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Effects of *Beauveria bassiana* on *Frankliniella occidentalis* (Thysanoptera: Thripidae) trough different routes of exposure

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Abstract: *Beauveria bassiana* is an entomopathogenic fungus widely used in biocontrol tactics over the world. Its potential has been tested on several pest species, included the Western Flower Thrips *Frankliniella occidentalis* Pergande. However, knowledge on *B. bassiana*-thrips interactions is limited. In laboratory bioassays, we exposed different developmental stages of *F. occidentalis* (first and second instar larvae, adults) to residual or topical applications of a *B. bassiana* commercial strain (strain ATCC 74040, Naturalis®). A moderate to high mortality was noticed for first instar larvae, late second instar larvae and adults when the two routes of exposure were combined. The results stress on the importance to favor the contact of thrips with *B. bassiana* to obtain satisfactory control of this pests. The significant reduction in thrips survival after *B. bassiana* applications suggests that the latter can be included in IPM tactics.

Key words: *Beauveria bassiana*, *Frankliniella occidentalis*, biological control

Introduction

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) is a Nearctic pest of various ornamentals and vegetables, in particular in greenhouses. It can cause both direct (by feeding and egg-laying) and indirect (transmission of tospoviruses, such as TSWV and INSV) damage. It can lead to a serious reduction of commercial crop value (Arzone *et al.* 1989). Insecticide use can be unsatisfactory against this pest because of insecticide resistance (Immaraju *et al.* 1992). Moreover, cryptic behavior of nymphs and adults can affect pest control. Current research focuses the attention on the control of *F. occidentalis* exploiting potential antagonists like predators (e.g., predatory mites, minute pirate bugs, rove beetles), parasitoids (e.g., Eulophids) and pathogens (a number of fungi and nematodes). *Beauveria bassiana* Vuill. (Hyphomycetes: Moniliaceae) is one of the most common and widely used insect-pathogenic fungi. It is endophyte and soil-inhabiting and capable of attacking a wide host range (~500 bio-target).

Several researchers have highlighted its pathogenicity against *F. occidentalis*, demonstrating an effective control in protected crops (e.g., Murphy *et al.* 1998, Shipp et *al.* 2003). Laboratory studies reported the susceptibility of some juvenile stages of this pest to *B. bassiana* infection (Ugine *et al.* 2005a, 2005b, Gouli *et al.*, 2008, Ansari *et al.*, 2007). However, the knowledge concerning the effect of *B. bassiana* infection on different thrips stages, as a function of different routes of exposure, is limited.

In this work we evaluated the susceptibility of different developmental stages of *F. occidentalis* (first instar larvae, second instar larvae and adults) to increasing levels of exposure to *B. bassiana*. Thrips were exposed to foliar deposits or to foliar deposits and a direct (topical) application of a *B. bassiana* solution in the laboratory. Preliminary results are reported herein.

Material and methods

Insect rearing

Thrips rearing was performed using cucumber fruits using a modified method described by De Graff *et al.* (2009). Cucumbers supplied both food source and oviposition sites. Rearing units were kept at room temperature (23±1°C) and 60-70% relative humidity, with a photoperiod of 16:8 (L:D). Thrips were collected from rearing units using a fine brush and assigned to each treatment according to their age (first instar larvae, early second instar larvae, late second instar larvae, and adult females).

Bioassay procedure

We used a *B. bassiana* conidia-based formulation (strain ATCC 74040, Naturalis®, Intrachem) applied at recommended dose (150ml hl^{-1}). The effectiveness of *B. bassiana* application was evaluated against first instar larvae, second instar larvae and adults. We also discriminated early from late second instar larvae according to their size of the abdomen relative to the head and thorax and behavioral traits (feeding and activity). In all trials we used bean leaves (*Phaseolus vulgaris*) as a substrate. Bean plants were cultivated in greenhouse at the Department of Environmental Agronomy and Crop Science, University of Padova. Before starting each trial, bean leaves were washed under water and then dipped in 1% NaClO solution for 5 min; leaves were rinsed under flushing water and then under sterilized deionized water. Finally they were left to dry in sterile conditions.

