

## Imidacloprid-Enhanced *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) Susceptibility to the Entomopathogen *Metarhizium anisopliae*

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**ABSTRACT** The effects of imidacloprid and the entomopathogen *Metarhizium anisopliae* (Metsch.) Sorokin on the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), were evaluated in a  $4 \times 3$  factorial experiment in both sterile and nonsterile loam soil. Termites were not susceptible to *M. anisopliae* when assays were conducted in nonsterile soil but were highly susceptible in sterile soil. Termite mortality after 21 d of continuous exposure to  $10^4$  conidia per gram of soil was zero and 41.6% in nonsterile and sterile soil, respectively. Termites were significantly more susceptible to sterile soil containing  $10^7$  conidia per gram than to the same soil containing  $10^4$  conidia per gram. In continuous exposure assays, termites were highly susceptible to imidacloprid-treated (5, 10, and 20 ppm) nonsterile and sterile soil containing no experimentally introduced *M. anisopliae*. Exposure of termites to imidacloprid enhanced their susceptibility to introduced *M. anisopliae* in nonsterile and sterile soil. Native entomopathogens recovered from termites exposed to imidacloprid-treated, nonsterile soil (i.e., no introduced *M. anisopliae*) included *Conidiobolus coronatus* (Constantin) Batko, *Cunninghamella echinulata* Thaxter, *Fusarium* spp., *Aspergillus* spp., and a naturally occurring strain of *M. anisopliae* variety *majus*.

**KEY WORDS** *Reticulitermes*, *Metarhizium anisopliae*, termites, imidacloprid, biological control, mycopathogen

THE CHLORONICOTINYL IMIDACLOPRID (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) interferes with postsynaptic acetylcholine receptors in the insect nervous system, resulting in an agonistic action at nicotinic acetylcholine receptors (Schroeder and Flattum 1984). Imidacloprid possesses excellent systemic properties and is effective against a broad range of agricultural pests (Elbert et al. 1990, 1991). The performance of imidacloprid in agricultural pest control has prompted its use in the control of urban pests such as termites (Boucias et al. 1996, Gatti and Henderson 1996), cockroaches (Kaakeh et al. 1997), fleas (Arther et al. 1997), and ants (Klotz and Reid 1993). Imidacloprid's development, mode of action, and current use patterns were reviewed by Leicht (1996).

In recent decades, many researchers have investigated the potential use of entomopathogens as microbial control agents for insects (Tanada and Kaya 1993). Much of this research has focused on the use of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium*

*anisopliae* (Metsch.) Sorokin. To date, the majority of work evaluating *M. anisopliae* for the biological control of insects has focused on agriculturally important pests (Zimmerman 1993). Grace (1997) provided a review of the biological control of termites and concluded that microbes, primarily entomopathogenic fungi, provide some potential in the biological control of Isoptera. A great deal of laboratory data are available on the efficacy of fungal pathogens for termite control, but very few field efficacy data currently exist. Application of very large quantities of 1 strain of *M. anisopliae* conidia directly into the nursery area of a mound building termite, *Coptotermes acinaciformis* (Froggatt), was successful in reducing termite populations (Milner and Staples 1996, Milner et al. 1998).

The concept of using a chemical stressor to enhance the efficacy of entomopathogens is not novel, yet limited data exist on this pest control tactic (Hassan and Charnley 1987, 1989). Boucias et al. (1996) reported that application of imidacloprid in a bait significantly enhanced the susceptibility of field-collected eastern subterranean termites, *Reticulitermes flavipes* (Kollar), to *B. bassiana*. Quintela and McCoy (1997, 1998) and Steinkraus and Tugwell (1997) likewise demonstrated imidacloprid-enhanced susceptibility to entomopathogens in the citrus root weevil, *Diaprepes abbreviatus* (L.), and the tarnished plant

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bug, *Lygus lineolaris* (Palisot de Bequvois), respectively. The objectives of our research were to document the individual and combined effects of commercially available imidacloprid (i.e., Premise 75 WP [wetttable powder]) and *M. anisopliae* (i.e., Bio-Blast Biological Termiticide) on *R. flavipes* mortality in both sterile and nonsterile loam soil.

### Materials and Methods

**Termites.** Termite traps consisted of polyvinyl chloride tubes (15 by 10 cm in diameter) buried in the ground with the top  $\approx 2$  cm below grade. A roll of moistened, corrugated cardboard was placed inside the tube and the tube was capped and covered with soil. Traps were checked weekly, termites collected, and the traps rebaited. Trapped termites were placed in plastic boxes (4.2 liter), returned to the laboratory, provided fresh, moistened cardboard, and held at 26°C until ready to use. Soldier termites were collected and identified as *R. flavipes* (N. Hostettler, personal communication). Termites used in this study were collected from a single trap in West Lafayette, IN, and at the time of assay had been in the laboratory for  $\leq 14$  d.

