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Effect of entomopathogenic fungi (EPF) on thrips (*Frankliniella occidentalis*) selected predators

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Introduction

In general thrips pest species are difficult to control. This is especially true for Western Flower Thrips (WFT), *Frankliniella occidentalis* (Thysanoptera: Thripidae). Thus, the most recommended management tool is through the use of Integrated Pest Management (IPM), i.e. by combination of different control strategies. A good example is the combination of different predatory antagonists with entomopathogenic fungi or nematodes, which could help to obtain synergistic effects, covering gaps in efficacy and reliability on single antagonistic species. Among the most successful antagonists of this pest are the predatory mite *Amblyseius cucumeris* (Acarina: Phytoseiidae) and the predatory bug *Orius laevigatus* (Hemiptera: Anthocoridae), both targeted on plant-dwelling developmental stages (EBSSA *et al.*, 2006) and *Hypoaspis aculeifer* (Canestrini) (Acarina: Laelapidae), targeted on soil-dwelling developmental stages of thrips (BERNDT *et al.,* 2004). Additionally, several studies report on entomopathogenic fungi, i.e. *Beauveria bassiana* (Bals.), *Metarhizium anisopliae* (Metsch.), and *Paecilomyces fumosoroseus* (Wize), as efficient thrips antagonists (JACOBSON *et al.,* 2001). Unfortunately, these fungi are generalists and therefore potentially able to infect non-target insects or mites, like predatory antagonists (HOLDER and KEYHANI, 2005). However, the virulence of different EPF strains shows considerably variation and therefore it is necessary to screen for negative impacts on each particular insect/mite species.

The main goal of the current study was to investigate if recently identified efficient strains of different entomopathogenic fungi have non-target effects on natural enemies of WFT. Therefore we studied (1) worst case scenarios (immersion tests, direct contamination) and (2) extended our studies to examine indirect effects of EPF on different antagonist species.

Material and Methods

All investigations were carried out under controlled laboratory conditions in climate chambers. Three previously selected EPF strains virulent to *F. occidentalis* were tested against selected natural enemies of thrips, i.e. the predatory mites *A. cucumeris* and *H. aculeifer* as well as the predatory bug *O. laevigatus*. Two different exposure scenarios of antagonists to EPFs were evaluated, (1) direct contamination by immersion in spore solutions and (2) indirect contamination with spores via substrate surface contact.

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(1) Direct contamination by immersion

Three different EPF species were tested for non-target effects on antagonists, (1) *B. bassiana* (Naturalis) (a commercial product with oily formulation), (2) *M. anisopliae* (M2539) and (3) *P. fumosoroseus* (FWA3). The final spore concentration in the test solutions was $1x10^7$ spores/ml, in the case of the non-formulated strains (*M. anisopliae*, *P. fumosoroseus*) with 0.05% Tween 80 as additive to reduce surface tension. *B. bassiana* (Naturalis) was tested at label dose, i.e. $1x10^4$ spores/ml. In all bioassays a 0.05% Tween 80 solution was used as control treatment. Preparation of all solutions was done immediately before experiments took place. Thrips antagonists were supplied by commercial breeders. Only adults were selected and immersed for 20s into the spore solution. Subsequently, arthropods were transferred to breeding arenas, supplied with food and stored under particular conditions for each of them (*A. cucumeris* and *H. aculeifer* : 25°C and 70% RH, *O. laevigatus* 19°C and 60%RH) in a climate chamber. During the next seven days mortality of arthropods was recorded on a daily basis.

(2) Indirect contamination by walking on substrates

Based on the results of the worst-case scenario (direct contamination) the indirect impact of EPFs on thrips antagonists by secondary spore pick-up through walking on a contaminated surface was investigated. Since *B. bassiana* showed non-target effects on both predatory mite species, i.e. *A. cucumeris* and *H. aculeifer*, in the first experiment both were tested for indirect effects. The spore concentrations were the same as in the first experiment and the solution was sprayed with a pump on glass vials. Subsequent to drying of the residues, mites were confined in the glass vials and exposed to the spore contaminated surface for 1, 2, or 3 days and then transferred to rearing arenas following the same procedure as described above. Again mortality of individuals was recorded on a daily basis for seven days.

Exploration of the results for the direct and indirect contamination experiments was done through survival analysis and Kaplan-Meier survival function estimators. In order to test for differences among treatments, the Log-Rank Test was applied. All calculations were done with the statistical software program SPSS (2001).