Three different treatments were compared according to an increasing level of *B. bassiana* exposure: 1) untreated control; 2) foliar deposits of *B. bassiana* (residual exposure); 3) foliar deposits plus topical *B. bassiana* applications (residual exposure + topical exposure). In treatment 2, bean leaves were dipped in the *B. bassiana* solution for 30 s and left to dry under sterile conditions. Then, leaves were added to the holding cells as substrate for thrips rearing. Holding cells were similar to those described by Dennehy *et al.* (1993) (see Duso *et al*. 2008 for details). In treatment 3, thrips were immersed in the *B. bassiana* solution using the micro-immersion bioassay (Dennehy *et al*. 1993; see also Castagnoli *et al*. 2005). Thrips were drawn into small pipette tips, after which the *B. bassiana* solution was drawn up, immersing the thrips for 30 s. The thrips were ejected from the pipette, dried on filter paper in sterile conditions then transferred singly into experimental cells using a fine brush. *B. bassiana* treated bean leaves were added to holding cells as a substrate for thrips. Five thrips were introduced into each cell, and maintained under controlled climatic conditions (23°C, 90% R. H.). Holding cells were daily monitored under a dissecting stereoscope to assess the number of alive and dead individuals, as well as their developmental stage. Dead thrips were checked to assess the identity of pathogens. Trials were carried out over 9-14 days depending on developmental stages involved. Each treatment comprised six replicates of five thrips each.

Statistical analysis

Mortality was calculated after three and eight days from *B. bassiana* applications and at the end of experiments. Data were analyzed by a one-way ANOVA (F test, $\alpha = 0.05$) and differences among treatments were calculated by applying Tukey-Kramer test ($\alpha = 0.05$). Prior to the analysis, data were checked for ANOVA assumptions. Corrected mortality was calculated for each developmental stage and route of exposure by Abbott's formula (1925).

Results and discussion

The mortality of *F. occidentalis* was not significantly affected three days after *B. bassiana* applications. In contrast, significant mortality effects were observed on all developmental stages eight days after applications (first instar larvae: $F = 50.38$; d.f. = 2, 15; $p < 0.001$; early second instar larvae: $F = 5.20$; d.f. = 2, 11; $p = 0.02$; late second instar larvae: $F = 12.56$; d.f. = 2, 12; $p = 0.001$; adults: $F = 10.39$; d.f. = 2, 13; $p = 0.002$). Mortality of first instar larvae was higher in treatment 3 (residual plus topical exposures) than in treatments 2 (residual exposure) and 1 (untreated control). Moreover, mortality in treatment 2 was higher than that recorded in treatment 1. Regarding early second instar larvae, mortality was higher in treatment 3 than in other treatments, while for late second instar larvae and adults mortality was higher in *B. bassiana* treatments compared to the control, independently from the route of exposure. At the end of trials, mortality in *B. bassiana* treatments was always higher than in the control (first instar larvae: $F = 60.98$; d.f. = 2, 15; $p < 0.001$; early second instar larvae: $F = 7.84$; d.f. = 2, 11; $p = 0.007$; late second instar larvae: $F = 17.63$; d.f. = 2, 12; $p < 0.001$; adults: $F = 17.56$; d.f. = 2, 13; $p < 0.001$), irrespectively of the routes of exposure, apart for first instar larvae where mortality was significantly higher in treatment 3 than in treatment 2. The highest values of corrected mortality were observed in treatment 3 (87.4% for first instar larvae, 49.2% for early second instar larvae, 68.8% for late second instar larvae and 75.4% for adults).

The results reported in this study show an effect of *B. bassiana* towards various developmental stages of *F. occidentalis*. Pathogenicity of *B. bassiana* appears to be affected by the routes of exposure. Mortality of larvae and adults exposed to foliar deposits was lower than that recorded in other studies (Ugine *et al.*, 2005a, 2005b; Gouli *et al.*, 2008). According to Ugine *et al*. (2005b) the effects of *B. bassiana* were higher on adults than on early second instar larvae. In this study, mortality induced by residual exposure appeared to depend on the mobility of thrips stages. The most motile stages (late second instar larvae and adults) were the most susceptible to *B. bassiana* through residual exposure. In this route of exposure, thrips can be contaminated through tarsi and feeding structures. Contamination appears to increase with thrips activity. First instar larvae and early second instar larvae were less affected in residual exposure alone, probably because of their low motility.