**Fungi and Termiticide.** *Metarhizium anisopliae* (strain ESC 1) was obtained from EcoScience Corporation (East Brunswick, NJ) as Bio-Blast Biological Termiticide (for review, see Quarles 1997). Each packet of Bio-Blast contained a minimum of  $4 \times 10^9$  viable conidia per gram of formulated product. Bio-Blast packages with the same lot number were used to minimize differences in conidia viability and concentration. Imidacloprid was obtained from Bayer Corporation (Kansas City, MO) as Premise 75 WP.

**Soil Collection and Sterilization.** Soil used in this study was collected from a turf plot on the Purdue Agronomy Research Center (West Lafayette, IN). Soil analysis (Midwest Laboratories, Omaha, NE) classified it as loam, with a structural composition of 36% sand, 40% silt, and 24% clay. The soil had a pH of 7.7, a cation exchange capacity of 17.8 meq/100 g, and an organic matter content of 2.8%. In previous years the plot was mowed (10 cm) weekly and received only natural rainfall. In September 1996 a fertilizer (N-P-K; 24-4-12) was applied at the rate of 0.454 kg/92.9 m<sup>2</sup>, and in the fall of 1993 and 1994 was sprayed with Trimec Classic to control broadleaf weeds. The plot had not been recently exposed to insecticides.

Soil was collected in the late afternoon, when top-soil moisture content was least (R.R., unpublished data) and if it had not rained during the previous 24 h. The top 7.5 cm of soil, beginning at the root zone, was collected from the turf plot by using a cup corer (10 cm in diameter), returned to the laboratory, and immediately divided in half; one-half was used in assays the day of its collection (nonsterile soil assays), whereas the other half was used 1 d later, after its sterilization (sterile soil assays). Soil was sterilized for 24 h with gamma irradiation ( $4.6 \times 10^6$  kilorads) by using a category I irradiator (Nordian International, Kanata, Ontario, Canada) (ANSI standard N433.1)

equipped with a cobalt 60 source with a half-life of 5.271 yr.

**Experimental Design and Assay.** In nonsterile and sterile soil, 2 factors were simultaneously evaluated in a  $4 \times 3$  factorial experiment. The quantitative factor imidacloprid was evaluated at 4 levels (0, 5, 10, and 20 ppm [wt:wt] imidacloprid in fresh soil), whereas the quantitative factor *M. anisopliae* was evaluated at 3 levels (0,  $10^4$ , and  $10^7$  conidia per gram of fresh soil); concentrations of both imidacloprid and *M. anisopliae* used were based on the weight of oven-dried soil. Each of the 12-factor-level combinations was replicated 3 times in each of 4 trials (1 trial per week during May 1997). Fresh soil was used for each trial.

For each factor-level combination, Premise 75 WP, Bio-Blast, or both was added to a beaker containing  $\approx 10$  ml of sterilized water (autoclaved for 30 min at 20 kg/cm<sup>2</sup> and 121°C). The beaker contents were then added to 100 g of fresh soil, providing a final soil moisture content of  $\approx 20\%$  because fresh soil contains native water. After mixing, the treated soil was then divided evenly among 3 glass petri dishes (60 by 15 mm) so that each dish was  $\approx 2/3$  full. Then, 25 *R. flavipes* workers were placed on the soil and a small piece of moistened cardboard was added as a source of food. Dishes were sealed with parafilm, wrapped in aluminum foil, and stored at 26°C. After 3, 7, 10, 14, and 21 d of incubation, each dish was opened and the number of live termites was counted and recorded; dead termites were removed from the dish and discarded. After counting, dishes were resealed and returned to the incubator.

**Identification of Native Entomopathogens.** Twenty-five dead termites were collected randomly from dishes containing imidacloprid-treated, nonsterile soil with and without introduced *M. anisopliae*. After they were completely dry, or when there was visible fungal growth, termites were individually sealed in 1.5-ml centrifuge tubes and sent to the USDA-ARS, Plant Protection Research Unit (Ithaca, NY) for fungal identification.

**Statistical Analysis.** Data across the 4 trials were pooled before analysis. The proportion dead was transformed by arcsine square root because mortality ranged from zero to 100% (Agresti 1990). For each soil type, a two-way analysis of variance (ANOVA) of the transformed response was conducted for each day that data were collected. After each two-way ANOVA, the percentage of variation in the data explained by each variable was calculated by dividing the sum of squares for each variable by the total sum of squares from the ANOVA table.

For each soil type, each factor was analyzed by day at fixed levels of the other factor (one-way ANOVA). Transformed means were separated with the Tukey honestly significant difference (HSD) and converted back to percentages. The SAS procedures were used in all statistical analyses (SAS Institute 1985, Schlotzhauer and Littell 1987).