Results and discussion

(1) Direct contamination by immersion

Compared to the control treatment the EPF strains *M. anisopliae* (M2539) and *P. fumosoroseus* (FWA3) did not affect survival of the three tested predatory antagonists (Fig. 1). In contrast, the commercial product Naturalis (*B. bassiana*) reduced survival of all antagonists significantly (Fig. 1) and effects were species specific. Compared to the control, survival probability recorded at the end of the seven day period was 4-times lower for the predatory bug *O. laevigatus* (18%) and 2-times lower for the two predatory mites *A. cucumeris* and *H. aculeifer* (38 and 43% respectively) (Fig. 1).

As expected mortality started few days after immersion of antagonists into the spore solution and increased steadily towards the end of the experiments. This is specifically true for *O. laevigatus* and *H. aculeifer*, which both suffer from entomopathogenic fungi infection. However, based on the shape of the *A. cucumeris* survival curve (Fig. 1a) mortality cannot be attributed exclusively to entomopathogenic fungi. Indeed the combined effect of *B. bassiana* and the oily formulation of the commercial product Naturalis is responsible for the high mortality recorded already during the first days (Arias, 2008).

The particular susceptibility of any organism to *B. bassiana* depends on several factors but is mainly directly related to the arthropods cuticle composition, i.e. the epicuticular layer and the biochemical processes needed for the conidia germination and subsequent host colonization (De

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Oliveira *et al*., 2004). Therefore it is likely that the different susceptibilities of the tested organisms are directly related to species-specific differences in the epicuticular layer of the cuticle.
On the other hand, the methodology used to apply the spore solution influences also the results. In the “worst-case” scenario, the organisms where dipped into a spore solution which maximized the area of the insect/mite body covered with spores assuring the development of a further fungal infection.

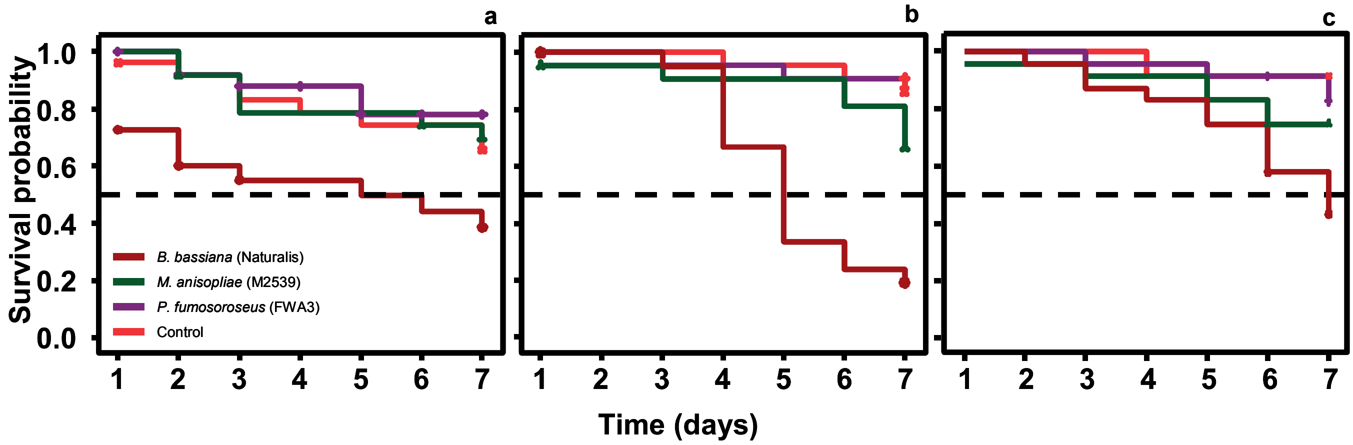

Figure 1. Kaplan-Meier Survival curve showing survival probability of adults of a) *A. cucumeris* and b) *O. laevigatus* and c) *H. aculeifer* during the 7-days after immersion in a spore solution . Statistical analysis was conducted by Log Rank (Mantel-Cox) test; a) χ^2 = 14.761, df = 3, P = 0.002, b) χ^2 = 39.374, df = 3, P < 0.001 and c) χ^2 = 15.448, df = 3, P < 0.001.

(2) Indirect contamination by walking on substrates
Since it was observed in the previous experiment that direct exposure to the commercial product Naturalis (*B. bassiana*) affected survival of thrips antagonists significantly, it was important to determine the effect of indirect contamination, i.e. by picking up spores while walking on a contaminated surface, e.g. after spraying. Results show that *H. aculeifer* was not affected by any of the exposure intervals. In contrast, compared to the control treatments exposition for a three-day period reduced survival of *A. cucumeris* significantly (Fig. 2). At the end of the 7-day period survival probability was reduced by 45%, which was 2-times higher than in the immersion test.

