one of three RHs (55, 75, and 95%) and counted daily. Mortality was recorded daily for 10 days after application. For each life stage there were five replicates for each level of humidity, with 10 predators per replicate (total of 50 mites exposed per life stage per RH level). The presence of fungal mycelia on dead mites was regarded as an indication of mycosis. Controls consisted of predators treated with distilled water. As no statistical differences were observed in rates of mortality among the three levels of RH for either egg hatch or nymphal or adult survival, we used 75.0 ± 2.0% RH only for all subsequent experiments.

2.3. Life table study of P. persimilis with B. bassiana applied at the egg stage

To determine the effects of exposure (during the egg stage only) on