Combining both residual and topical exposure, a substantial increase in thrips mortality was observed. Above all, mortality was higher in first instar larvae and adults. High percentage of mortality was reported for early second instar larvae through topical exposure (Ugine *et al*. 2005a). In topical exposure, integumental penetration of the fungus is promoted. Different levels of thickening and/or hardening of cuticle may explain the major susceptibility of first instar larvae to topical exposure. Further research is needed to fully understand the role of exposure on the fungal activity. The outcomes of this study confirm that *B. bassiana* is a valuable antagonist of *F. occidentalis.* Moreover, this research highlights the importance of routes of exposure to the fungus in affecting its potential in biological control tactics.

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References

Abbott, W. S. 1925: A method for computing the effectiveness of an insecticide. J. Econom. Entomol. 18: 265–267.

Ansari, M. A., Brownbridge, M., Shah, F. A. & Butt T. M. 2008: Efficacy of entomopathogenic fungi against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis*, in plant-growing media. Entomologia Experimentalis et Applicata 127: 80-87.

Arzone, A., Alma, A. & Rapetti, S. 1989: *Frankliniella occidentalis* (Perg.) (Thysanoptera: Thripidae) nuovo fitomizo delle serre in Italia. Informatore Fitopatologico 39: 43- 48.

Castagnoli, M., Liguori, M., Simoni, S. & Duso, C. 2005: Toxicity of some insecticides to *Tetranychus urticae*, *Neoseiulus californicus* and *Tydeus californicus*. BioControl 50: 611–622.

DeGraff, H. E. & Wood, G. M. 2009: An improved method for rearing western flower thrips *Frankliniella occidentalis*. Florida Entomologist 92: 664-666.

Dennehy, T. J., Farnham, A. W. & Denholm, I. 1993: The microimmersion bioassay: a method for topical application of pesticides to spider mites. Pesticide Science 39: 47–54.

Duso, C., Malagnini, V., Pozzebon, A., Buzzetti, F. M. & Tirello, P. 2008: A method to assess the effects of pesticides on the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) in the laboratory. Biocontrol Sci. Technol. 18: 1027-1040.

Gouli, S., Gouli, V., Skinner, M., Parker, B., Marcelino, J. & Shternshis, M. 2008: Mortality of western flower thrips, *Frankliniella occidentalis*, under influence of single and mixed fungal inoculations. J. Agricult. Technol. 4: 37-47.

Immaraju, J. A., Paines, T. D., Bethke, J. A., Robb, K. L. & Newman, J. P., 1992: Western flower thrips (Thysanoptera: Thripidae) resistance to insecticides in coastal California greenhouses. J. Econom. Entomol. 85: 9-14.

Murphy, B. C., Morisawa, T. A., Newman, J. P., Tjosvold, S. A. & Parrella, M. P. 1998: Fungal pathogen controls thrips in greenhouse flowers. California Agriculture 52:32-36.

Shipp, J. L., Zhang, Y., Hunt, D. W. A. & Ferguson, G. 2003: Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. Environmental Entomology 32: 1154-1163.

Ugine, T. A., Wraight, S. P. & Sanderson, J. P. 2005a: Development of a novel bioassay for estimation of median lethal concentrations (LC_{50}) and doses (LD_{50}) of the entomopathogenic fungus *Beauveria bassiana*, against western flower thrips, *Frankliniella occidentalis*. J. Invert. Pathol. 89: 210-218.

Ugine, T. A., Wraight, S. P. & Sanderson, J. P. 2005b: Acquisition of lethal doses of *Beauveria bassiana* conidia by western flower thrips, *Frankliniella occidentalis*, exposed to foliar spray residues of formulated and unformulated conidia. J. Invert. Pathol. 90: 10-23.