NOTE

In Vitro Germination and Growth Response of Two Entomogenous Fungi to Imidacloprid[1]

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Synergistic interactions between the insecticide imidacloprid and the conidia of entomopathogenic fungi have been detected in several insects, including the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar), the German cockroach, *Blatella germanica* (L.), the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), and the root weevil *Diaprepes abbreviatus* (L.) (Boucias et al., 1996, Plfanzenshutz-Nachr. Bayer 44: 113–136; Kaakeh et al., 1997, J. Econ. Entomol. 90: 473–482; Steinkraus and Tugwell, 1997, J. Entomol. Sci. 32: 79–90; Quintela and McCoy, 1997, Environ. Entomol. 26: 1173–1182). The synergism typically manifests itself in mycosis incidence and mortality levels elevated above those obtained following exposure to the insecticide or the fungal conidia alone. Boucias et al. (1996) indicated that the synergistic interaction detected in termites was due to decreased social grooming activities in treated termites, an activity that effectively removes fungal conidia from the insect cuticle. The study reported herein was undertaken to further delineate the possible causes of the observed synergistic interactions through *in vitro* assessments of the effects of imidacloprid on the germination of the conidia and growth of mycelia of two entomopathogenic fungi—*Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin.

Imidacloprid used in these *in vitro* assays was provided by Bayer Corporation (Kansas City, MO) as technical grade (BAY NTN 33893, 95.8%, Batch No. 17129-90). *Beauveria bassiana* used in the assays was originally isolated from a pecan weevil, *Curculio caryae* (Horn), adult in Georgia. The *M. anisopliae* was isolated from *D. abbreviatus* in Florida and was designated MADA strain. Conidia of the two fungi were harvested from cultures produced on Sabouraud's dextrose agar (SDA) fortified with yeast extract (0.5%). Harvested conidia were stored in liquid nitrogen until used in the assays.

Assay methods were modified from those of Gardner and Storey (1985, J. Econ.

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Table 1. *In vitro* response of *B. bassiana* and *M. anisopliae* conidial germination and mycelial growth to imidacloprid.*

Fungus	Concn (ppm)	Number of conidia germinated per 100	Dry mycelial weight (mg)
B. bassiana	0	85.1 ± 1.6	119.3 ± 15.9
	0.001	83.9 ± 3.4	108.8 ± 10.4
	0.01	84.3 ± 3.0	119.4 ± 9.1
	0.1	87.0 ± 2.4	123.2 ± 18.4
	1.0	79.5 ± 5.6	124.5 ± 9.6
	10.0	83.0 ± 2.8	112.8 ± 5.2
	100.0	86.0 ± 1.8	121.7 ± 4.9
M. anisopliae	0	87.9 ± 1.8	209.5 ± 18.3
	0.001	86.6 ± 3.4	183.8 ± 22.6
	0.01	87.7 ± 3.2	168.2 ± 23.9
	0.1	85.6 ± 1.9	175.4 ± 31.5
	1.0	89.0 ± 2.1	160.7 ± 19.2
	10.0	78.0 ± 6.1	164.5 ± 21.4
	100.0	87.9 ± 2.3	144.2 ± 28.4

*Response expressed in means ± SEM. No significant difference detected with General Linear Models procedure of the Statistical Analysis System.

Entomol. 78: 1275–1279) in their *in vitro* assays of the sensitivity of *B. bassiana* to selected herbicides. In each of the current assays, a stock solution of imidacloprid was prepared, serially diluted, and filtered through a sterile Millex®-HA 0.45 µm filter unit (Millipore Corp., Bedford, MA) into either autoclaved liquefied agar (SDA) held at a constant 60°C in a water bath or autoclaved broth (SDB) cooled to room temperature for the respective assays of conidial germination and mycelial growth. The imidacloprid was dispensed so as to achieve final concentrations of 0.001, 0.01, 0.1, 1.0, 10.0 and 100.0 ppm in the agar and the broth. The agar/imidacloprid mixtures were poured into Petri dishes measuring 35 × 10 mm (10 mls per dish) and allowed to solidify by cooling to room temperature. The broth/imidacloprid mixtures were dispensed into 125-ml Erlenmeyer flasks (50 mls per flask). Appropriate controls were established using mixtures of sterile distilled water and either agar or broth.

Conidial suspensions of each fungus were prepared in sterile distilled water and Tween® 80 (Fisher Chemical, Fair Lawn, NJ) at 0.01% (v/v) to a final concentration of 10^6 conidia per ml for the germination assays and 10^7 conidia per ml for the growth assays. Aliquots of 0.05 ml each of the conidial suspension containing 10^6 conidia per ml were drop plated onto the agar in the Petri dishes. These dishes were incubated at 27°C. One day later, 100 conidia on each plate were microscopically examined. Numbers of ungerminated and germinated conidia were recorded with only those conidia producing germ tubes being scored as germinated. Similarly, 0.1-ml aliquots

of the conidial suspension containing 10^7 conidia per ml were dispensed into each of the Erlenmeyer flasks containing the broth mixtures creating a final conidial concentration of 2×10^6 conidia per ml. The flasks were incubated on a rotary shaker at 27°C for 7 d. After incubation, the contents of each flask were filtered by vacuum. Mycelial dry weights were obtained by oven drying and weighing the filtrates.

The assays were designed so that each concentration of imidacloprid was replicated five times within each assay, and each assay was repeated 10 times for *B. bassiana* and eight times for *M. anisopliae*. Germination response and mycelial growth response were analyzed separately for each fungus using the General Linear Models procedure of the Statistical Analysis System (SAS Institute Inc., 1985, Cary, NC).

No significant differences in the *in vitro* response of either conidial germination or mycelial growth of the two fungi to the imidacloprid concentrations ranging between 0.001 and 100 ppm were detected (Table 1). These results demonstrate that the active ingredient (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) in imidacloprid commercial formulations does not enhance the conidial germination or the mycelial growth of either *B. bassiana* and *M. anisopliae in vitro* at these concentrations and, thus, apparently is not responsible for the reported synergism between the insecticide and entomogenous fungi against certain insects. These findings, however, do not preclude the possibility that inert components of commercial formulations can enhance germination, as was indicated by Quintela (1996, Ph.D. Diss., Univ. Florida), or growth of entomogenous fungi.

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