Results

In both nonsterile (Table 1) and sterile (Table 2) loam soil, each main effect (*M. anisopliae* and imidacloprid) was highly significant; the interaction of these 2 variables was not significant in nonsterile and sterile soil until 10 and 14 d after exposure, respectively. In both soils, imidacloprid accounted for more of the variability in mortality than did *M. anisopliae*, the interaction or the error term (Fig. 1). In nonsterile soil, the interaction term and the main effect *M. anisopliae* each accounted for <10% of the variation in the response in each analysis, whereas the variation explained by imidacloprid increased with each successive analysis; by day 21, imidacloprid accounted for 90.5% of the total variation in the response (Fig. 1A) in nonsterile soil. Likewise, in sterile soil imidacloprid accounted for more variation than any of the other variables, but peaked at day 10 (71.8%), then declined, and by day 21 accounted for only 45% of the total variation. This decrease was due mainly to the increase in variation accounted for by the interaction of the 2 variables (Fig. 1B).

**Influence of Fungi on Termite Mortality.** In nonsterile soil, continuous exposure of termites to *M. anisopliae* alone resulted in only minimal mortality. After 21 d, mortality of termites exposed to the highest rate of *M. anisopliae* was 0.50% (Table 3). In contrast, continuous exposure of termites to low and high rates of *M. anisopliae* alone in sterile soil resulted in 41.6 and 100% mortality, respectively, after 21 d (Table 4).

Table 1. Two-way ANOVA, by day, of the effect of *M. anisopliae* and imidacloprid on mortality of *R. flavipes* workers in nonsterile soil

Day	Source	Sum of squares	Mean square	F	P
3	Ma	9,612.5	4,806.3	8.6	0.0003
	Imid	16,051.6	5,350.5	9.5	0.0001
	Ma*Imid	5,522.9	920.5	1.6	0.1415
	Error	74,174.0	561.9		
	Total	105,361.0			
7	Ma	22,147.8	11,073.9	12.9	0.0001
	Imid	80,732.8	26,910.9	31.3	0.0001
	Ma*Imid	9,465.2	1,577.5	1.8	0.0967
	Error	113,403.5	859.1		
	Total	225,749.3			
10	Ma	10,615.4	5,307.7	11.1	0.0001
	Imid	157,133.4	52,377.8	109.6	0.0001
	Ma*Imid	12,736.8	2,122.8	4.4	0.0004
	Error	63,097.9	478.0		
	Total	243,583.4			
14	Ma	4,990.1	2,495.1	8.8	0.0002
	Imid	188,149.7	62,716.6	222.2	0.0001
	Ma*Imid	12,202.8	2,033.8	7.2	0.0001
	Error	37,252.3	282.2		
	Total	242,595.0			
21	Ma	1,159.0	579.5	4.4	0.0137
	Imid	200,948.0	66,982.7	512.1	0.0001
	Ma*Imid	2,648.4	441.4	3.4	0.0040
	Error	17,266.4	130.8		
	Total	222,021.7			

For each two-way ANOVA, df = 2, 3, 6, 132, and 143 for Ma, Imid, Ma\*Imid, Error, and Total, respectively. Ma, *M. anisopliae*; Imid, imidacloprid.

Table 2. Two-way ANOVA, by day, of the effect of *M. anisopliae* and imidacloprid on mortality of *R. flavipes* workers in sterile soil

Day	Source	Sum of squares	Mean square	F	P
3	Ma	5,533.4	2,766.7	7.0	0.0012
	Imid	17,781.5	5,927.2	15.0	0.0001
	Ma*Imid	3,716.9	619.5	1.6	0.1591
	Error	51,903.1	393.2		
	Total	78,934.9			
7	Ma	46,265.0	23,132.5	28.9	0.0001
	Imid	88,434.2	29,478.1	36.9	0.0001
	Ma*Imid	7,157.0	1,192.8	1.5	0.1855
	Error	105,500.6	799.2		
	Total	247,356.8			
10	Ma	11,995.6	5,997.8	18.0	0.0001
	Imid	153,191.4	51,063.8	152.9	0.0001
	Ma*Imid	4,021.7	670.3	2.0	0.0691
	Error	44,092.7	334.0		
	Total	213,301.4			
14	Ma	8,960.5	4,480.2	15.0	0.0001
	Imid	131,821.2	43,940.4	147.4	0.0001
	Ma*Imid	10,925.0	1,820.8	6.1	0.0001
	Error	39,338.7	298.0		
	Total	191,045.4			
21	Ma	12,716.2	6,358.1	37.8	0.0001
	Imid	58,135.3	19,378.4	115.2	0.0001
	Ma*Imid	36,102.8	6,017.1	35.8	0.0001
	Error	22,208.6	168.2		
	Total	129,163.0			

For each two-way ANOVA, df = 2, 3, 6, 132, and 143 for Ma, Imid, Ma\*Imid, Error, and Total, respectively. Ma, *M. anisopliae*; Imid, imidacloprid.