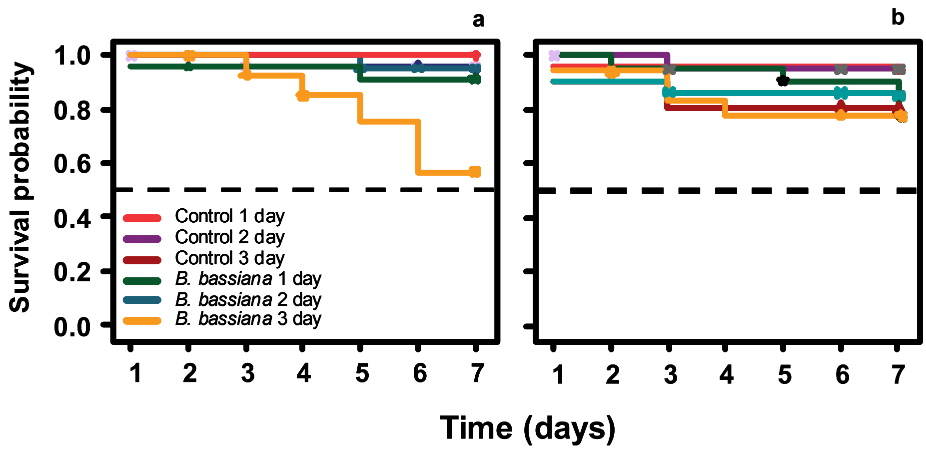

Figure 2. Kaplan-Meier Survival curve showing survival of adult *A. cucumeris* (a) and *H. aculeifer* during 7-days after 1, 2 or 3 day exposure *B. bassiana* (Naturalis) contaminated surfaces. Statistical analysis of the model using Log Rank (Mantel-Cox) test; (*A. cucumeris*: χ^2 = 21.772, df = 5, P < 0.001), (*H. aculeifer*, n.s.).

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The survival probability of *A. cucumeris* was slightly lower than in the direct contamination experiments, but statistically significant different to the control. Nevertheless, the mortality was quite high even though it was a secondary contamination. Under natural conditions, this kind of contamination should generate a lower impact on survival of any non-target organism, since less amounts of spores come into contact with the body surface. Furthermore, the spores picked up usually reach highly sclerotized body parts (i.e. legs), where the infection could hardly take place. Probably, the strong effect observed in the present experiment can be attributed to a better adhesion of the spores to the mite body due to the oily formulation of the product, the permanent contact of the mite with the contaminated surface without any possibility to avoid or escape from it or to the high humidity achieved inside the glass vials which could have prompted to a faster germination and infection of the mites.

Conclusions and outlook

Whenever the use of entomopathogenic fungi for WFT control is intended, questions about compatibility with natural enemies arise. Under the worst-case scenario, all predators suffered increased mortality when treated with the commercial product Naturalis (*B. bassiana*) compared to the control, but not when *M. anisopliae* or *P. fumosoroseus* strains were used. Particularly, *A. cucumeris* started to die already during the first day after treatment. This suggested most likely an effect of the additives present in the formulation of the product on the mite mortality. Indeed a killing contact effect of the oily formulation of Naturalis (*B. bassiana*) alone could be confirmed by additional immersion experiments with *A. cucumeris* (Arias, 2008). Additional experiments are necessary to clarify the effect of the formulation on other predatory antagonists, since a synergistic effect of the formulation and the strain might be possible.

Finally, based on our current results it seems to be safe to recommend the combined use of *Metarhizium* or *Paecilomyces* with any of the predators without negative impact on adult mortality. However in the case of *Beauveria* extended laboratory studies are necessary to issue final conclusions.

References

ARIAS, E. 2008 Effect of different strains of entomopathogenic fungi (EFP) on thrips (*Frankliniella occidentalis*) and selected natural enemies (NE). Master thesis, University of Hannover Germany, 92 pp.

BERNDT, O.; MEYHÖFER, R.; POEHLING, H-M. 2004. The edaphic phase in the ontogenesis of *Frankliniella occidentalis* and comparison of *Hypoaspis miles* and *Hypoaspis acuilefer* as predators of soil-dwelling thrips stages. Biological Control 30(2004): 17-24.

EBSSA, L.; BORGEMEISTER, C., POEHLING, H-M. 2006. Simultaneous application of entomopathogenic nematodes and predatory mites to control western flower thrips *Frankliniella occidentalis*. Biological Control 39: 66-74.

JACOBSON, R.; CHANDLER, D.; FENLON, J.; RUSSELL, K. 2001. Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to Control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on Cucumber Plants. Biocontrol Science and Technology 11: 391-400.

OLIVEIRA, R.; NEVES, P.; ALVES, L. 2004. Seleção de Fungos Entomopatogênicos Para o Controle de *Oligonychus yothersi* (McGregor) (Acari: Tetranychidae), na Cultura da Erva-Mate (*Ilex paraguariensis* St. Hill.). Neotropical Entomology 33(3):347-351.

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