Susceptibility of the Convergent Lady Beetle (Coleoptera: Coccinellidae) to Four Entomogenous Fungi

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ABSTRACT Many entomogenous fungi used as biological control agents of insect pests have broad host ranges and may infect nontarget organisms, potentially causing unanticipated environmental effects. We tested the susceptibility of a predatory beetle, *Hippodamia convergens* Guérin-Méneville, to five entomogenous fungi, all of which are being considered or used for pest control; *Metarhizium anisopliae, Paecilomyces fumosoroseus, Nomuraea rileyi,* and two strains of *Beauveria bassiana.* First-instar beetle larvae were exposed in laboratory bioassays to five concentrations of fungal preparations ranging from 10^4 to 10^8 conidia/ml. *M. anisopliae* caused up to 97% mortality, an aphid-derived strain of *B. bassiana* caused up to 95% mortality, a beetle-derived strain of *B. bassiana* caused up to 75% mortality, and *P. fumosoroseus* caused up to 56% mortality. The rate of mortality was dependent on exposure concentration. No infection was detected with *N. rileyi.* We conclude that *M. anisopliae, B. bassiana,* and *P. fumosoroseus* have the potential to infect *H. convergens* if used in crops where this predator occurs, whereas *N. rileyi* does not. The original host species of the fungi did not indicate their degree of virulence toward the beetle, probably because most of the fungi tested have very broad host ranges in general.

KEY WORDS *Hippodamia convergens,* microbial pesticides, nontarget effects of pesticides

MANY ENTOMOGENOUS FUNGI have wide host ranges, and the possibility for nontarget effects should be carefully tested before their widespread use to avoid disruption of ecological systems that currently provide biological control of potential pests. Both the U.S. Environmental Protection Agency (EPA) and the International Organization for Biocontrol have developed guidelines for registration of entomogenous fungi that include testing the susceptibility of nontarget predacious and parasitic invertebrates (Hall et al. 1982, Pesticides Testing Guidelines 1989). As part of a project to develop testing methods for the EPA, we tested the susceptibility of the coccinellid *Hippodamia convergens* Guérin-Méneville to the following five fungi: (1) a beetle-derived strain of *Beauveria bassiana* (Balsamo) Vuillemin (ARSEF 252), (2) an aphid-derived strain of *B. bassiana* (ARSEF 2883), (3) *Metarhizium anisopliae* (Metschnikoff) Sorokin (strain ARSEF 683, originally isolated from a Scarabaeidae larva), (4) *Nomuraea rileyi* (Farlow) Samson (strain ARSEF 323, originally isolated from *Spodoptera*), and (5) *Paecilomyces fumosoroseus* (Wize) Brown & Smith (strain ATCC 20874, patented to control whiteflies, mealybugs, thrips, and mites). All these fungi were obtained from the U.S. Department of Agriculture's Collection of Entomopathogenic Fungal Cultures, Ithaca, NY, except for *P. fumosoroseus* which was obtained from Fred Genthner, EPA Environmental Research Laboratory, Gulf Breeze, FL.

All of these fungi are being considered for use as pest control agents. Interest in fungi for this purpose is, in part, due to the hardiness of conidia, which are infective. *Paecilomyces* and *B. bassiana* conidia have been shown to remain active in soil for 2–3 yr and *M. anisopliae* for at least 40 d (McCoy et al. 1988). Survival above the soil varies greatly. For example, Daoust & Pereira (1986) found *B. bassiana* conidia survived for at least 16 wk in host cadavers under protected conditions but for only 2 wk when exposed to sunlight and rain. In an alfalfa field, we were able to recover 10^6 *B. bassiana* conidia/g of plant (dry biomass) 28 d after it was treated at a rate of 10^7 conidia per g of plant (R.R.J. & B. L., unpublished data). This constitutes a 90% loss in viability; however, a large number of spores were still present. The long-term persistance of some conidia increases the risk of nontarget insects to these fungal pathogens.

