PATHOGENICITY OF THE FUNGI *METARHIZIUM ANISOPLIAE* AND *BEAUVERIA BASSIANA* TO THE WESTERN FLOWER THRIPS, *FRANKLINELLA OCCIDENTALIS*.

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Summary

The pathogenicity of several isolates of the hyphomycete fungi *Metarhizium anisopliae* and *Beauveria bassiana* to *Franklinella occidentalis* was investigated. Of the three *M. anisopliae* isolates tested, isolate CBS caused the highest mortality of thrips, both females and first stage larvae. *B. bassiana,* recently isolated from thrips, caused higher mortality of thrips than *M. anisopliae* isolates.

INTRODUCTION

Western flower thrips, *Franklinella occidentalis* (Pergande), is a serious pest on ornamental and vegetable crops (Lewis, 1997). It causes damage directly through feeding, and indirectly through the transmission of lethal plant viruses (Peters *et al.*, 1991). Because of its large host range it has a world-wide pest status. Growers frequently use insecticides to control *F. occidentalis* in their crops, but increased tolerance or even resistance to the chemical compounds will necessitate them to search for other control methods (Brødsgaard, 1994). Next to predators like *Orius spp.* and *Amblyseius spp.* entomopathogenic fungi are a good, environmental friendly alternative for chemical insecticides. A new concept in biological control is "Attract and Infect": first an insect is attracted to a trap, within the trap the insect becomes infested with a pathogen than the insect leaves the trap and will contaminate the rest of the insect population with this pathogen (Smits, this issue).

The aim of this study is to find entomopathogenic fungi which can be used in the "Attract and Infect" concept. Of several *Metarhizium anisopliae* isolates and a new isolate of *Beauveria bassiana* the pathogenicity to *F. occidentalis* is compared.

METHODS

Maintenance of fungal and insect cultures

The isolates V38 and 275 of *M. anisopliae* were obtained from S. Vestergaard, Denmark. The isolate CBS (# 160.96) of *M. anisopliae* was obtained from CBS, Baarn, The Netherlands. The isolate IPO (#9901) of *B. bassiana* was isolated from *F. occidentalis* on *Arabidopsis dahliani* at IPO, Wageningen, The Netherlands. The isolates were cultured on SDAY (Sabouraud dextrose agar + 2g/l yeast) at 25°C in the dark.

A rearing of *F. occidentalis* was maintained on potted, flowering chrysanthemum plants, *Dendranthema grandiflora* Tzelev, of the susceptible cultivar 'Sunny Cassa' in a green house at 25°C and 70% RH.

Bioassays

Conidia were harvested from 14 day old cultures by flooding the plates with sterile 0.01% aqueous (w/v) Triton X-100 and agitating with a glass rod. The suspension was

filtered through tissue paper followed by centrifugation for 5 min. at 3000 rpm. The pellet was washed twice with sterile 0.01% Triton X-100 with intervening centrifugation steps. The concentration of the inoculum was adjusted to 10^6 conidia ml^{-1} with 0.01% Triton X-100.

The viability of the conidia was assessed according to the method of Hall (1976) and usually exceeded 95%.

The containers used for the bioassays were made from clear polystyrene (Greiner) of 68 mm diameter, 66 mm height and 190 ml content. Each container had 4 round holes (Ø 22 mm) on the side, closed with gauze (Monodur 80 µm mesh). On the bottom of the container a layer of about 25 ml 1.5% water agar was poured, a leaf disc (Ø 40 mm) from a first leaf of a bean plant (cv. Bataaf) was placed on the agar and the edge of the leaf disc was sealed to the agar with hot wax. The leaf disc was treated with 100µl conidia suspension or, as a control, 100µl 0.01% Triton X-100. Per container 10 female thrips or first stage larval thrips were added using a fine, moist brush and a small amount of pine tree pollen was added. The containers were closed with a lid and sealed with parafilm. The containers were incubated at 25°C and 16:8 h photoperiod. The relative humidity (RH) was maintained at 71% by a saturated solution of NaCl or at 94% by a saturated solution of KNO_3.

