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Entomopathogenic activity of a whitefly-derived isolate of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against the Russian wheat aphid, *Diuraphis noxia* (Hemiptera: Sternorrhyncha: Aphididae) with the description of an effective bioassay method

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Microbial control, hyphomycete fungus, bioassay method, *Paecilomyces fumosoroseus*, *Diuraphis noxia*, Russian wheat aphid

Abstract. This is the first report of the potential of the hyphomycete fungus, *Paecilomyces fumosoroseus* (Wize) Brown & Smith, for the microbial control of aphids. The LD_{50} and LD_{90} of a strain of *P. fumosoroseus* isolated from *Bemisia tabaci* (Gennadius) from Multan, Pakistan and bioassayed against third instar *Diuraphis noxia* (Mordvilko) were $1.78 \cdot 10^3$ conidia/cm^2 and $1.43 \cdot 10^4$ conidia/cm^2, respectively. Strong dosage-mortality and dosage-mycosis responses were evident with larger proportions of individuals dying within the first three days post-treatment at the higher conidial dosages ($1.25–3.75 \cdot 10^4$ conidia/cm^2) than was observed for dosages equal to or lower than $3.75 \cdot 10^3$ conidia/cm^2. The LT_{50}s for *D. noxia* treated with $3.75 \cdot 10^4$ conidia/cm^2 and $3.75 \cdot 10^3$ conidia/cm^2 were 2.06 and 7.50 days, respectively. The entomopathogenic activity of *P. fumosoroseus* against *D. noxia* compares favorably with other Hyphomycetes reported in the literature. Based on the results reported here, further investigation of the potential of *P. fumosoroseus* for the microbial control of *D. noxia* and other aphids is warranted.

In addition to data on the efficacy of *P. fumosoroseus* as a microbial control agent of *D. noxia*, a simple, but effective bioassay method for the evaluation of fungi against cereal aphids is described.

INTRODUCTION

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) is believed to have originated from the region encompassing southern Russia, Iran, and Afghanistan, but is now widespread throughout many wheat growing regions of the world (Aalbersberg et al., 1987; Puterka et al., 1993). In geographic areas where it has been recently introduced, most notably the U. S., it is responsible for multi-million dollar agricultural losses. Wheat yield losses attributed to *D. noxia* in the 1991–1992 growing season in the western U. S., for example, were estimated at nearly $75 million (Legg & Amoson, 1993). In an effort to achieve sustainable control of this pest, an integrated approach that includes biological control and use of resistant varieties of wheat, among other interventions, has been the focus of several research groups (compiled by Webster & Treat, 1994). In order to provide potential biological control agents of *D. noxia*, including

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entomopathogenic fungi, the USDA-ARS European Biological Control Laboratory (EBCL) in Montpellier, France has conducted foreign exploration for natural enemies of the aphid throughout Europe and Asia.

Screening of fungi that have been collected from aphids and other Hemiptera against *D. noxia* revealed elevated levels of pathogenic activity of *Paecilomyces fumosoroseus* (Wize) Brown & Smith that had been isolated from the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). The objective of the research presented here was to provide background on the pathogenic activity and microbial control potential of *P. fumosoroseus* for *D. noxia*.

MATERIAL AND METHODS

Insect

The aphid used for bioassays, *D. noxia*, originated from wheat fields in the vicinity of Montpellier, France and has been maintained in a laboratory colony at the EBCL since 1991. The individuals used for testing were reared in the laboratory on young barley plants (*Hordeum vulgare* L. var. "Clarine"), according to the method described by Gruber & Dureseau (1978). The rearing conditions were: 22–24°C, 60–70% relative humidity, and 16 h photoperiod. Fifteen adult females (viviparous) were transferred with a fine camel's hair brush to 15 Petri plates (9 cm diameter) containing three barley leaves (5 cm long) the bases of which were wrapped in moistened cotton. The insects were kept in these conditions for 24 h to obtain nymphs of a known age. After 24 h, the young aphids were transferred onto young barley plants (6–8 cm high) until they matured to third instars. Rearing and identification of this stage were based on development time for the species, relative size of the nymphs and the number of antennal segments (Aalbersberg et al., 1987).

Fungus

The strain of *P. fumosoroseus* used for bioassays was isolated in 1992 from *B. tabaci* in Multan, Pakistan (Lacey et al., 1993). This isolate has been added to the EBCL collection (strain 92117) and the USDA-ARS Entomopathogenic Fungal Culture Collection in Ithaca, NY (ARSEF 3877). It was selected for further study after preliminary tests at EBCL revealed elevated pathogenic activity for *D. noxia*.