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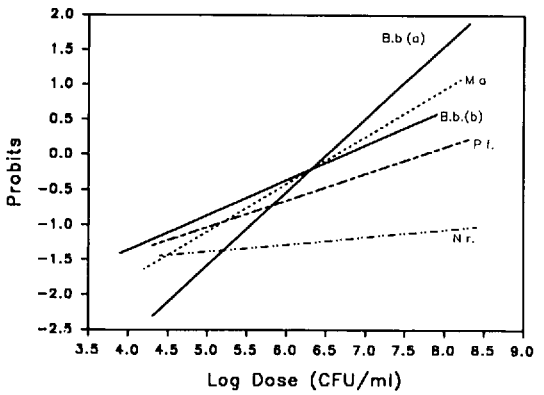

Fig. 1. Fitted probit models for mortality of neonate *Hippodamia convergens* exposed to different concentrations of conidia of B.b. (a), *Beauveria bassiana* ARSEF 2883; B.b. (b), *B. bassiana* ARSEF 252; M.a., *Metarhizium anisopliae;* P.f., *Paecilomyces fumosoroseus;* and N.r., *Nomuraea rileyi.*

H. convergens was selected as a nontarget test insect because it is a widespread and abundant predator of aphids and other Homoptera in many agricultural settings and thus has the potential to be exposed to these fungi if they were used for pest control in field crops.

Materials and Methods

To obtain conidia for the tests, *B. bassiana*, *M. anisopliae*, and *N. rileyi* were cultured on Sabouraud Maltose Agar with Yeast (Difco Laboratories, Detroit, MI); *P. fumosoroseus* was cultured on Potato Dextrose Agar (BBL Microbiological Systems, Cockeysville, MD). With the exception of *B. bassiana* ARSEF 252, conidia had been collected by scraping them off the top of 4-wk-old cultures the day before the experiment. *B. bassiana* ARSEF 252 conidia were previously collected and stored dry at −80°C for 10 mo. Dry conidia were suspended in sterile deionized water using a tissue grinder. The conidial preparations were diluted to obtain the following exposure concentrations: 10^4, 10^5, 10^6, 10^7, and 10^8 conidia/ml. Conidia viability was determined by plate count.

Insects were obtained from laboratory cultures as indicated previously by James & Lighthart (1992). To obtain large numbers of eggs and synchronize their hatch, eggs were collected daily and stored at 12°C. Eggs hatched after 3 d incubation at 25°C; no eggs older than 1 wk were used. Neonate larvae were placed in 20-ml glass scintillation vials with foam stopper tops and fed live pea aphids. Larvae were then placed at 25°C, 70% RH, and 14:10 (L:D) h for 24 h just before the experiment. Individual larvae were dipped for 10 s in one of the conidial suspensions or in sterile deionized water as a control. A vortex mixer was used to keep the conidia in suspension throughout the treatment period. Forty larvae were used per treatment for a total of 200 larvae per fungus.

Larvae were incubated at 96% RH just after being exposed to the fungus to allow proper conditions for germination of the conidia and after 72 h they were transferred to 70% RH to minimize the detrimental effects of humidity on their viability. Mortality was recorded every 24–48 h for 10 d. The statistics package SAS was used to construct probit models for each fungus to determine the effect of conidia concentration on mortality. Abbott's adjustment (Abbott 1925) was used in the probit analysis because some mortality was caused by exposure to high humidity.

Results and Discussion

M. anisopliae, *P. fumosoroseus*, and the two strains of *B. bassiana*, were found to be pathogenic towards *H. convergens*. However, *N. rileyi* had very little effect on larval mortality (Fig. 1). The LC_{50}s for *M. anisopliae* and both strains of *B. bassiana* were similar, but the slope of the probit line for the aphid-derived strain of *B. bassiana* (ARSEF 2883) was much greater (Table 1). Thus, a given increase in exposure rate led to a greater increase in mortality for this strain. However, it also means that this strain caused less mortality at low exposure rates but greater mortality at high exposure rates (Fig. 1). Mortality associated with exposure to *P. fumosoroseus* was not as high as for *M. anisopliae* and *B. bassiana*.