In imidacloprid-treated nonsterile soil, mortality was highest in termites exposed to the highest rate of *M. anisopliae* (Table 3). After 7 d, termites concurrently exposed to any quantity of imidacloprid and the highest rate of fungi was 90.4–96.1%. After 10 d, termites exposed to either 10 or 20 ppm of imidacloprid and the low rate of fungi was 98.1 and 99%, respectively; after 14 and 21 d, mortality of termites concurrently exposed to 5 ppm of imidacloprid and the low rate of fungi was 79 and 100%, respectively. Similar results were found in sterile soil (Table 4). After 7 d, mortality in termites concurrently exposed to imidacloprid and the highest rate of fungi was 100%, and after 10 d, termites concurrently exposed to imidacloprid and the low rate of fungi was from 90.8 to 100%.

**Influence of Imidacloprid on Termite Mortality.** Mortality of termites exposed to imidacloprid-treated, nonsterile soil was highest in treatments containing the most imidacloprid (Table 3). One hundred percent mortality of termites exposed to either 20 or 10 ppm of imidacloprid occurred after 10 and 14 d, respectively; after 21 d, mortality of termites exposed to 5 ppm was 86.5%. The trend in sterile soil was similar (Table 4); 95.7% mortality of termites exposed to 20 ppm of imidacloprid occurred after 10 d. Mortality of termites exposed to 5 and 10 ppm was 92.1 and 96.4%, respectively, after 14 d. By 21 d, all termites exposed to imidacloprid-treated sterile soil were dead.

In both nonsterile and sterile soils, on any day of sampling, termite mortality was highest in those groups of termites concurrently exposed to the highest

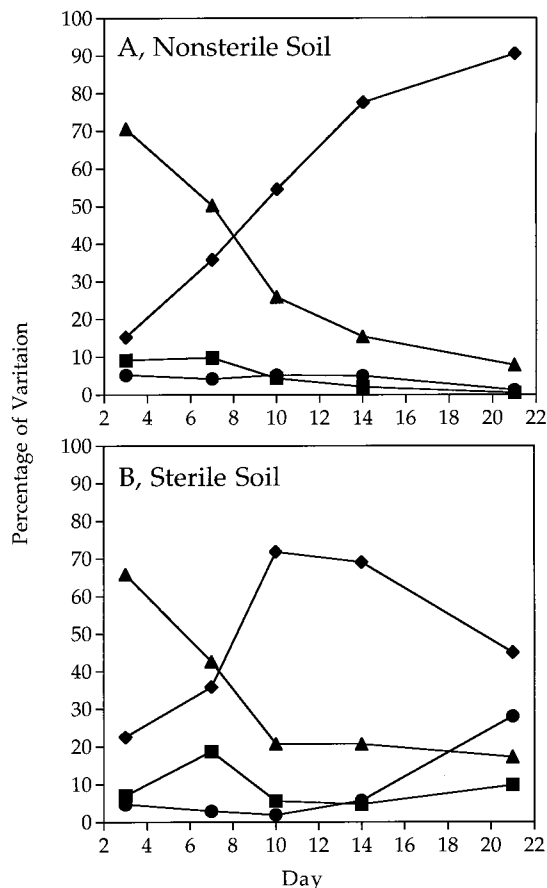


Fig. 1. Percentage of variation in percent mortality explained by the treatments *M. anisopliae* (■), imidacloprid (◆), their interaction (●), and experimental error (▲) in 4 × 3 factorial experimental assays conducted in (A) nonsterile and (B) sterile soil. Percentages were obtained by dividing the sum of squares for each variable by the total sum of squares in the appropriate ANOVA table (Tables 1 and 2 for nonsterile and sterile analyses, respectively).

rates of imidacloprid and both rates of *M. anisopliae*. In nonsterile soil (Table 3), at the highest rate of fungi, differences among rates of imidacloprid in termite mortality were manifested after just 3 d. From day 7 to 21, mortality of termites concurrently exposed to the highest rate of fungi and any rate of imidacloprid were not significantly different but were significantly higher than the control (i.e., highest rate of fungi and 0 ppm imidacloprid). At the low rate of fungi, differences among rates of imidacloprid in termite mortality were not apparent until after 7 d of continuous exposure. From day 7 to 21, mortality of termites concurrently exposed to the low rate of fungi and any rate of imidacloprid was significantly higher than that of the control (i.e., low rate of fungi and 0 ppm of imidacloprid); termites exposed to either 10 or 20 ppm of imidacloprid and the low fungi rate exhibited significantly higher mortality than that of termites concurrently exposed to the low fungi rate and 5 ppm of

imidacloprid. By day 21, mortality of termites exposed to the low rate of fungi and any rate of imidacloprid was not different.

The trends in sterile soil (Table 4) were similar to those in nonsterile soil. Differences in termite mortality among rates of imidacloprid were manifested after just 3 d for cohorts exposed to either rate of fungi. From day 7 to 21, termite mortality among rates of imidacloprid application was not significantly different for either rate of fungi.