Preparation of inoculum

The fungus was cultured on SMDAY medium (neopeptone: 10 g; dextrose: 20 g; maltose: 20 g; brewer's yeast: 20 g; agar: 10 g; sterile deionized and distilled water: 1 l), at 24°C. The isolate was passed through the aphid, and, after sporulating on the surface of cadavers on water agar, was subcultured on SMDAY at 24°C for 12–14 days. A stock suspension was prepared by scraping conidia from the surface of the cultures in 3.5 cm diameter Petri plates into 10 ml of sterile distilled water. This suspension was added to a bead mill (60 ml volume containing 70–80 four mm glass beads) and agitated for 5 minutes at 700 oscillations/min (10 cm of vertical travel), without surfactant in a Dangoumau mechanical agitator. A 10^{-2} dilution of the stock suspension was used for determining spore count in a Newbauer hemacytometer. Dilutions of the stock were then made to produce suspensions ranging from 3×10^5 to 10^8 conidia/ml.

To determine viability, 0.1 ml of a suspension of 10^6 conidia/ml was plated onto SMDAY in a 9 cm diameter Petri plate. The plate was then incubated at 24°C, for 18–20 h. The % germination was determined by observation of four lots of 100 conidia using a Olympus Vanox phase contrast microscope (200 ×) and an ocular grid. Conidia were considered to have germinated when a germ tube longer than the diameter of the spore was observed.

Bioassay procedure

The Potter spray tower (Potter, 1952) was used for applying suspensions of *P. fumosoroseus* conidia. The tower was calibrated by spraying 2 ml suspensions of conidia (5×10^7 or 10^8 conidia/ml) onto 9 cm diameter Petri plates containing water agar. Three Petri plates were sprayed for each of 15 suspensions. The spores in 10 separate 0.25 mm^2 areas on each plate were counted at a magnification of 200 × using an Olympus Vanox phase contrast microscope.

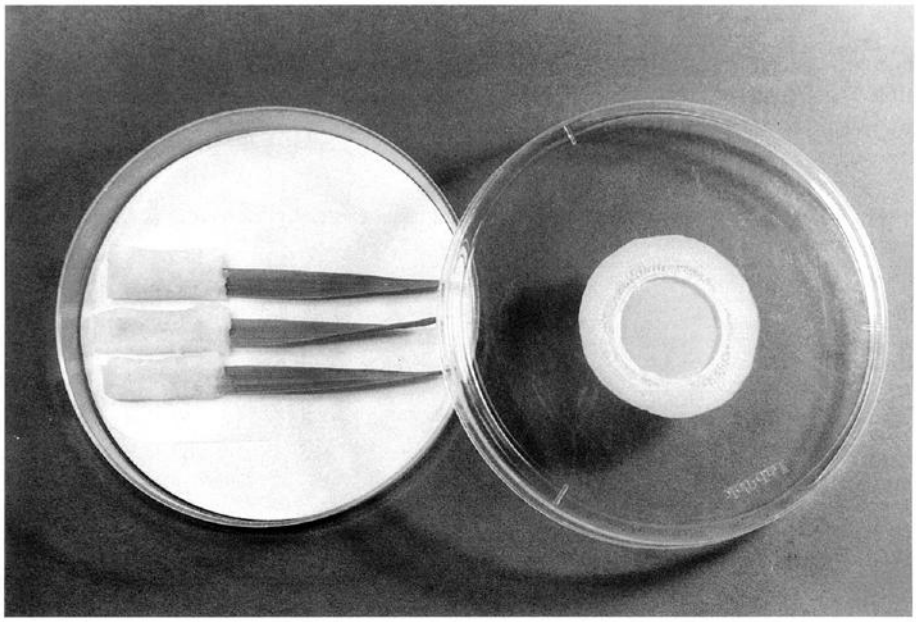

Fig. 1. Bioassay system used for evaluation of entomopathogenic fungi against aphids. Ventilated cover prevents excessive humidity and eliminates condensation.

For bioassays, fifteen third-instar aphids were placed on three barley leaves in 28 Petri plates as described above. A disk of filter paper (8.5 cm diameter; Watman grade 1) was placed on the bottom of each Petri plate before the addition of barley leaves. A rectangle of parafilm (5 cm × 3.5 cm) was placed under the wet cotton that was used to provide water to the leaves to avoid moistening the filter paper. Four replicated lots of 15 aphids were used for each concentration and control. Six concentrations of conidia were tested ranging from 3×10^5 to 10^8 conidia/ml which corresponded to a coverage of 1.12×10^2; 3.75×10^2; 1.13×10^3; 3.75×10^3; 1.25×10^4; and 3.75×10^4 conidia/cm^2 when 2 ml of spore suspension were used. Controls were sprayed with sterile deionized water. Applications of the conidial suspensions were made in the spray tower at a pressure of 10 lbs/in.^{2} (51.7 cm Hg) with a fine droplet spray nozzle (0.7 mm internal diameter).