Table 1. Probit model parameters for tests of the effect of fungal conidia concentration (CFU per ml) on the mortality rate of neonate *Hippodamia convergens*

Fungus	Log LC_{50} (limits)[a]	Slope ± SEM	y-Intercept ± SEM	Model χ^2
Metarhizium anisopliae	6.62 (5.43–8.48)	0.68 ± 0.16	−4.47 ± 1.06	10.9[b]
Beauveria bassiana ARSEF 2883	6.51 (5.93–6.86)	1.04 ± 0.21	−6.80 ± 1.49	3.9
Beauveria bassiana ARSEF 252	6.73 (6.33–7.23)	0.50 ± 0.08	−3.34 ± 0.47	0.7
Paecilomyces fumosoroseus	7.73 (7.18–8.66)	0.38 ± 0.07	−2.90 ± 0.48	2.4
Nomuraea rileyi	18.60 —[d]	0.10 ± 0.17	−1.88 ± 1.15	12.1[c]

[a] 95% fiducial limits.
[b] All variances and covariances were multiplied by a heterogeneity factor of 3.63 because the χ^2 was large ($P = 0.012$).
[c] All variances and covariances were multiplied by a heterogeneity factor of 4.02 because the χ^2 was large ($P = 0.007$).
[d] No fiducial limits could be calculated because of the poor fit of the probit model.

The host species from which a fungus was isolated did not indicate its degree of virulence toward the nontarget organism. *M. anisopliae, P. fumosoroseus,* and *B. bassiana* are all known to have very broad host ranges (Goettel et al. 1990, Osborne et al. 1990), and it may be more difficult to predict the virulence of such pathogens toward a particular nontarget organism on the basis of phylogenetic relationships between target and nontarget organisms. *B. bassiana, M. anisopliae,* and *P. fumosoroseus* all show some degree of virulence toward *H. convergens* and possibly other coccinellids as well. Magalhaes et al. (1988) found *Coleomegilla maculata* and *Eriopis connexa* to also be highly susceptible to *B. bassiana.* However, further research is needed to determine how direct effects detected in the laboratory play out in the field where multiple species interact.

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References Cited

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

Daoust, R. A. & R. M. Pereira. 1986. Survival of *Beauveria bassiana* (Deuteromycetes: Moniliales) conidia on cadavers of cowpea pests stored outdoors and in laboratory in Brazil. Environ. Entomol. 15: 642–647.

Goettel, M. S., T. J. Poprawski, J. D. Vandenberg, Z. Li & D. W. Roberts. 1990. Safety to non-target invertebrates of fungal biocontrol agents, pp. 209–223. *In* M. Laird, L. A. Lacey & E. W. Davidson [eds.], Safety of microbial insecticides. CRC, Boca Raton, FL.

Hall, R. A., G. Zimmermann & A. Vey. 1982. Guidelines for the registration of entomogenous fungi as insecticides. Entomophaga 27: 121–127.

James, R. R. & B. Lighthart. 1992. Protocol for testing the effects of fungal pesticides on non-target beetles using *Hippodamia convergens.* National Technical Information Service publication no. PB92–217–488. Springfield, VA.

Magalhaes, B. P., J. C. Lord, S. P. Wraight, R. A. Daoust & D. W. Roberts. 1988. Pathogenicity of *Beauveria bassiana* and *Zoophthora radicans* to the coccinellid predators *Coleomegilla maculata* and *Eriopis connexa.* J. Invertebr. Pathol. 52: 471–473.

McCoy, C. W., R. A. Samson & D. G. Boucias. 1988. Entomogenous fungi, pp. 151–236. *In* C. M. Ignoffo [ed.], CRC handbook of natural pesticides, vol. 5. Microbial insecticides, part A entomogenous protozoa and fungi. CRC, Boca Raton, FL.

Osborne, L. S., G. K. Storey, C. W. McCoy & J. F. Walter. 1990. Potential for controlling the sweetpotato whitefly, *Bemsia tabaci,* with the fungus *Paecilomyces fumosoroseus,* pp. 386–390. *In* Fourth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, 20–24 August 1990. Society of Invertebrate Pathology, Bethesda, MD.

Pesticide Testing Guidelines, Subdivision M. 1989. U.S. Dept. of Commerce, National Technical Information Service no. PB89–211–676. Springfield, VA.

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