**Fungi-Imidacloprid Combination.** The combination treatment of imidacloprid and *M. anisopliae* played an important role in the mortality of *R. flavipes*. In both nonsterile (Table 3) and sterile (Table 4) soils, imidacloprid enhanced termite susceptibility to introduced *M. anisopliae*. In both soils, mortality of termites concurrently exposed to *M. anisopliae* and imidacloprid was higher than mortality of termites exposed to either treatment alone. Furthermore, at each concentration of imidacloprid, the difference was more pronounced in those termites exposed to the highest rate of *M. anisopliae*. After 10 d of continuous exposure in nonsterile soil, mortality of termites concurrently exposed to the highest rate of *M. anisopliae* and 5 ppm imidacloprid (96.5% mortality) was significantly higher than that of termites exposed to either the highest rate of *M. anisopliae* alone (0.10% mortality) or 5 ppm imidacloprid alone (20.3% mortality) (Table 3); mortality of termites concurrently exposed to the low rate of *M. anisopliae* and 5 ppm imidacloprid was 38.2%.

**Native Entomopathogens.** Fungi identified from termite cadavers exposed to imidacloprid-treated nonsterile soil included *Conidiobolus coronatus* (Constantin) Batko, *Cunninghamella echinulata* Thaxter, *Fusarium* spp., *M. anisopliae* variety *majus*, *M. anisopliae* variety *anisopliae* (Bio-Blast), and *Aspergillus* spp. *M. anisopliae* variety *majus* was identified from 2 termites exposed to only imidacloprid-treated soil, indicating that this was a naturally occurring strain.

## Discussion

Mortality was higher in termites exposed to *M. anisopliae* introduced into sterilized soil (Table 4) than in termites exposed to *M. anisopliae* introduced into freshly collected, nonsterile soil (Table 3) that contained native microbes. The evidence provided herein, and supported by additional literature (Cook et al. 1995, Inglis et al. 1998), indicates that native soil microbes successfully competed with introduced *M. anisopliae* and rendered it less effective. Mortality of female grasshoppers was higher in individuals ovipositing into sterile sandy-loam and clay-loam soils amended with *B. bassiana* than in soils treated similarly but that were nonsterile (Inglis et al. 1998). In their study, of 27 species of fungi identified from nonsterile soil, the most common taxa were *Fusarium*, *Gliocladium*, *Penicillium*, and *Trichoderma*; the most common species of bacteria were in the genera *Bacillus*, *Paenibacillus*, and *Pseudomonas*. *Pseudomonas* has been studied extensively for its antagonistic ac-

**Table 3.** ANOVA by day, of the effect of *M. anisopliae* at fixed levels of imidacloprid, and the effect of imidacloprid at fixed levels of *M. anisopliae* on *R. flavipes* mortality in nonsterile soil