After spraying, the lids of the Petri plates were hermetically sealed with strips of parafilm to maintain saturated humidity and the plates were incubated at 24°C for 24 h. Following incubation in high humidity, the lids were replaced with others with a gauze (0.1 mm mesh) covered 2 cm hole that allowed aeration (Fig. 1). The plates were then held at 22–24°C, 70–80% relative humidity, and 16 h photoperiod for the next six days. After three days, the sprayed barley leaves in each Petri were replaced with fresh ones; aphids were transferred to new leaves using a camel's hair brush. The number of dead insects in each plate was recorded daily over the seven days of the test. Cadavers were removed each day and placed in Petri plates on water agar at 24°C. Infection with *P. fumosoroseus* was confirmed by observation of characteristic sporulation on the surface of the cadavers.

Statistical analysis

Probit analysis for determination of LD_{50-90} and LT_{50-90} was conducted on the bioassay data (total mortality six days after initial exposure) after correction for control mortality using Abbott's formula followed by log transformation using the log-probit analysis software (CIRAD, 1989). The effect of different dosages of *P. fumosoroseus* were analyzed using ANOVA (general linear model procedure) and Tukey's studentized multiple range test after arcsine-square root transformations on the percentage mortality (SAS Institute Inc., 1989).

RESULTS

The *P. fumosoroseus* conidia used for bioassays were 95% viable. When 2 ml of a 10^8 conidia/ml suspension was applied onto Petri plates containing water agar using the Potter spray tower, an average of 375.2 ± 18.72 (s.e.) conidia/mm^2 were detected on the surface of the agar.

TABLE 1. Mortality and incidence of mycosis in third-instar *Diuraphis noxia* six days after treatment with six dosages of the fungus, *Paecilomyces fumosoroseus.*

Dosages (spores/cm^2)	Average mortality[a] % ± s.e.	Mycosis[a] % ± s.e.
3.75×10^4	95.00 ± 3.19 a	93.32 ± 4.71 a
1.25×10^4	91.67 ± 3.18 a	88.35 ± 4.19 a
3.75×10^3	73.33 ± 8.61 ab	63.33 ± 6.39 b
1.13×10^3	46.65 ± 6.09 bc	33.35 ± 6.09 c
3.75×10^2	21.65 ± 6.32 cd	18.33 ± 5.68 cd
1.12×10^2	18.32 ± 3.21 cd	6.67 ± 2.72 d
control	11.67 ± 5.69 d	–

$LD_{50} = 1.78 \times 10^3$ conidia/cm^2 (95% C.I. $1.29 - 2.45 \times 10^3$)[b]
$LD_{90} = 1.43 \times 10^4$ conidia/cm^2 (95% C.I. $0.89 - 2.32 \times 10^4$)[b]

[a] Means in the same column followed by the same letter are not significantly different ($P > 0.05$) as determined by Tukey's test for separation of means.

[b] LC_{50-90} determined using the probit analysis program DL_{50} (CIRAD 1989). (Regression equation $y = 1.41x + 0.41$)

The LD_{50} and LD_{90} for the Multan isolate against third-instar *D. noxia* and the effects of individual dosages are reported in Table 1. Cumulative mortality of *D. noxia* over time and at different concentrations of *P. fumosoroseus* conidia is presented in Fig. 2. Strong dosage-mortality and dosage-mycosis responses were evident with larger proportions of individuals dying within the first three days post-treatment at the higher conidial dosages ($1.25–3.75 \times 10^4$ conidia/cm^2) than was observed for dosages equal to or lower than 3.75×10^3 conidia/cm^2. At the highest two concentrations, maximum mortality was observed five days after treatment. In the remaining treatments, maximum mortality was observed six days after treatment. Time intervals required for 50 and 90% (LT_{50-90}) mortality of third instar *D. noxia* following treatment with three dosages of *P. fumosoroseus* are presented in Table 2.

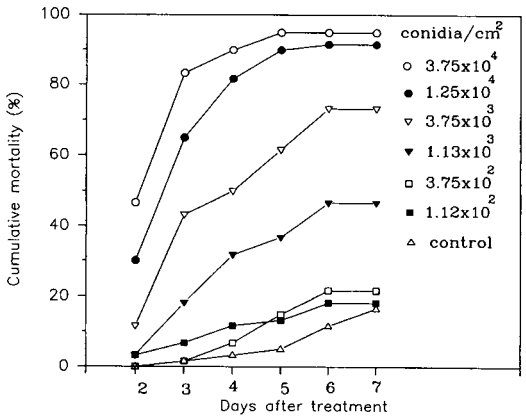

Fig. 2. Cumulative mortality of *Diuraphis noxia* after treatment of third-instar nymphs with six dosages of conidia of *Paecilomyces fumosoroseus.*

TABLE 2. Time intervals required for 50 and 90% (LT_{50-90}) mortality of third-instar *Diuraphis noxia* following treatment with three dosages of *Paecilomyces fumosoroseus*.