The mortality and the number of offspring was assessed every day for seven days and every day the produced larvae were removed after anaesthetising the thrips with carbondioxide. Per treatment three containers were used and the experiments were done in three replicates.

M. anisopliae isolates were tested on thrips females and first stage larvae while the new isolate of *B. bassiana* was tested on females only.

Results were statistically analysed by analysis of variance. Pairwise comparison were made by least-significant-difference-test (LSD).

Table 1. Mortality, 7 days post inoculation, of female and first stage larvae (L1) of *F. occidentalis* exposed to different isolates of *M. anisopliae* at different relative humidity (10^6 conidia/ml, 25°C).

Isolate	mortality of female 94% RV	mortality of female 71% RV	mortality of L1 71% RV
control	10%	11%	6%
CBS	80%	71%	34%
V38	67%	35%	12%
275	57%	42%	26%

RESULTS

Mortality of thrips caused by *M. anisopliae* started after 3 or 4 days (Fig. 1). Of the three *M. anisopliae* isolates CBS performed best (LSD α=0.01), on both adults and larvae (table 1). Pathogenicity against female thrips was higher than against first stage thrips larvae, for all isolates (table 1). CBS did not perform much better at higher humidity (94%) but the other two isolates did (table 1). *B. bassiana* performed better than the best *M. anisopliae* isolate (CBS) (Fig. 2).

Control mortality was due to natural mortality, no fungus infection was observed.

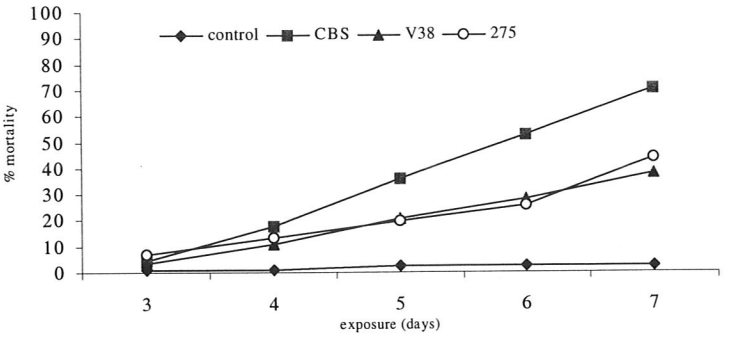

Figure 1. Mortality of female *F. occidentalis* exposed to different isolates of *M. anisopliae* at 71% RH

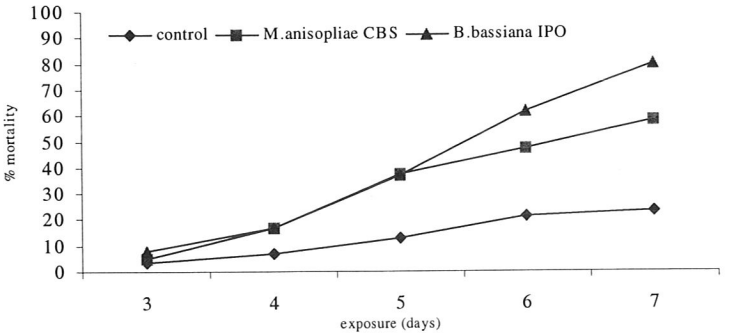

Figure 2. Mortality of *F. occidentalis* exposed to *M. anisopliae* isolate CBS and *B. bassiana* isolate IPO at 71% RH.

CONCLUSIONS

M. anisopliae isolates differ in their pathogenicity against thrips. This makes it useful to select for more virulent strains and isolates. A highly virulent pathogen will perform better as a biological control agent.

The new isolate of *B. bassiana* caused higher thrips mortality than the *M. anisopliae* isolates. This isolate was recently isolated from thrips. Whether this high virulence is lost after several cultivation's on artificial medium has to be tested.

Most entomopathogenic fungi request a rather high humidity to infect and kill an insect. In the greenhouse a high humidity also causes growth of plantpathogenic fungi, so a high humidity is not desirable. *M. anisopliae* isolates differ in their tolerance for low humidity. This makes it possible to select for an isolate which is still virulent at low humidity levels.

Screening and selection of entomopathogenic fungi for RH tolerance and high virulence can improve biological control of thrips.

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