Day	Ma <sup>a</sup>	Mortality (mean ± SE) <sup>b</sup> at concn. of imidacloprid in soil, ppm				ANOVA results		
		0	5	10	20	F	P	R <sup>2</sup>
3	0	0.0 ± 0.0aB	0.4 ± 0.1aAB	2.5 ± 0.4aAB	10.1 ± 1.8abA	3.3	0.0290	18.3
	10 <sup>4</sup>	0.0 ± 0.0aA	0.3 ± 0.1aA	7.4 ± 2.1aA	5.6 ± 1.9bA	1.6	0.2000	9.9
	10 <sup>7</sup>	0.0 ± 0.0aB	5.7 ± 1.8aAB	38.9 ± 4.6aA	56.1 ± 4.0aA	5.5	0.0027	27.3
		F = 0.5 P = 0.6111 R <sup>2</sup> = 2.9	F = 1.6 P = 0.2267 R <sup>2</sup> = 8.6	F = 3.1 P = 0.0601 R <sup>2</sup> = 15.7	F = 4.1 P = 0.0249 R <sup>2</sup> = 20.0			
7	0	0.0 ± 0.0aB	18.9 ± 3.8bAB	56.4 ± 4.7aA	63.3 ± 3.0abA	6.1	0.0014	29.4
	10 <sup>4</sup>	0.0 ± 0.0aB	19.7 ± 3.8bAB	67.6 ± 2.5aA	41.6 ± 2.9bA	7.0	0.0006	32.1
	10 <sup>7</sup>	0.1 ± 0.1aB	90.4 ± 2.0aA	95.4 ± 1.0aA	96.1 ± 2.0aA	33.2	0.0001	69.4
		F = 1.1 P = 0.3564 R <sup>2</sup> = 6.1	F = 6.6 P = 0.0038 R <sup>2</sup> = 28.6	F = 2.5 P = 0.0967 R <sup>2</sup> = 13.2	F = 4.4 P = 0.0204 R <sup>2</sup> = 21.0			
10	0	0.0 ± 0.0aB	20.3 ± 3.7bB	73.0 ± 3.9aA	99.6 ± 0.2aA	21.8	0.0001	59.7
	10 <sup>4</sup>	0.0 ± 0.0aC	38.2 ± 3.8bB	98.1 ± 0.9aA	99.0 ± 0.3aA	38.2	0.0001	72.2
	10 <sup>7</sup>	0.1 ± 0.1aB	96.5 ± 1.0aA	99.1 ± 0.5aA	100.0 ± 0.0aA	131.6	0.0001	90.0
		F = 1.1 P = 0.3564 R <sup>2</sup> = 6.1	F = 8.1 P = 0.0014 R <sup>2</sup> = 32.8	F = 3.4 P = 0.0439 R <sup>2</sup> = 17.2	F = 1.6 P = 0.2174 R <sup>2</sup> = 8.8			
14	0	0.0 ± 0.0aC	35.5 ± 4.2bB	100.0 ± 0.0aA	99.9 ± 0.1aA	53.7	0.0001	78.5
	10 <sup>4</sup>	0.0 ± 0.0aC	79.0 ± 4.1abB	100.0 ± 0.0aA	100.0 ± 0.0aA	51.1	0.0001	77.7
	10 <sup>7</sup>	0.3 ± 0.1aB	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	3,343.2	0.0001	99.6
		F = 2.0 P = 0.1479 R <sup>2</sup> = 10.9	F = 7.7 P = 0.0018 R <sup>2</sup> = 31.9	F = · P = · R <sup>2</sup> = ·	F = 1.0 P = 0.3788 R <sup>2</sup> = 5.7			
21	0	0.0 ± 0.0bC	86.5 ± 3.8aB	100.0 ± 0.0aA	100.0 ± 0.0aA	57.2	0.0001	79.6
	10 <sup>4</sup>	0.0 ± 0.0abB	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	8,572.5	0.0001	99.8
	10 <sup>7</sup>	0.5 ± 0.1aB	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	2,758.9	0.0001	99.5
		F = 3.3 P = 0.0483 R <sup>2</sup> = 16.8	F = 3.7 P = 0.0371 R <sup>2</sup> = 18.1	F = · P = · R <sup>2</sup> = ·	F = · P = · R <sup>2</sup> = ·			

Means within a column (row), separated by day, and followed by the same lower (upper) case letter are not significantly different. Tukey HSD (SAS Institute 1985). For each ANOVA, R<sup>2</sup> = model sum of squares ÷ total sum of squares. df = 2, 33 for each comparison of Ma at fixed rates of imidacloprid. df = 3, 44 for each comparison of imidacloprid at fixed rates of Ma.

<sup>a</sup> Spores of *M. anisopliae* per gram of soil.

<sup>b</sup> Each mean based on n = 12 replicates (3 replicates for each of 4 trials). There were 25 termites per replicate.

tions against soil fungi (Cook et al. 1995). Zoberi and Grace (1990b) identified 40 species of fungi associated with *R. flavipes*, including many of the same microbes reported in our study and by Inglis et al. (1998). Although no data were provided, Zoberi and Grace (1990b) and Zoberi (1995) speculated on the impact of native soil microbes, and other antagonists, on the efficacy of entomopathogens against subterranean termites, suggesting that interspecific fungal interactions might benefit *R. flavipes*. Our data support their speculation.

The fungistatic properties of termites have been documented (Boucias et al. 1996, Rosengaus et al. 1998, Wiltz et al. 1998), but the impact of soil and the competitive effect of its native microbe fauna on entomopathogen efficacy against subterranean termites has not been adequately addressed. A review of assay techniques from available literature indicates that, in most cases, soil was removed as a variable when evaluating the efficacy of entomopathogens against subterranean termites. In most studies, pathogenicity investigations were typically performed in petri dish assays containing only termites and the candidate pathogen. Studies on efficacy screenings (Lai et al. 1982, Zoberi and Grace 1990a, Milner 1992, Wells et al. 1995, Zoberi 1995, Jones et al. 1996, Milner and Staples

1996, Rath and Tidbury 1996, Suzuki 1996, Milner et al. 1998) and the ecology of infection (Kramm et al. 1982, Zoberi and Grace 1990a, Grace and Zoberi 1992, Zoberi 1995, Boucias et al. 1996, Jones et al. 1996) of various entomopathogens, including various strains of *M. anisopliae*, against subterranean termites have been conducted almost exclusively in the absence of soil, and thus competing microbes.