Dosages (spores/cm^2)	Regression equation [a]	LT_{50} days after treatment	95% confidence interval	LT_{90} days after treatment	95% confidence interval
3.75×10^4	$y = 3.69x + 3.84$[b]	2.06	1.71 – 2.47	4.57	3.97 – 5.26
1.25×10^4	$y = 4.08x + 3.19$[c]	2.77	2.45 – 3.13	5.70	4.97 – 6.55
3.75×10^3	$y = 0.91x + 4.20$[d]	7.50	[d]	[e]	

[a] LT_{50} and LT_{90} calculated with 95% confidence intervals (C.I.) on mortality assessed 1–6 days following treatment.

[b] Linearity of data confirmed ($\chi^2 = 6.35$; prob. of χ^2 17.48%; non-significant).

[c] Linearity of data confirmed ($\chi^2 = 4.93$; prob. of χ^2 29.49%; non-significant).

[d] Non-linearity of data confirmed ($\chi^2 = 34.42$; prob. of 0.00%; significant), hence C.I. not calculated.

[e] Data not observed.

DISCUSSION

Under certain environmental conditions entomopathogenic fungi are extremely important natural control agents of aphids (Latgé & Papierok, 1988). Some species of Entomophthorales, such as *Pandora* (*Erynia*) *neoaphidis* Remaudière & Hennebert, are responsible for spectacular epizootics in aphid populations, including *D. noxia* (Feng et al., 1990a, 1991a, 1991b, 1992; Wraight et al., 1993). Unfortunately, epizootics often occur in peak *D. noxia* populations later in the growing season after economic thresholds have been surpassed. Although inoculation of insect populations with entomopathogenic fungi has provided classical biological control of some pests, currently the most reliable method of employing fungi for insect control in general is through inundative means.

In most cases, the production of entomophthoralean fungi on artificial media is expensive and the shelf life of spores that can rapidly infect the target insect is often too short to enable large scale production and inundative application of these fungi. Alternatively, several species of fungi in the Hyphomycetes offer good potential for production on inexpensive artificial media and have relatively good shelf life.

Although *P. fumosoroseus* is an entomopathogenic fungus with a fairly broad host range (Samson, 1974; Smith, 1993), it has not been reported from aphids under natural conditions. During the past 6–7 years, concerted effort has been expended in the development of this fungus as a microbial control agent of *Bemisia* spp. and other whiteflies (Lacey et al., 1995). Its production on artificial media, relative stability in storage, and virulence toward *Bemisia argentifolii* Bellows & Perring and other species of whiteflies, make it one of the best candidates for commercial development. The activity against *D. noxia* reported here also indicates that it is an ideal candidate for further development as a microbial control agent of aphids. Additional studies conducted at EBCL on the interaction between *P. fumosoroseus* and *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae), a common parasitoid of *D. noxia*, indicate the existence of a high degree of compatibility between these two agents, with reduced antagonistic interaction (Mesquita & Lacey, unpubl. data).

Feng & Johnson (1990) and Feng et al. (1990b) assayed several isolates of two other Hyphomycetes, *Beauveria bassiana* (Balsamo) Vuillemin and *Verticillium lecanii* (Zimm) Viegas, against *D. noxia* and other cereal aphids. Although all were infective, the lowest LC_{50} (8.2×10^4 conidia/ml) was observed with an aphid derived isolate of *B. bassiana*. The

LC_{50} of *V. lecanii* was 4.1×10^5 conidia/ml. The dosage of *P. fumosoroseus* that corresponds with 50% mortality of *D. noxia* (1.78×10^3 conidia/cm^2) compares favorably with that of Feng & Johnson (1990) and Feng et al. (1990b) for *B. bassiana*. In their assays, the aphids came into direct contact with suspensions whereas in those reported here less than 2% of the inoculum in the suspensions reached the Petri plate at the bottom of the spray tower. A more precise comparison requires evaluation of the two fungi under identical conditions.

Knudsen et al. (1990, 1991) obtained further improvement in *B. bassiana* activity against cereal aphids through formulation that improved application, growth and sporulation of the fungus. Operational research with these objectives for *P. fumosoroseus* is also warranted.

The immersion method for exposing aphids to fungal inoculum does not permit a precise method of measuring the amount of conidia actually coming into contact with the aphids unless a sample of the treated insects is washed and the spores in the resultant suspension are counted. The bioassay method and system described herein, permit calculation of conidia per square area and provide assay conditions in which humidity is not unrealistically high.

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