The combination of imidacloprid and *M. anisopliae* killed termites at a faster rate than either treatment alone (Tables 3 and 4). A mounting body of evidence supports our data. Incorporation of *B. bassiana* in soil, even at extremely high concentrations, resulted in <5% termite mortality after 2 wk of continuous exposure (Boucias et al. 1996). Concurrent exposure of termites to *B. bassiana*-amended soil and filter paper discs dipped in a 0.001% imidacloprid solution, however, greatly enhanced susceptibility to the pathogen. For instance, after 2 wk of continuous exposure, mortality of termites exposed to either 10<sup>5</sup> conidia per gram of soil or 10<sup>5</sup> conidia per gram of soil and filter paper dipped in 0.001% imidacloprid was 1 and 99%, respectively (Boucias et al. 1996).

The primary defense against entomopathogen infection in *R. flavipes* is their social behavior of grooming (Smythe and Coppel 1966, Boucias et al. 1996).

Table 4. Analysis of variance, by day, of the effect of *M. anisopliae* at fixed levels of imidacloprid, and the effect of imidacloprid at fixed levels of *M. anisopliae* on *R. flavipes* mortality in sterile soil

Day	Ma <sup>a</sup>	Mortality (mean $\pm$ SE) <sup>b</sup> at concn. of imidacloprid in soil, ppm				ANOVA results		
		0	5	10	20	F	P	R <sup>2</sup>
3	0	0.0 $\pm$ 0.0aB	0.4 $\pm$ 0.2aB	1.0 $\pm$ 0.1aB	16.0 $\pm$ 2.3aA	5.3	0.0034	26.5
	10 <sup>4</sup>	0.0 $\pm$ 0.0aB	0.1 $\pm$ 0.1aB	0.0 $\pm$ 0.0aB	8.8 $\pm$ 1.6aA	5.0	0.0043	25.6
	10 <sup>7</sup>	0.0 $\pm$ 0.0aB	1.5 $\pm$ 0.2aB	14.6 $\pm$ 4.1aAB	53.0 $\pm$ 3.3aA	6.6	0.0009	30.9
		F = 1.0 P = 0.3788 R <sup>2</sup> = 5.7	F = 1.3 P = 0.2962 R <sup>2</sup> = 7.1	F = 2.9 P = 0.0697 R <sup>2</sup> = 14.9	F = 3.1 P = 0.0587 R <sup>2</sup> = 15.8			
7	0	0.0 $\pm$ 0.0aB	22.9 $\pm$ 3.6bAB	38.5 $\pm$ 3.1bA	49.0 $\pm$ 3.5bA	4.5	0.0074	23.6
	10 <sup>4</sup>	0.0 $\pm$ 0.0abB	62.6 $\pm$ 4.5bA	78.4 $\pm$ 3.5abA	81.1 $\pm$ 4.1abA	8.8	0.0001	37.6
	10 <sup>7</sup>	7.3 $\pm$ 1.7aB	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	98.3	0.0001	87.0
		F = 4.1 P = 0.0267 R <sup>2</sup> = 19.7	F = 10.7 P = 0.0003 R <sup>2</sup> = 39.3	F = 9.1 P = 0.0007 R <sup>2</sup> = 35.5	F = 6.2 P = 0.0052 R <sup>2</sup> = 27.3			
10	0	0.0 $\pm$ 0.0bC	57.7 $\pm$ 3.0bB	88.4 $\pm$ 2.6bAB	95.7 $\pm$ 0.7bA	23.9	0.0001	62.0
	10 <sup>4</sup>	0.1 $\pm$ 0.1abC	90.8 $\pm$ 2.1abB	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	96.9	0.0001	86.9
	10 <sup>7</sup>	7.3 $\pm$ 1.7aB	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	98.3	0.0001	87.0
		F = 3.8 P = 0.0331 R <sup>2</sup> = 18.7	F = 7.3 P = 0.0023 R <sup>2</sup> = 30.7	F = 4.7 P = 0.0162 R <sup>2</sup> = 22.1	F = 6.7 P = 0.0038 R <sup>2</sup> = 28.7			
14	0	0.0 $\pm$ 0.0bB	92.1 $\pm$ 1.6aA	96.4 $\pm$ 1.1aA	100.0 $\pm$ 0.0aA	73.6	0.0001	83.4
	10 <sup>4</sup>	0.1 $\pm$ 0.1bB	97.7 $\pm$ 1.1aA	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	200.7	0.0001	93.2
	10 <sup>7</sup>	54.2 $\pm$ 5.1aB	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	10.7	0.0001	42.0
		F = 12.6 P = 0.0001 R <sup>2</sup> = 43.3	F = 2.3 P = 0.1190 R <sup>2</sup> = 12.1	F = 3.2 P = 0.0531 R <sup>2</sup> = 16.3	F = . P = . R <sup>2</sup> = .			
21	0	0.0 $\pm$ 0.0cB	99.9 $\pm$ 0.1aA	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	2,068.3	0.0001	99.3
	10 <sup>4</sup>	41.6 $\pm$ 4.9bB	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	15.1	0.0001	50.8
	10 <sup>7</sup>	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	100.0 $\pm$ 0.0aA	.	.	.
		F = 37.1 P = 0.0001 R <sup>2</sup> = 69.2	F = 1.0 P = 0.3788 R <sup>2</sup> = 5.7	F = . P = . R <sup>2</sup> = .	F = . P = . R <sup>2</sup> = .			

Means within a column (row), separated by day, and followed by the same lower (upper) case letter are not significantly different. Tukey HSD (SAS Institute 1985). For each ANOVA, R<sup>2</sup> = model sum of squares  $\div$  total sum of squares. df = 2, 33 for each comparison of Ma at fixed rates of imidacloprid. df = 3, 44 for each comparison of imidacloprid at fixed rates of Ma.

<sup>a</sup> Spores of *M. anisopliae* per gram of soil.

<sup>b</sup> Each mean based on  $n = 12$  replicates (3 replicates for each of 4 trials). There were 25 termites per replicate.

The ability of termites to protect themselves from entomopathogen infection is disrupted by exposure to imidacloprid and results in enhanced susceptibility. Imidacloprid treatment did not alter the number of conidia that were able to attach to the termite cuticle, or alter the phagocytic, cellular defense reaction to a foreign body compared with normal termites (Boucias et al. 1996). Collectively, this evidence indicates that imidacloprid did not disrupt termite cellular defense mechanisms, and further suggests that social behaviors are the primary defense against pathogen infection. When termites were exposed to imidacloprid for 3 d, then dipped in a conidial suspension and held in groups, conidia were not removed (Boucias et al. 1996). Furthermore, imidacloprid-treated termites held either in groups or individually suffered similar, high rates of mortality after 2 wk of continuous exposure to soil containing 10<sup>2</sup> to 10<sup>7</sup> conidia per gram (Boucias et al. 1996).

Imidacloprid-enhanced susceptibility to both *B. bassiana* and *M. anisopliae* also was demonstrated in *D. abbreviatus* larvae (Quintela and McCoy 1997, 1998). Larval mortality was  $\leq 10\%$  1 wk after constant exposure to soil containing either 50  $\mu\text{g}$  of imidacloprid per gram or up to  $5 \times 10^5$  *M. anisopliae* conidia per gram. The addition of 50  $\mu\text{g}$  of imidacloprid per gram of soil

enhanced termite susceptibility to  $5 \times 10^3$ ,  $5 \times 10^4$ , and  $5 \times 10^5$  conidia per gram of soil by  $\approx 70$ ,  $\approx 80$ , and  $\approx 90\%$ , respectively (Quintela and McCoy 1998). Quintela (1996) attributed the enhanced susceptibility to behavioral changes in *D. abbreviatus* larvae. It seems that untreated larvae move through the soil with enough force to physically remove attached conidia. Imidacloprid-intoxicated larvae exhibit reduced mobility, preventing the physical removal of conidia, resulting in enhanced susceptibility. Similarly, in field tests, mortality in *L. lineolaris* was significantly higher after exposure to a combination treatment of *B. bassiana* and imidacloprid than either treatment alone; 5 d after treatment, mortality of plant bugs exposed to imidacloprid, *B. bassiana*, or the combination was 67.3, 52.0, and 97.9%, respectively (Steinkraus and Tugwell 1997).

There are many potential fungal entomopathogens associated with subterranean termites. Zoberi and Grace (1990b) surveyed the microbial fauna associated with *R. flavipes* in Ontario, Canada, and report several of the same fungi in common to those reported herein, most notably *C. echinulata*, *Fusarium* spp., and *Aspergillus* spp. Because the entomopathogens identified in our study were taken from assays conducted

in nonsterile soil, it remains unclear whether the microbes were soil- or termite-borne.

In laboratory assays, *A. niger* Van Tieghem, *A. flavus* Link, and *C. echinulata* were pathogenic to subterranean termites, but *Fusarium proliferatum* (Matsushima) Nirenberg was not (Beal and Kais 1962, Smythe and Coppel 1966, Suzuki 1996). High termite mortality was correlated with the presence of *C. coronatus* (Boucias et al. 1996). *C. coronatus* was typically detected within 4 d, and often killed all termites in the petri dish. The presence of *C. coronatus* also seemed to be positively correlated with imidacloprid dosage. Boucias et al. (1996) suggested that *C. coronatus* was associated with the termites because sterile conditions were used throughout their studies. In petri dish assays, *C. coronatus* was lethal when termites were forced to walk across a hyphal mat of the pathogen (Wells et al. 1995).

Microbes, especially fungi, exhibit some potential for the biological control of termites (Grace 1997). Although a wealth of data exists on the laboratory efficacy and ecology of infection of fungal pathogens against subterranean termites, success in their biological control with entomopathogenic fungi has been limited because of competition from naturally occurring microbes and the inherent social behaviors (grooming) of the target pest. In addition to being acutely toxic to termites, the chloronicotinyl imidacloprid alters the social behaviors of termites and renders them susceptible to entomopathogens.

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